CHAPTER 4
RESULTS AND DISSCUSION

4.1 *P. emblica* fruits extraction

The fresh fruits of *P. emblica* were washed, dried, and ground to powder. The yield value of the dried powder of the plant was 20.0%. The powder of dried fruits of *P. emblica* was macerated with 95% ethanol. The macerate was collected and then evaporated under reduced pressure until dryness. The crude extract of *P.emblica* fruits was obtained. After that, the crude extract was fractional extracted with n-hexane, chloroform, ethyl acetate and n-butanol, respectively. The fractional extract of each solvent was evaporated to dryness. The yield values of crude and fractional extracts were shown in Table 3.

Table 3 Yield values of *P. emblica* crude and fractional extracts

Crude extract	**% Yield**
P. emblica extract	21.23±1.04
Fractions*	**% Yield**
n-Hexane	5.37±0.33
Chloroform	1.24±0.64
Ethyl acetate	16.00±1.75
Butanol	26.78±0.85
Water residue	14.55±1.85

* The yield values of the fractional extracts were calculated based on the crude extract

As showed in Table 3, the crude extract of *P. emblica* fruits had the yield value of 21.23±1.04 %. This result was in accordance with that reported in the previous study (17) which demonstrated that the yield value of the *P. emblica* fruit extract was about 20%. The butanol fractional extracts of *P. emblica* fruits had the highest yield value of 26.78±0.85%, followed by ethyl acetate, water, n-hexane and

chloroform fractional extracts, respectively. The yield values of the fractional extracts revealed in this study were slightly different from the previous study (7) which reported that the highest yield was found in the ethyl acetate fraction among the water, butanol, benzene, and hexane fractions. The highest yield of butanol fractional extract found in this study might result from partitioning of water into butanol layer during partition extraction and thus made the evaporation of this fraction difficult and not complete.

The appearance of the *P. emblica* fruit extracts were shown in Figure 12. The appearance of *P. emblica* crude extract was viscous semi-solid with dark brown in colour. The butanol and water fractions were dark brown solid. The ethyl acetate fraction was light brown semi-solid, whereas the hexane extract was green semi-solid because of chlorophyll.

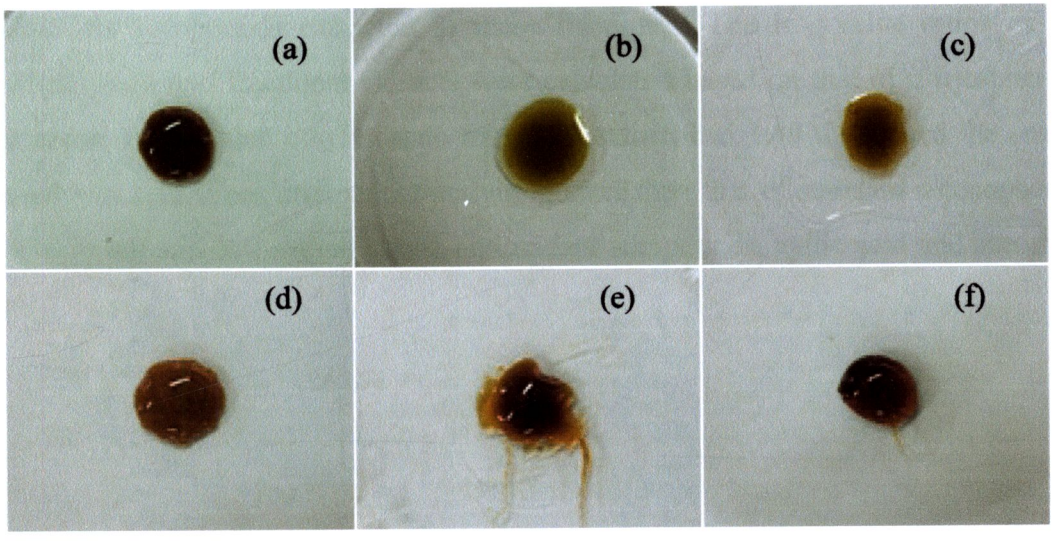
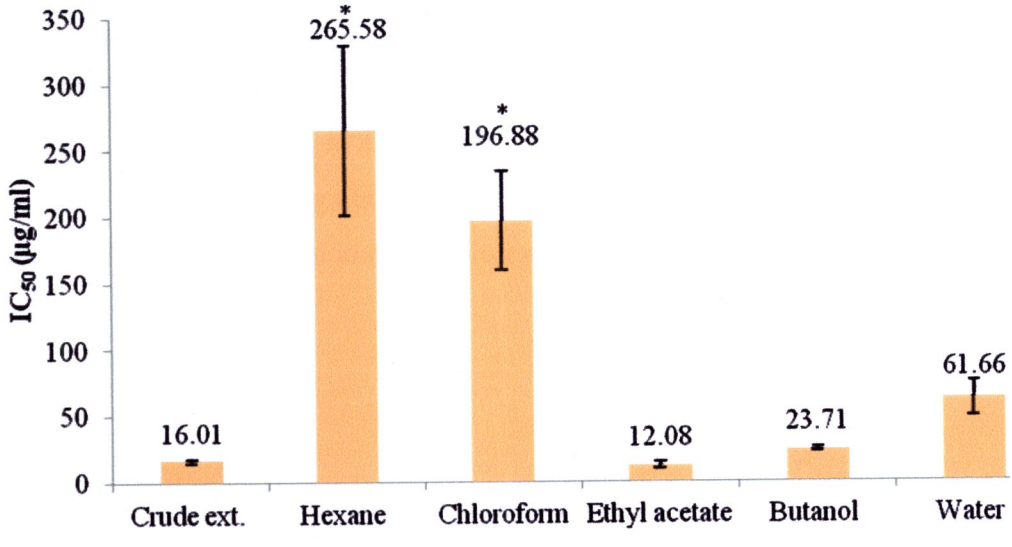


Figure 12 Appearance of *P. emblica* crude extract (a), n-hexane (b), chloroform (c), ethyl aceate (d), butanol (e), and water residue (f) fractional extracts

4.2 Antioxidant activity of *P. emblica* crude and fractional extracts

4.2.1 DPPH radical scavenging activity (DPPH method)

The antioxidant activity of *P. emblica* fruit extracts on radical scavenging activity via DPPH method was evaluated and the results were shown in Figure 13 and summarized with the IC_{50} values of the standard gallic acid, ascorbic acid (vitamin C), and α-tocopherol (vitamin E) in Table 4. The ethyl acetate fractional extract had the highest antioxidant activity with the lowest IC_{50} value of 12.08±2.62 μg/ml. Its value was equivalent to 75.45% and 67.14% of the IC_{50} of the crude extract and α-tocopherol. All other fractions had higher IC_{50} value than that of the crude extract and α-tocopherol. The hexane and chloroform fractional extracts had the highest IC_{50} values or the lowest antioxidant activity. Their values were significantly different ($p<0.05$) from those of the other extracts, indicating that there was less active antioxidant compounds extracted in these fractions. The IC_{50} value ratios of *P. emblica* crude and fractional extracts were calculated based on that of α-tocopherol. The result showed that ethyl acetate fractional extract had 1.49 times, and the crude extract had 1.12 times antioxidant activity greater than that of standard α-tocopherol (Table 5) but still lower than strong antioxidant compounds, gallic acid and ascorbic acid.



(*) $p<0.05$, multiple comparison by LSD

Figure 13 IC_{50} values on DPPH method of *P. emblica* crude and fractional extracts

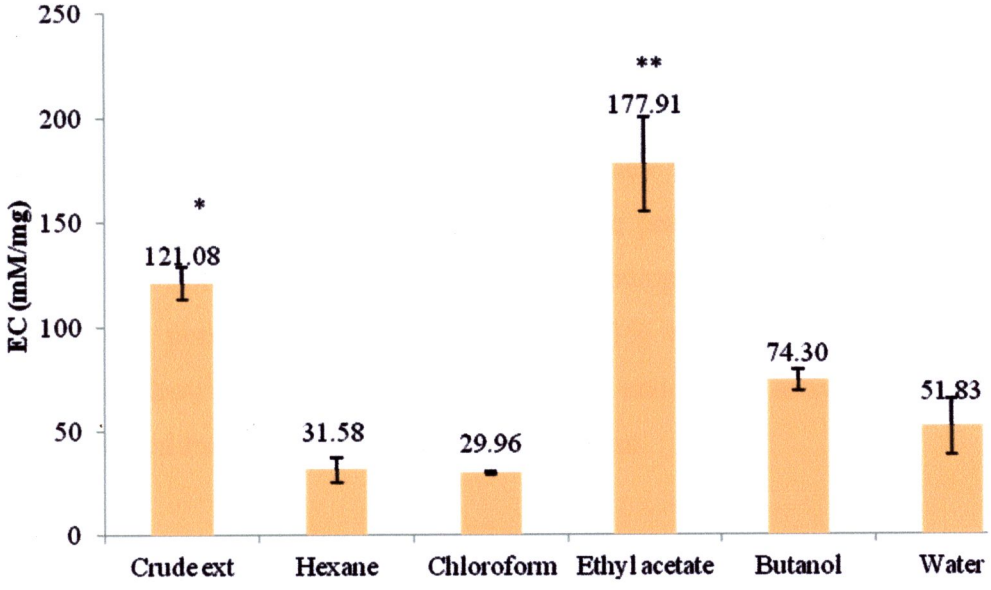
Table 4 IC_{50} values of *P. emblica* crude and fractional extracts and IC_{50} value ratios based on that of α-tocopherol

Samples	IC_{50}	IC_{50} ratio α-tocopherol /ext.
Crude ext.	16.01±1.83	1.12
Hexane	265.58±63.98*	0.07
Chloroform	196.88±37.18*	0.09
Ethyl acetate	12.08±2.62	1.49
Butanol	23.71±1.76	0.76
Water	61.66±13.20	0.29
std. Gallic acid	2.48±0.89	7.25
std. Ascorbic acid	7.86±1.12	2.29
std. α-Tocopherol	17.99±0.94	1.00

(*) $p<0.05$, multiple comparison by LSD among *P. emblica* crude and fractional extracts

4.2.2 Reducing ability on FRAP method

The antioxidant activity of *P. emblica* crude and fractional extracts on FRAP method was shown in Figure 14. The ferrous sulphate ($FeSO_4$) was used as a standard reducing agent and the standard curve was shown in Figure 15. The results was expressed in term of EC value; the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$. The fraction of ethyl acetate showed the highest reducing ability with EC value of 177.91±22.43 mM/mg. The EC values of ethyl acetate fraction and crude extract were significantly higher than other fractions ($p< 0.05$).



