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# Fractionation of *Phyllanthus emblica* extract for encapsulated products

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## INTRODUCTION AND OBJECTIVES

The extract of *Phyllanthus emblica* shows many pharmacological effects against many diseases such as cancer, diabetes, liver injury, heart disease, ulcer and anemia (Khan 2009). It enriches with vitamin C and polyphenolic compounds such as gallic acid, emblicanin A, emblicanin B, pedunculagin and punigluconin (Bhattacharya 2002) which have a high antioxidant activity and thus are beneficial for pharmaceutical and cosmetic applications. However, the crude extract of *P. emblica* has an obstruction on product development due to its unpleasant appearance and low stability (Liu 2008). Fractionated extraction was carried out in this study to purify the crude extract in order to improve the appearance and the antioxidant activity. The appropriate fraction will be selected for encapsulation to enhance the stability in the future.

## MATERIALS AND METHOD

### *Preparation of P. emblica fraction*

Fresh fruits of *P. emblica* were dried and ground to powder then extracted with 95% ethanol. The crude extract was evaporated to dryness. The extract was dissolved in water and then fractional extracted with different solvents namely hexane, chloroform, ethyl acetate and butanol, respectively according to solvent polarity from non-polar to polar. The solvent fractions were collected and evaporated to dryness.

### *DPPH radical scavenging activity*

The free radical scavenging activity of the crude extract and its fractions was determined by DPPH method. The samples were dissolved with ethanol to prepare various concentration solutions. 20 µl of each sample solution was added with 180 µl of ethanolic solution containing DPPH (1,1-diphenyl 2-picrylhydrazyl) radicals at a concentration of 100 mM DPPH and kept in the dark for 30 min at room temperature. The absorbance was measured at 540 nm using Microplate reader (Bio-Rad Model 680, USA). The IC50 values, the effective concentration of sample to obtain 50% antioxidant activity, were determined.

### *Ferric reducing antioxidant power (FRAP) assay*

The reducing power of the crude extract and its fractions was determined by FRAP assay. The FRAP reagent containing 1 ml of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 1 ml of 20 mM $FeCl_3$ and 10 ml of acetate buffer pH 3.6, was freshly prepared. The crude or the fractional extract was dissolved in ethanol to a concentration of 150 µg/ml. An aliquot (20 µl) of the extract solution was mixed with 180 µl of FRAP reagent, and the absorbance of the mixture was measured at 540 nm using Microplate reader (Bio-Rad Model 680, USA). The $FeSO_4$ solutions were used to obtain the calibration curve. The reducing power was expressed as equivalent concentration ($EC_1$); the concentration of antioxidant having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$.

### *Total phenolic content*

The total phenolic contents of *P. emblica* crude and fractional extracts were determined with Folin-Ciocalteu's reagent (FCR). An aliquot of 20 µl of the crude and fractional extract solution (400 µg/ml) was mixed with 45 µl of FCR followed by 135 µl of 2% w/v $Na_2CO_3$ solution. The absorbance was then measured at 790 nm using Microplate reader (Bio-Rad Model 680, USA) after incubation at room temperature for 2 hr. Gallic acid was used as a standard. Result was expressed in term of gallic acid equivalent (GAE) mg/g dry extract.

### *HPLC analysis*

The crude and fractions of *P. emblica* extract were characterized by HPLC (Hewlett packard/hp1100, USA) analysis on a reverse phase $C_{18}$ column (5 µm, 4.0x250 mm, Hypersil ODS, Agilent, USA) using UV-detector operating at 220 nm. A solvent system was acetonitrile: 0.05% phosphoric acid (isocratic; 10:90) at a flow rate of 0.5 ml/min. All extracts were dissolved in methanol (1mg/ml) and filtered through a membrane filter 0.45 µm before injection (10 µl) into HPLC system. Gallic acid and ascorbic acid were used as standards.

## RESULTS AND DISCUSSION

### *Yield values and appearance of P. emblica extracts*

The dried crude extract of the *P. emblica* fruits showed the yield value of 21.23±1.04%. For fractional extraction, butanol, ethyl acetate, hexane and chloroform fractions had the yield values of 26.78±0.85%, 16.00±1.75%, 5.37±0.33%, 1.24±0.64%, respectively. The water residue from fractional extraction after drying had a yield value of 14.55±1.85%. The crude extract, butanol and water fractions were dark brown in color. The color of ethyl acetate fraction was light brown, whereas the hexane extract was green because of the chlorophyll.

### *Antioxidant activity of P. emblica extracts*

The IC50 and EC1 values of all extracts are shown in Table 1. Ethyl acetate fractional extract had the highest antioxidant activity with the IC50 value of 12.08±2.62 µg/ml. Its value was equivalent to 75.45% and 67.14% of

the IC50 of the crude extract and α-tocopherol, respectively. All other fractions had higher IC50 than that of the crude extract and α-tocopherol. The fraction of ethyl acetate also showed the highest reducing ability with EC1 value of 177.91±22.43 mM/mg. The EC1 values of ethyl acetate fraction and crude extract were significantly higher than other fractions.

**Table 1: Antioxidant activity of the fractional extract**

| Type of sample | IC50 (µg/ml) | EC1 (mM/mg) |
|---|---|---|
| Crude extract | 16.01±1.83 | 121.08±7.68* |
| Hexane | 265.58±63.98 | 31.58±5.72 |
| Chloroform | 196.88±37.18 | 29.96±0.88 |
| Ethyl acetate | 12.08±2.62 | 177.91±22.43** |
| Butanol | 23.71±1.76 | 74.30±4.86 |
| Water residue | 61.66±13.20 | 51.83±13.14 |
| α-Tocopherol | 17.99±0.04 | |