(*) $p<0.05$

Figure 14 EC values of *P. emblica* crude and fractional extracts

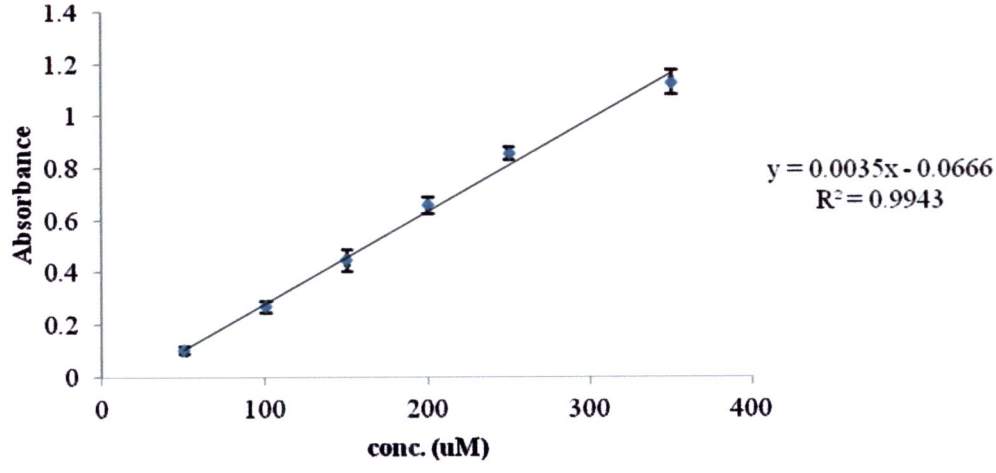


Figure 15 Standard curve of $FeSO_4$

4.3 Total phenolic content of *P. emblica* crude and fractional extracts

The total phenolic contents of *P. emblica* crude and fractional extracts were determined in this study by Folin-ciocaltue method and the results are shown in Figure 16. The ethyl acetate fraction had the highest total phenolic content with the GAE value of 51.65±7.69 mg/g dry extract. The previous studies showed that the extract of *P. emblica* is composed of phenolic compounds, namely emblicanin A, emblicanin B, punigluconin and pedunculagin (16) with gallic acid as substituent groups(21). Thus, it can be indicated that the high antioxidant activity of ethyl acetate fraction resulted from the high total phenolic content.

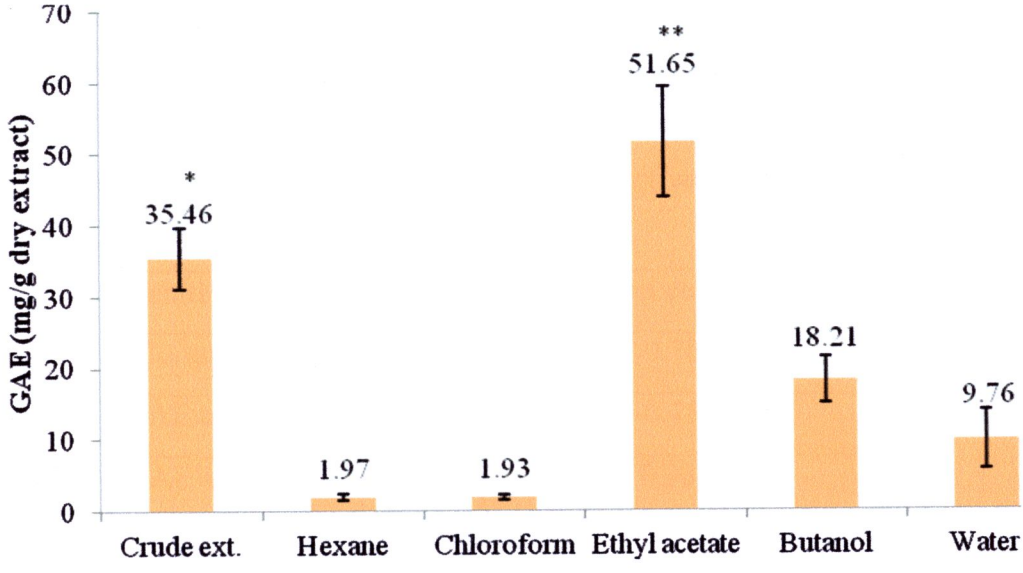


Figure 16 GAE values of *P. emblica* extracts

4.4 Correlations between antioxidant activity and total phenolic content

In this study, the correlations between individual antioxidant activity and the total phenolic content of the crude and fractional extracts were evaluated and the results are shown in Table 5. The correlation coefficients (r) of all relationships were more than 0.9 or less than -0.7, indicating good correlations between these parameters.

It was previously reported that polyphenols found in dietary and medicinal plants are able to inhibit oxidative stress (37, 55) and the direct linear relationship between the total phenolic content and total antioxidant activity was observed (56). From the results, it was highly possible that polyphenolic compounds in *P. emblica* extracts were responsible for the antioxidation activity via two mechanisms i.e., free radical scavenging and reducing ability.

Table 5 The correlation coefficients of antioxidant activity and total phenolic content of *P. emblica* crude and fractional extracts

Relationship	**Correlation coefficients (r)**
IC_{50} and EC	-0.704**
GAE and IC_{50}	-0.750**
GAE and EC	0.981**

**$p<0.01$

4.5 HPLC analysis of the *P. emblica* crude and fractional extracts

The HPLC chromatogram of *P. emblica* crude extract (Figure 17) demonstrated 2 major peaks at the retention times of 4.470 min and 6.339 min, corresponding to ascorbic acid and gallic acid, respectively. The HPLC chromatograms of the ethyl acetate fraction showed that polyphenols, particularly gallic acid were substantially extracted by ethyl acetate. The chromatogram of the water fraction demonstrated the large peak of ascorbic acid, indicating that this substance remained in the water after organic solvent extraction due to its high polarity (Figure 18). There was no gallic acid or ascorbic acid peaks in the chromatograms of hexane and chloroform fractions, while only the small peak of gallic acid was observed in the chromatogram of butanol fraction (Figure 19). The result in Figure 20 demonstrated that ethyl acetate fractional extract was composed of gallic acid more that on fourth of the net weight (270.16±2.55 µg per 1 mg of the extract).

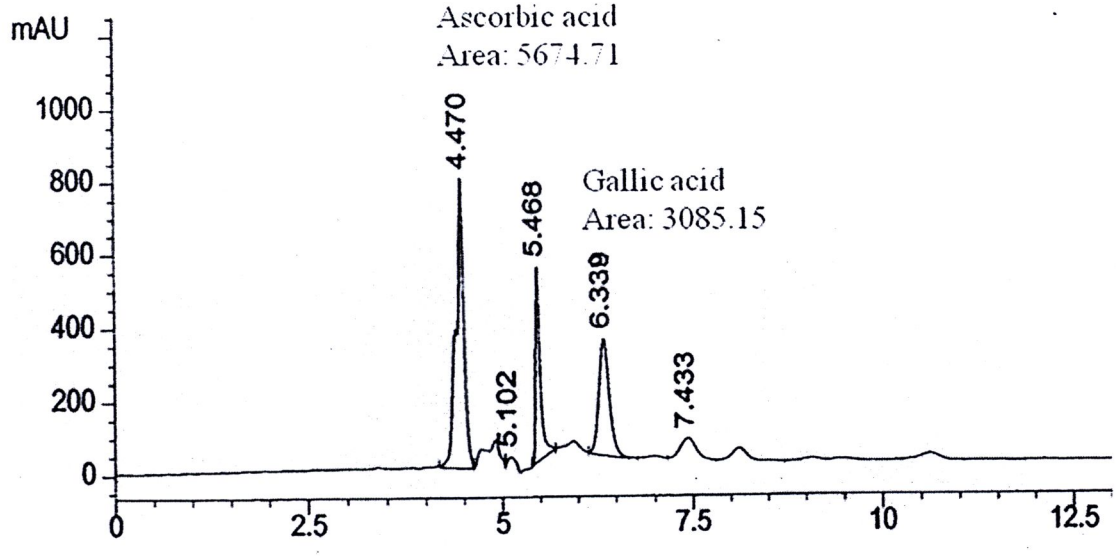


Figure 17 HPLC chromatogram of *P. emblica* crude ethanol extract

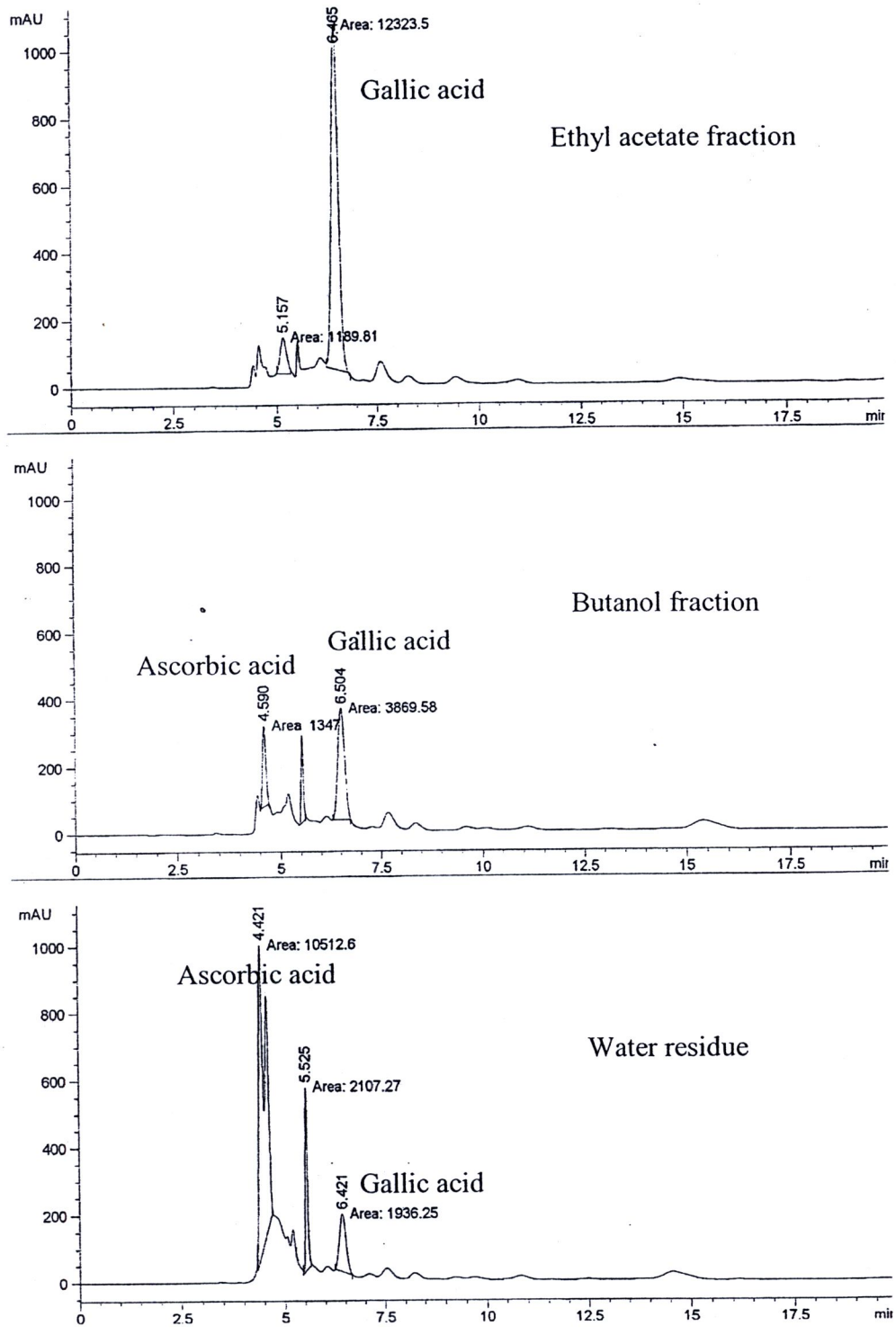
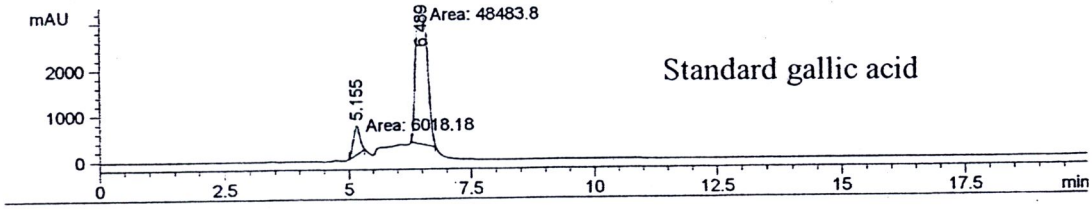
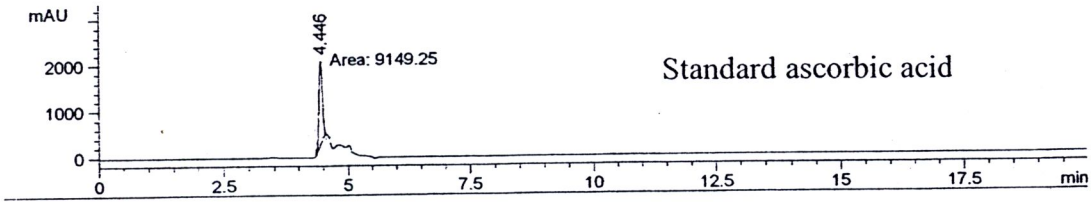


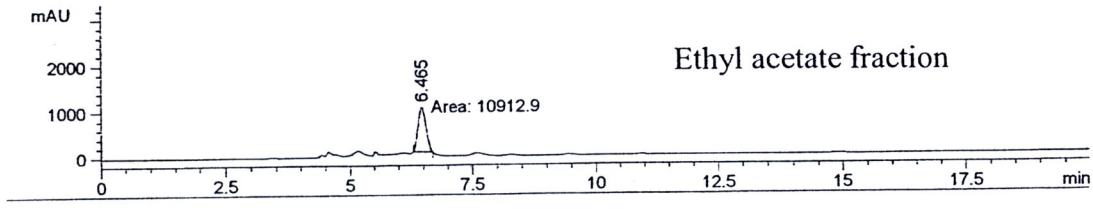
Figure 18 The HPLC chromatogram of *P. emblica* fractional extracts



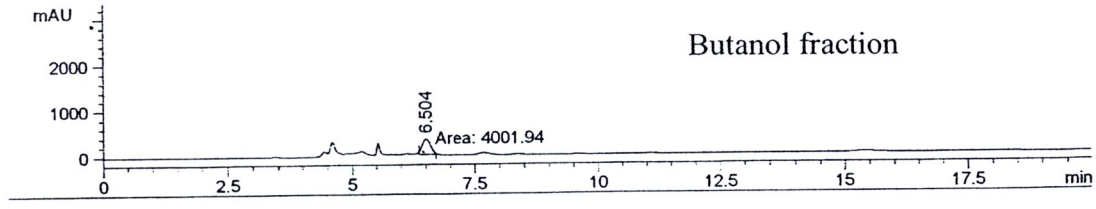
Standard gallic acid



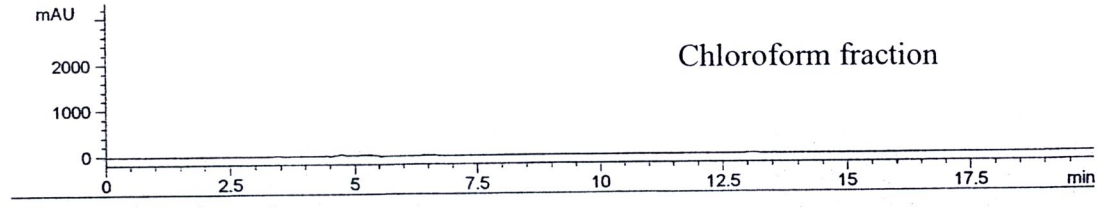
Standard ascorbic acid



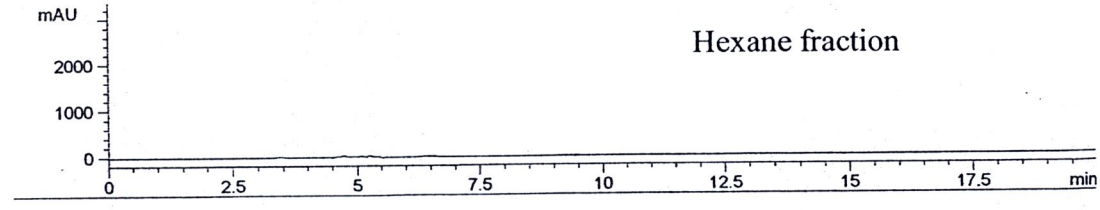
Ethyl acetate fraction



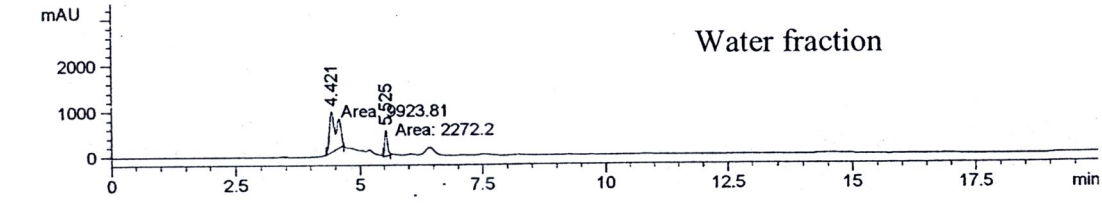
Butanol fraction



Chloroform fraction



Hexane fraction



Water fraction

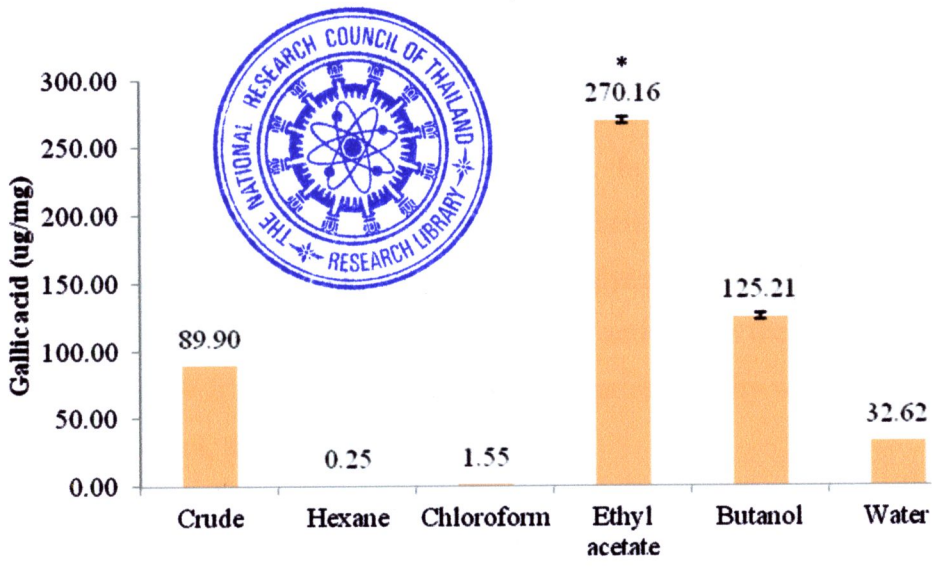


Figure 20 Gallic acid contents in the *P. emblica* crude and fractional extracts

The previous studies on active compounds in *P. emblica* fruit extract were controversial. Bhattacharya and coworkers (16) demonstrated that this fruit extract had the hydrolysable tannins namely emblicanin A, emblicanin B, punigluconin, pedunculagin. However, Kumaran and colleagues (17) found only gallic acid, methyl gallate, corilagin, furosin, and geraniin in the *P. emblica* fruit extract. The recent study by Majeed and coworkers and coworkers (20) showed the content of β-glucogallin an mucic acid 1,4-lactone 5-*O*-gallate instead of emblicanin A, and emblicanin B. The studies of Raghu et al. (19) and Scartezzini et al. (18) revealed that the *P. emblica* fruit extract had high vitamin C content. In this study, gallic acid and ascorbic acid were found in the *P. emblica* fruit extracts by HPLC analysis. As for gallic acid peak in the chromatograms, it is plausible that this peak were the combination of various gallic acid derivatives because the HPLC condition used in this study was isocratic, while most of the previous studies used gradient condition. Therefore, the separation of gallic acid derivatives was not achieved in this study. However, this condition was sufficient to compare the total amount of gallic acid derivatives found in each fractional extract. The HPLC results were corresponding to the total phenolic content assay as well as the antioxidant activity. It can be concluded that *P. emblica* fruit extract was a high source of ascorbic acid and polyphenolic compounds which were gallic acid derivatives. These substances are believed to contribute to the high antioxidant activity of this fruit extract.

4.6 Antiglycation activity of *P. emblica* crude and fractional extracts

The inhibitory activity of *P. emblica* fractional extracts on AGEs formation was studied and the results were shown in Figure 21. In this study, the hexane and the chloroform fractions were not included because of their low yield value and defect in solubility in PBS, the solvent used for *in vitro* antiglycation study. The ethyl acetate fraction of *P. emblica* had the highest % inhibitory activity on the glycation reaction with IC_{50} value of 19.20±1.99 µg/ml and its antiglycation activity was higher than that of the positive control, aminoguanidine (AG).

The studies of importance of glycation reaction and AGEs formation on diabetic complications have been reported (42, 43). During glycation reaction occurred *in vivo,* free radicals are generated (26) and they can damage proteins, lipids, and nucleic acids and might contribute toward tissue damage and complications in diabetes The relationship between antioxidant and antiglycation activity was studied, and the compound which had antioxidant activity tended to have antiglycation activity(50). This was similarly observed in our results, the ethyl acetate fractional extract exhibited the highest antioxidant activity along with antiglycation activity.

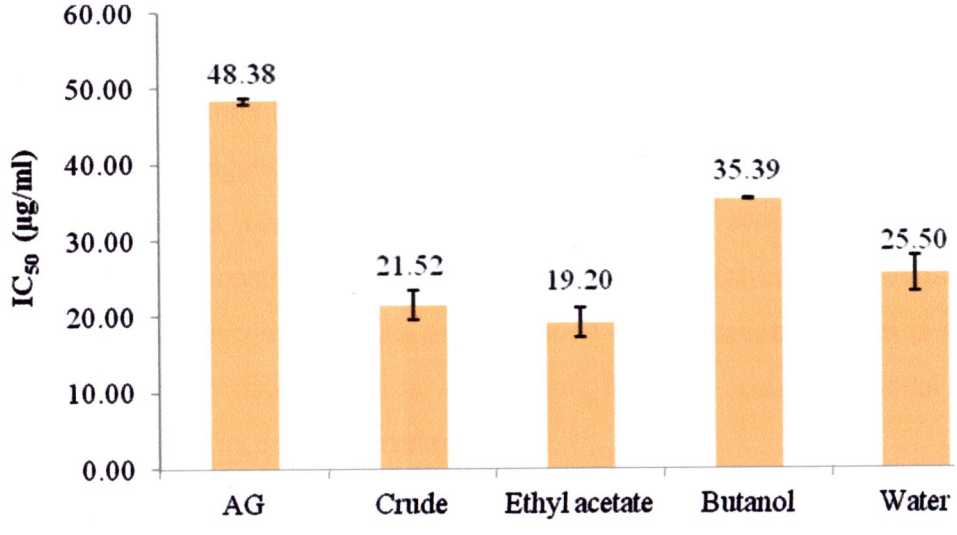


Figure 21 IC_{50} values on antiglycation activity of *P. emblica* extracts and aminoguanidine

Figure 21 IC_{50} values on antiglycation activity of *P. emblica* extracts and aminoguanidine

According to the results of this study, the ethyl acetate fractional extract of *P. emblica* fruits showed the highest antioxidant and antiglycation activities corresponding to the highest phenolic compound content which was gallic acid and its derivatives and its appearance was lighter in color. This suitable fraction was selected for further tablet formulation in the following study.

4.7 Formulation of *P. emblica* ethyl acetate fractional extract (PEF) tablets

4.7.1 Preformulation study on rheological properties of *P. emblica* crude and fractional extracts

The ethyl acetate fractional extract of *P. emblica* fruits was investigated for the rheological properties compared with the crude extract. The viscosity and rheological behavior of the extracts were shown in Table 6 and the rheograms of *P. emblica* crude and fractional extracts were demonstrated in Figure 22. The ethyl acetate fractional extract had significantly lower viscosity than that of the crude extract. It can be suggested that ethyl acetate fraction extraction could eliminate inactive and viscous components and resulted in the semi-purified bioactive compounds that demonstrated higher antioxidant and antiglycation activities. In addition, the highly viscous materials might cause a disintegration problem in a tablet formulation in the subsequent process. As a result, the ethyl acetate fractional extract offered superior benefits over the crude extract.

Table 6 Viscosity of *P. emblica* crude extract and ethyl acetate fractional extracts

Extract	Average Eta [Pas]
Crude	242.64±107.06
Ethyl acetate	127.00±36.26

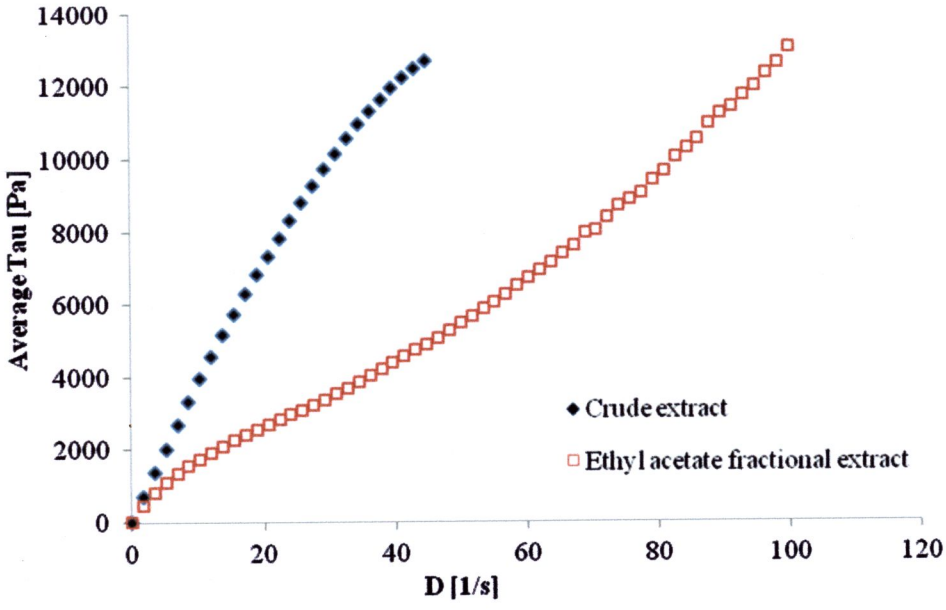


Figure 22 Rheograms of *P. emblica* crude extract and ethyl acetate fractional extract

4.7.2 Dosage calculation of PEF tablet

The dose of the PE tablet was calculated equivalent to the antioxidant activity of the daily recommended dose of vitamin E (α-tocopherol) via DPPH method. The dose of vitamin E used for dietary supplement is 400-1000 IU (international unit) or 266.7-666.7 mg per day. The ethyl acetate fraction showed the antioxidant activity 1.49 times higher than vitamin E, so the dose of PEF tablet should be within the range of 176.97 to 447.43.mg per day. The details of the calculation were shown in the appendix. In this study, we selected the content of the PEF in a tablet at 250 mg, that is equivalent to 372.5 mg of vitamin E and therefore the recommended consumption of the PEF tablet was 1-2 tablets per day.

4.7.3 Evaluation of diluents

Certain diluents that are commonly used in tablet formulation i.e., lactose, corn starch, rice starch, calcium hydrogen phosphate dihydrate (Emcompress®) and microcrystalline cellulose (Avicel® PH101), were gradually added and mixed with PEF until the suitable wet mass was obtained. All diluents could produce good wet

PEF until the suitable wet mass was obtained. All diluents could produce good wet mass when combined with PEF. However, the wet mass produced from calcium hydrogen phosphate dihydrate was very sticky and could not be pressed through the sieve. The characteristics of the obtained wet mass and the resulting dry granules prepared from various diluents except calcium hydrogen phosphate dihydrate were shown in Figure 23. The ratio of ethyl acetate (EtOAc) fractional extract of *P. emblica* to diluent that could produce good wet mass were shown in Table 7. This ratio was used to estimate the tablet weight of PEF tablet containing 250 mg of *P. emblica* ethyl acetate fractional extract. In general, the tablet weight should be minimum to provide the consumers with ease when administration.

The ratio of PEF: lactose and Emcompress® were 1:9.3, and 1:6.60. This result suggested the large amount of diluents required in the formulation to obtain the suitable wet mass. As a result, the wet mass produced from these diluents had lighter brown color than those produced from other diluents as can be seen in Figure 23. In addition, the calculated tablet weights for these diluents were more than 1,000 mg. Tablets with such a high weight were considered too large for administration per oral and these diluents were not considered suitable.

The ratios of PEF: corn starch (1:1.22), rice starch (1:0.88) and Avicel® PH101 (1:0.78) were closed to 1:1. These diluents thus could produce the tablets which had the total weights of about 500 mg which were not considered too large for oral administration. Thus, these three diluents were chosen as suitable candidates for further pharmaceutical property studies, namely moisture content, hardness, and disintegration time (DT).

The dry granules prepared from corn starch, rice starch and Avicel® PH101 were compressed into tablets at a compression force of 9800 N (1 ton). The pharmaceutical properties of the resulting tablets were shown in Table 8 and the pictures of the tablets were demonstrated in Figure 24.

Figure 23 Wet mass and dried granules of PEF prepared from various diluents: lactose (a), corn starch (b), rice starch (c), and Avicel® PH101 (d)

Table 7 Ratios of ethyl acetate (EtOAc) fractional extract of *P. emblica* and diluents and the estimated tablet weight calculated based on 250 mg of the extract

Diluent	**EtOAc fraction:Diluent**	**Tablet weight (mg)**
Lactose	1:9.33	2,583
Corn starch	1:1.22	554
Rice starch	1:0.88	471
Emcompress®	1:6.60	1,900
Avicel® PH101	1:0.78	445

Table 8 Some pharmaceutical properties of PEF tablets using different diluents and compressed at 9800 N (1 ton)

Diluent	**Tablet weight (mg)**	**Moisture content* (%)**	**Hardness (N)**	**DT (min)**
Corn starch	554.17±18.76	1.36±0.19	3.27±2.83	7.60±0.4
Rice starch	470.83±7.22	1.25±0.25	18.63±0.98	13.30±1.1
Avicel®PH101	465.83±14.43	0.93±0.01	61.46±4.53	27.30±1.8

* the moisture contents of tablets after storage at 30°C and 75% RH for 24 hr.

Figure 24 Appearance of PEF tablets prepared from corn starch (a), rice starch (b), and Avicel®PH101 (c) as a diluent

All tablets produced from corn starch, rice starch and Avicel® PH101 had low moisture adsorption property. This result suggested that was not very hygroscopic, and thus the control of relative humidity in the environment during the manufacturing process is considered not important. The hardness of tablets using Avicel®PH101 as a diluent was very high (61.46±4.53 N), as a result of the high compressibility property of microcrystalline cellulose, However, these tablets showed very slow disintegration times (27.30±1.8 min), although they contained high amount of microcrystalline cellulose in the formulation. Tablets made from corn starch had the fastest disintegration time but the lowest hardness, whereas tablets using rice starch had intermediate compressibility and disintegrating properties. To produce *P. emblica* ethyl acetate fractional extract tablets with all acceptable pharmaceutical properties, mixed diluents between Avicel® PH101 and corn starch in several ratios was investigated and the results were shown in Table 9.

Tablet 9 Pharmaceutical properties of PEF tablets using mixed diluents between Avicel® PH101 and corn starch at various ratios

Diluent	Diluents used per 1 g. extract	Tablet weight (mg)	Moisture content (%)	Hardness (N)	DT (min)
Avicel:Corn starch (1:1)	0.67	417.5	1.47±0.01	8.83±3.54	10.2±2.1
Avicel:Corn starch (1:0.75)	0.84	460.0	1.35±0.01	18.96±1.13	14.0±0.4
Avicel:Corn starch (1:0.5)	0.87	467.5	1.02±0.03	29.42±4.90	19.5±1.0
Avicel:Corn starch (1:0.25)	0.78	445.0	1.06±0.19	37.59±2.83	20.8±2.5

Tablets produced from the mixed diluents between Avicel® PH101 and corn starch in general gave good tablet weights (lower than 500 mg) and low moisture adsorption property. When increasing the concentration of corn starch, the disintegration time of tablet was improved but the hardness was impaired. In summary, there was no formulation exhibited the acceptable hardness (>40 N) and disintegration time (< 15 min) from this approach. As a result, the formulation containing only Avicel® PH101 as a diluent was selected and addition of superdisintegrant was considered to enhance the disintegration property of the tablets.

4.7.4 Evaluation of superdisintegrant concentrations

From the previous study, PEF tablets prepared using only Avicel® PH101 as a diluent had suitable pharmaceutical properties except for the disintegration times which were longer than 15 min. Hence, croscarmellose sodium (Ac-Di-Sol®) was added into a formulation at various concentrations (1, 3 and 5%) to improve the disintegration property. In this process, 2% talcum and 0.5% magnesium stearate were added as a glidant and a lubricant to investigate the effect of adding lubricants simultaneously. Purified talcum and magnesium stearate have a water resistance property, and they might reduce tablet hardness. To compensate this effect, the compaction force used for production of the tablets in this step was investigated at two compression forces 9,800 N (1 ton) and 14,700 N (1.5 ton). The effects of adding

Ac-Di-Sol® at various concentrations with and without lubricants (purified talcum and magnesium stearate) on pharmaceutical properties were shown in Figures 25-27.

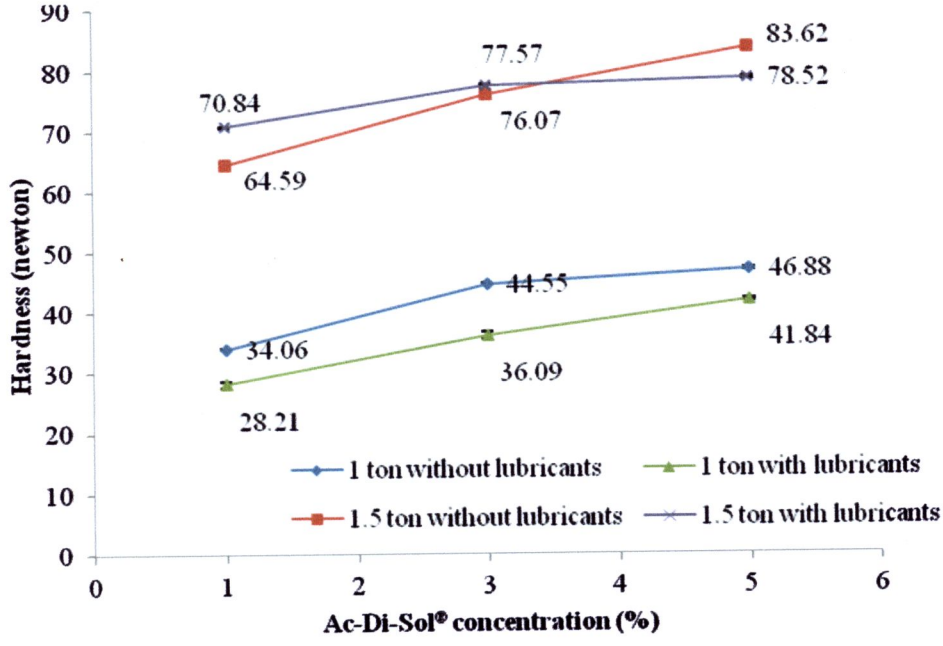


Figure 25 Hardness of the PEF tablets containing Ac-Di-Sol® at various concentrations

The hardness of the tablets compressed at 14,700 N (1.5 ton) was markedly greater than those compressed at 9800 N (1 ton). When adding purified talcum and magnesium steatrate, the tablet hardness was slightly reduced at both compression forces (Figure 25). In the following experiments, the effect of adding a superdisintegrant was evaluated in PEF tablets produced with 14,700 N compaction forces. The results of the disintegration study of PEF tablets containing different percentages of Ac-Di-Sol® were shown in Figure 26. Tablets disintegrated faster when they contained higher concentrations of Ac-Di-Sol®. The effect of adding lubricant was slight but noticeable. Tablets which had 5% Ac-Di-Sol® as a superdisintegrant and contained no purified talcum and magnesium steatrate had the fastest disintegration time of 11.69±0.33 min. The results of moisture adsorption study illustrated in Figure 27 revealed that addition of superdisintegrant slightly increased the moisture adsorption property of the tablets. This finding indicated the

hygroscopicity nature of a disintegrant. However, the moisture contents of all formulations were considered low.

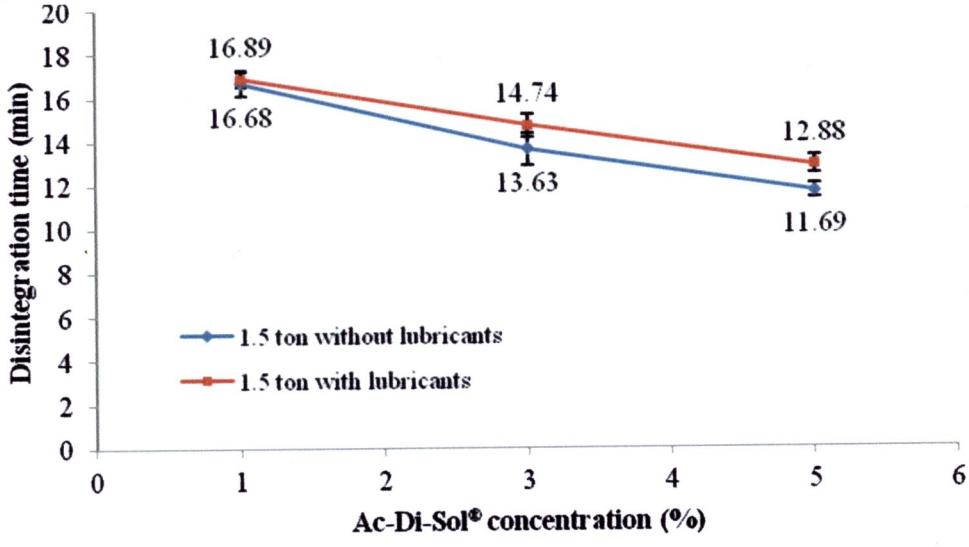


Figure 26 Disintegration time of PEF tablets containing different concentrations of Ac-Di-Sol® compressed at 14,700 N (1.5 ton)

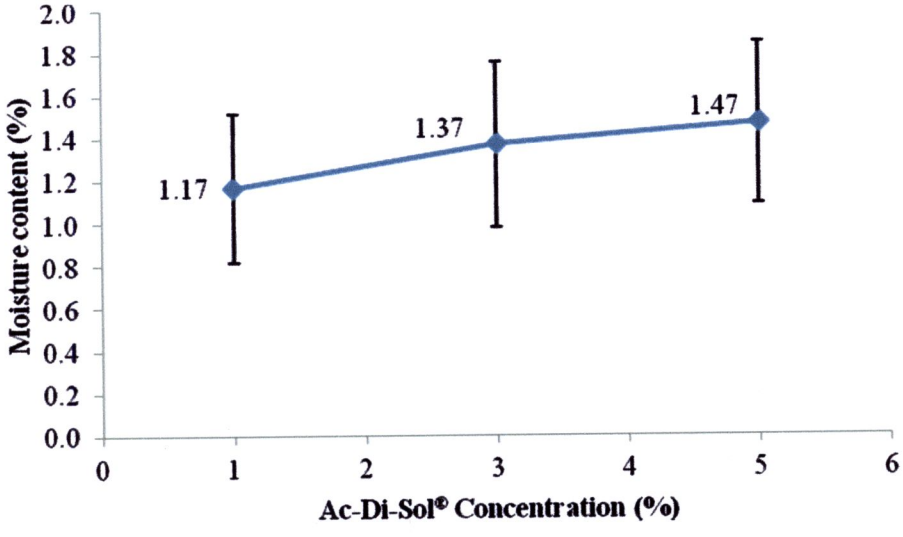


Figure 27 Moisture content of PEF tablets containing different concentrations of Ac-Di-Sol® after storage at 30°C, 75% RH for 24 hr

The amount of gallic acid content in PEF tablets was investigated. The results showed that gallic acid found in the PEF tablet was 63.13 mg per tablet, while the gallic acid content in the PEF used for 1 tablet (250 mg) before formulation was 67.54 mg. It could be indicated that the gallic acid content in PEF was only slightly reduced during the formulation process.

The suitable PEF tablets formulation was suggested in Table 10. The appearance of the obtained tablets in comparison with tablets prepared from the crude extract under the same formulation and tableting condition was shown in Figure 28. It can be seen that the color of the crude extract tablets was obviously darker than the ethyl acetate fractional extract tablets. The crude extract tablets were likely to be much more hygroscopic and finally the good quality tablets were not obtained.

Tablet 10 The appropriate formulation of PEF tablet

	Amount (mg)	**%**
P. emblica fractional extract	250	53.09
Avicel®PH101	188.05	39.93
Ac-Di-Sol®	21.9	4.65
Talcum	8.76	1.86
Mg stearate	2.19	0.47
total	470.9	100.00

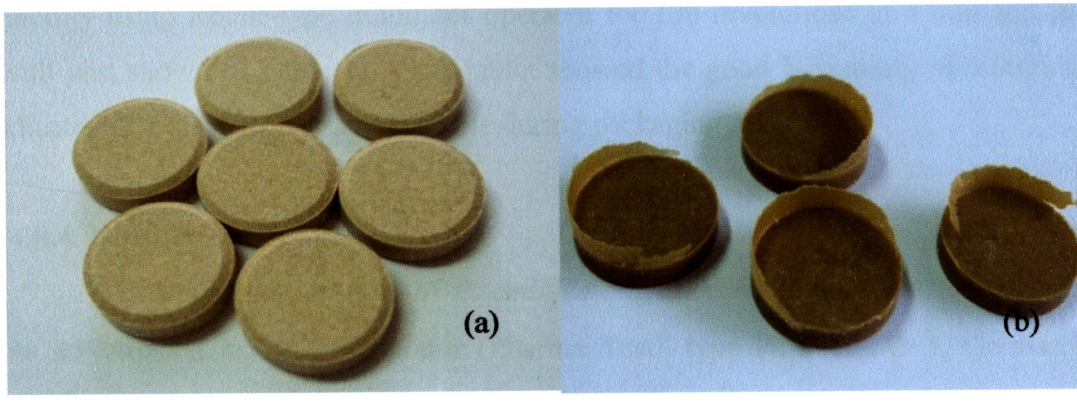


Figure 28 Appearances of PEF tablets (a) and crude extract tablets (b)

4.8 Quality control of the finished product

The preparation of *P. emblica* ethyl acetate fractional extract tablets was scaled up by using the single stroke tableting machine (HANSEATEN Wilhelm Fette, Germany) instead of the hydraulic press. Then, they were evaluated for quality control by studying the weight variation, thickness and diameter, hardness, friability test, disintegration time and dissolution test.

4.8.1 Weight variation, Diameter, Thickness

Twenty tablets were measured and weighed individually and the results were shown in Table 11. The diameter and the thickness of the PEF tablets were 12.64±0.01 and 3.34±0.01 mm, respectively. The average weight of the tablet was 514.9±9.3 mg. All tablets had their weights within the acceptable range of 5% of the average weight (489.2-540.7 mg)

4.8.2 Hardness

The hardness of PEF tablets was studied and the results were shown in Table 11. The average tablet hardness was 67.72±4.95 N. The appropriate hardness of the tablet should primarily be more than 40 N to provide the tablets which is not fractured during packaging process and storage.

4.8.3 Friability test

The *P. emblica* ethyl acetate fractional extract tablets were studied for friability using Roche-type friabilator operated for 100 revolutions in 4 min and the result was shown in Table 11. The tablet showed the good %friability of 0.0058%, indicated that these tablets would stable during packaging and storage

4.8.4 Disintegration time

The disintegration time of *P. emblica* ethyl acetate fractional extract tablets was investigated using disintegrator (Pharma Test® type PTZ-AUTO 3, Germany) with discs and the result was shown in Table 11. The average disintegration time of the *P. emblica* extract tablet was 19.19±2.32 min, slightly slower than the tablets prepared by a hydraulic press (12.88±0.41 min).

Table 11 Pharmaceutical properties of PEF tablets

Pharmaceutical properties	Values
Weight (mg)	514.9±9.3
Diameter (mm)	12.64±0.01
Thickness (mm)	3.34±0.01
Hardness (N)	67.72±4.95
Friability (%)	0.0058
Disintegration time (min)	19.19±2.32

4.8.5 Dissolution test

The dissolution of the PEF tablets was studied using a dissolution tester (Pharma test, PTW 600, Germany) with apparatus 2, paddle method. The amounts of gallic acid dissolved were investigated at 5, 10, 15, 30, 45, 60 min and the dissolution profile of the *P. emblica* extract tablet was shown in Figure 29. Gallic acid could dissolve more than 90% in phosphate buffer pH 6.8 within 60 minutes, providing that it is ready for absorption in the gastrointestinal tract after administration.

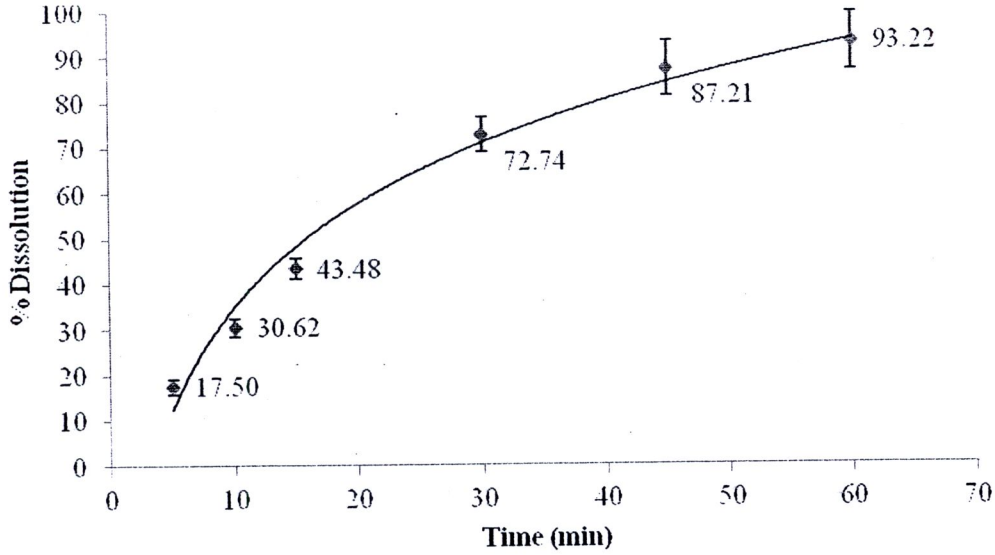


Figure 29 Dissolution profile of PEF tablets

4.9 Stability of PEF tablet

The stability of PEF tablets were studied by storage the tablet in an ambient condition (30°C, 65% RH) and accelerated condition (45°C, 75% RH) for 120 days. After predetermined time, tablets were taken to investigated for the antioxidant activity, total phenolic content, antiglycation activity, and HPLC finger print at days 0, 7, 15, 30, 60, 90, and 120.

4.9.1 Stability of antioxidant activity of PEF tablets

4.9.1.1 Scavenging activity on DPPH method

The stability of antioxidant activity of PEF tablets in scavenging the free radical, DPPH, was studied. The results were showed in term of average IC_{50} (Table 12). The average IC_{50} values tended to increase from 52.80±1.81 µg/ml at day 0 to 55.75±1.80 µg/ml at 30°C, 65%RH (105.59±3.41%), and 71.36±2.12 µg/ml (135.15±4.02%) at 45°C, 75% RH on day 120. The average IC_{50} value of PEF tablets which were kept at 45°C was higher than that of 30°C. It suggested that the antioxidant activity of the tablet was reduced at elevated temperature. The % increasing of IC_{50} value of the tablets form day 0 to day 120 was shown in Figure 30.

Table 12 Average IC_{50} values of PEF tablet after storage at 30°C, 65% RH and 45°C, 75% RH for 120 days

Day	IC_{50} values (µg/ml)	
	30°C, 65% RH	**45°C, 75% RH**
0	52.80±1.81	52.80±1.81
7	54.06±1.64	53.60±4.21
15	56.53±1.32	59.55±4.25
30	54.02±2.71	59.04±5.67
60	50.47±3.79	54.73±2.61
90	55.40±2.02	60.48±3.81
120	55.75±1.80	71.36±2.12

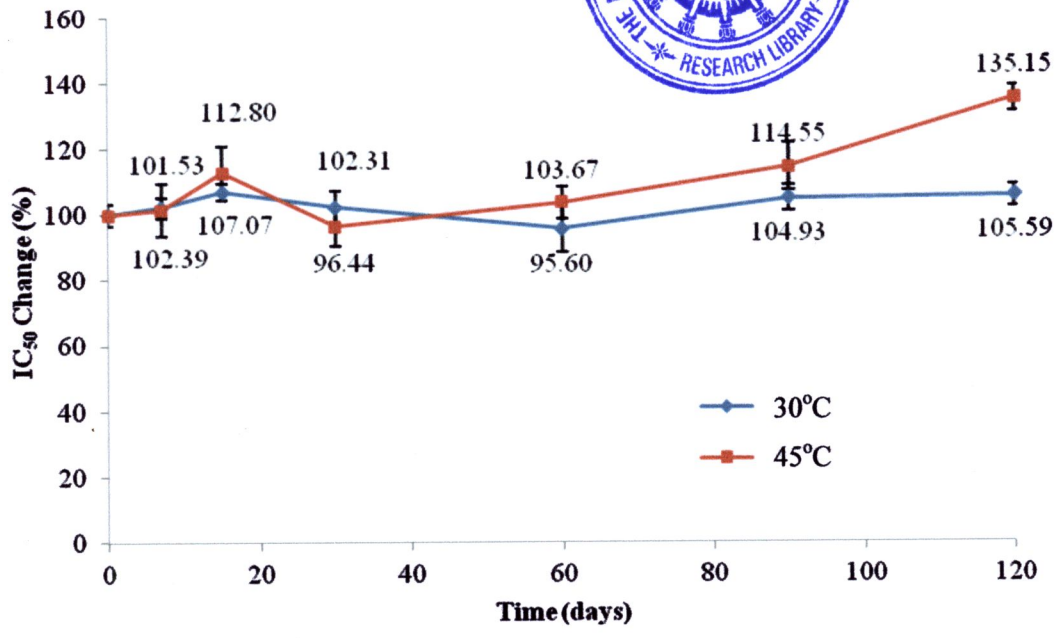


Figure 30 The percentage increase of IC_{50} values of PEF tablets after storage at 30°C, 65% RH and 45°C, 75% RH for 120 days on antioxidant activity

4.9.1.2 Reducing ability on FRAP method

The reducing ability of *P. emblica* ethyl acetate fractional extract tablets was studied for its stability. The results were shown in term of EC values in Table 13 and Figure 31. It was found that the EC values of the tablets were reduced from 282.13±17.39 mM/mg extract to 242.91±11.98 mM/mg extract (86.10±7.79 %) when kept at 30°C, 65% RH for 120 days. The tablets kept under the condition of 45°C, 75% RH showed the lower EC values and the lowest value was observed at day 120 with EC value of 207.17±16.06 mM/mg (73.43±5.69 %). It could be indicated that the antioxidant activity of *P. emblica* ethyl acetate fractional extract tablets measured by FRAP method reduced faster at high temperature; however, the antioxidant activity of the tablets still remained at high percentage, particularly at ambient temperature.

Table 13 EC values of the PEF tablets after storage at 30°C, 65% RH and 45°C, 75% RH for 120 days

Days	EC value (mM/mg extract)	
	30°C, 65% RH	**45°C, 75% RH**
0	282.13±17.39	282.13±17.39
7	247.69±10.99	236.52±9.61
15	236.15±4.05	264.22±10.87
30	239.32±4.68	257.98±7.48
60	260.11±11.24	247.92±8.45
90	249.73±3.20	240.74±7.69
120	242.91±11.98	207.17±16.06

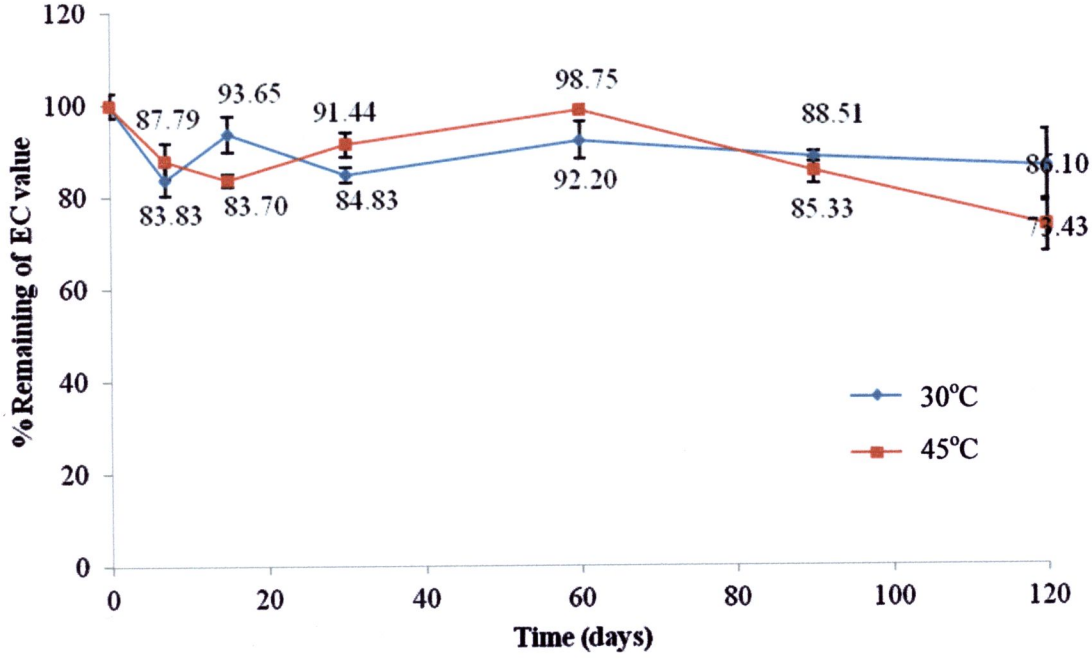


Figure 31 The % remaining of EC value of the PEF tablets after storage at 30°C, 65% RH and 45°C, 75% RH for 120 days

4.9.2 Stability of total phenolic content

The total phenolic content in the *P. emblica* ethyl acetate fractional ectract tablets was investigated after storage in studying conditions for 120 days and the results were showen in Table 14 and Figure 32. The gallic acid equivalence (GAE) of the tablets was 23.90±1.47 mg/g extract at day 0. After storage for 120 days, the GAE values were reduced to 21.69±0.62 mg/g extract (90.75±2.58%) at 30°C, 65% RH and 20.65±0.10 mg/g extract (86.41±0.41%) at 45°C, 75% RH. The results indicated that the total phenolic content in the PEF tablets was slightly reduced over the 120 days of storage.

Table 14 GAE values of PEF tablets after storage at 30°C, 65%RH and 45°C, 75%RH for 120 days

Day	GAE value (mg/g extract)	
	30°C, 65%RH	**45°C, 75%RH**
0	23.90±1.47	23.90±1.47
7	23.82±0.35	24.37±0.11
15	21.71±0.06	20.86±0.02
30	22.66±0.08	21.04±0.04
60	23.41±0.18	23.63±0.23
90	23.20±0.34	21.42±0.16
120	21.69±0.62	20.65±0.10

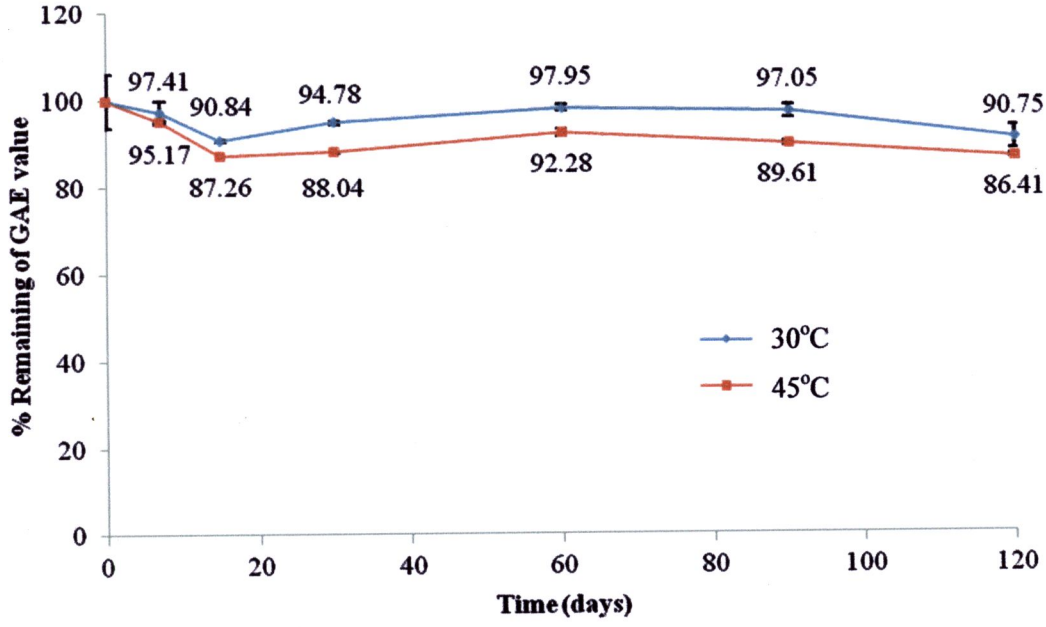


Figure 32 The % remaining of GAE values of the PEF tablet after storage at 30°C, 65% RH and 45°C, 75% RH

4.9.3 Stability of antiglycation activity

The antiglycation activity of *P. emblica* ethyl acetate fractional extract tablets was also investigated over the stability period of 120 days. The IC_{50} value of the tablets trended to increase over time from 48.06±1.50 µg/ml at day 0 to 68.38±1.40 µg/ml at 30°C, 65%RH and 69.32±2.49 µg/ml at 45°C, 75% RH (Table 15). The % increase in IC_{50} values was shown in Figure 33. They were increased to 142.29±2.92% at 30°C, 65%RH and 144.23±1.58% at 45°C, 75% RH. It was likely that the antiglycation activity of the tablet was reduced at a higher rate than the antioxidant activity and the total phenolic content.

Table 15 IC_{50} values of PEF tablets on antiglycation activity after storage at 30°C, 65%RH and 45°C, 75%RH for 120 days

Day	The IC_{50} value (µg/ml)	
	30°C, 65% RH	**45°C, 75% RH**
0	48.06±1.50	48.06±1.50
7	45.51±1.87	46.45±1.18
15	51.49±5.68	53.62±4.96
30	52.54±3.27	51.22±3.07
60	53.85±3.24	56.21±2.69
90	55.15±3.17	57.49±4.70
120	68.38±1.40	69.32±2.49

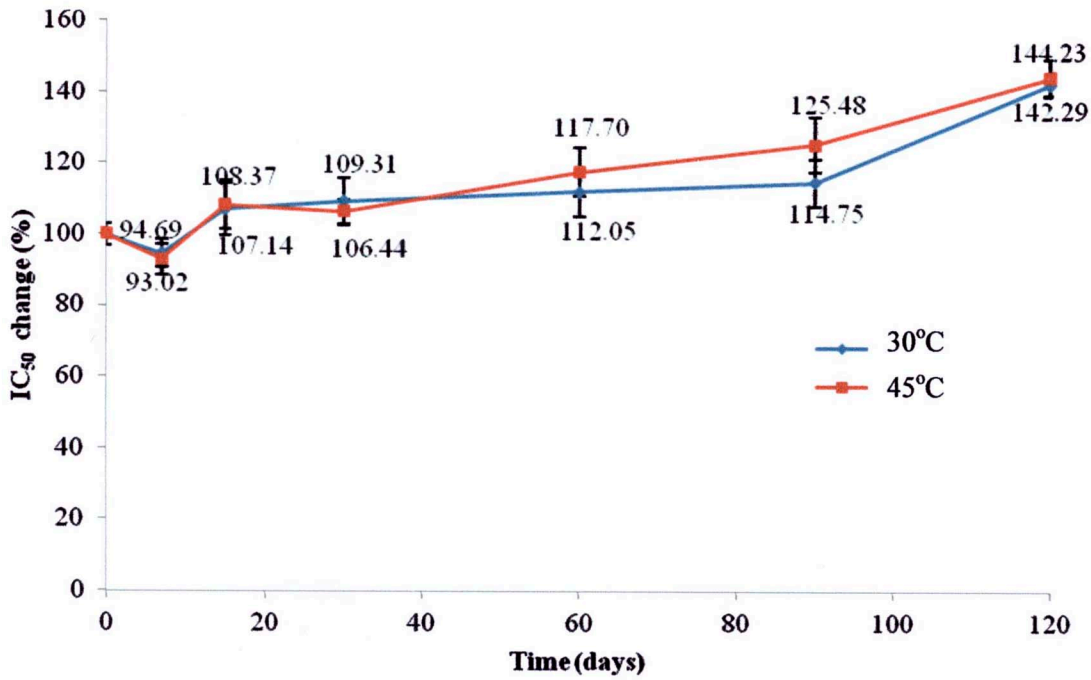


Figure 33 The percentage increase of IC_{50} values of PEF tablets after storage at 30°C, 65% RH and 45°C, 75% RH for 120 days on antiglycation activity

4.9.4 HPLC analysis

The amount of gallic acid in *P. emblica* ethyl acetate fractional extract tablets was studied for by HPLC analysis on day 0 and after storage at 30°C, 65%RH and 45°C, 75% RH for 120 days. The HPLC chromatogram pattern of *P. emblica* ethyl acetate fractional extract tablet was similar to that of the *P. emblica* ethyl acetate fractional extract (Figure 34). The HPLC chromatograms of the tablets stored at 30°C, 65%RH and 45°C, 75% RH were not different from that of the day 0. The gallic acid contents of tablets before and after storage were shown in Table 16. After storage at 30°C, 65%RH for 120 day, the gallic acid content was decreased from 63.10±2.30 to 50.86±1.09 mg per tablet (% decrease = 19.39±1.74%) and at 45°C, 75% RH to 45.54±3.00 mg per tablet (% decrease = 27.28±4.75%).

After storage at the ambient and accelerated conditions for 120 days, it could be suggested that the active compounds, gallic acid deeivatives were relatively stable. The developed formula was suitable to be used as a dietary supplement for antioxidant and antiglycation activities, but the fractional extract should be added in an extra amount into the formulation to replace the loss from the degradation.

Table 16 The amount of gallic acid in PEF tablet (250 mg of PEF)

	Gallic acid content (mg)
Tablets; day 0	63.10±2.30
Tablets; day 120, 30°C, 65%RH	50.86±1.10
Tablest; day 120, 45°C, 75% RH	45.55±3.00

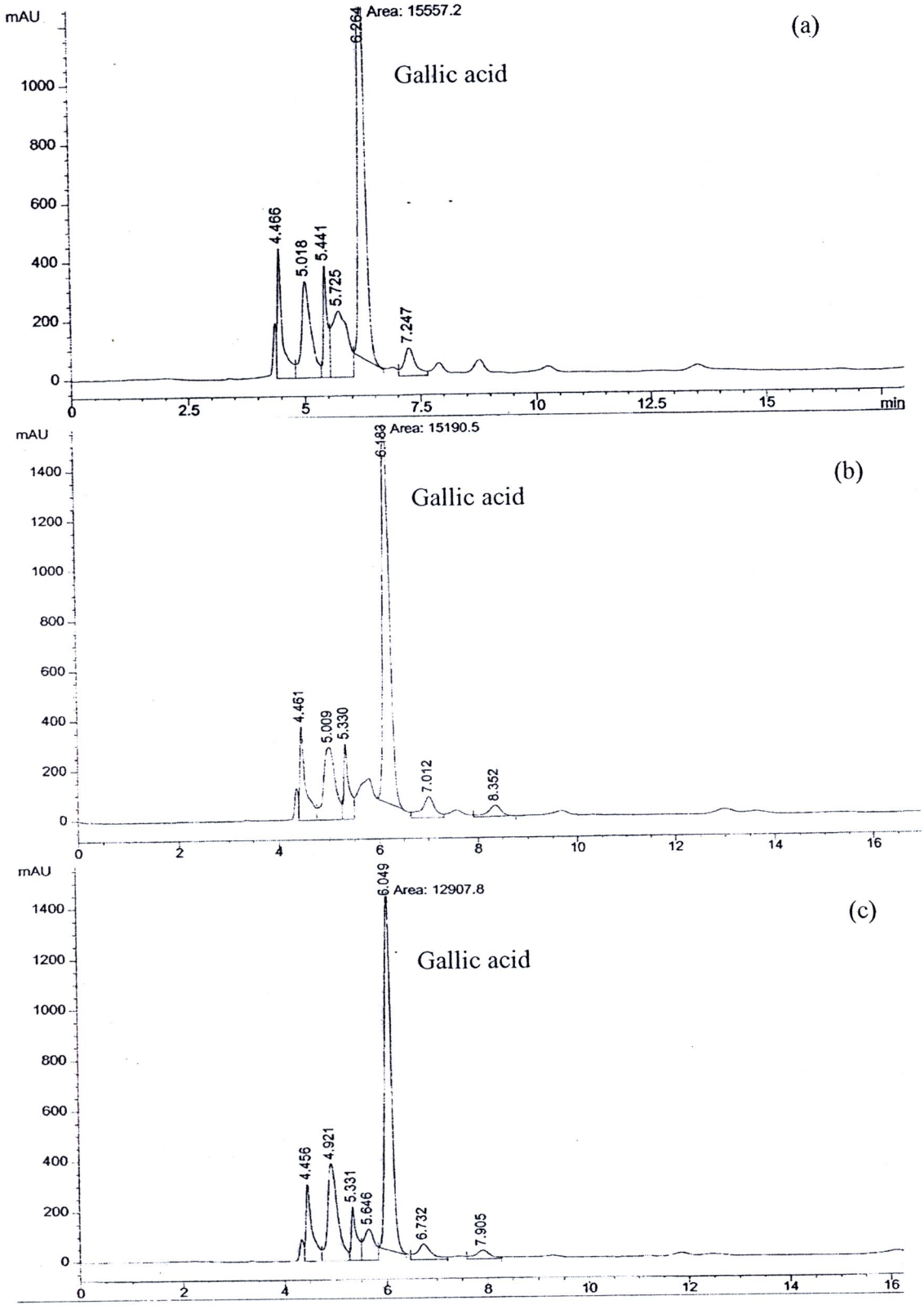


Figure 34 The HPLC chromatogram of PEF tablets at day 0 (a), day 120 when stored at 30°C, 65%RH (b), and 45°C, 75%RH (c)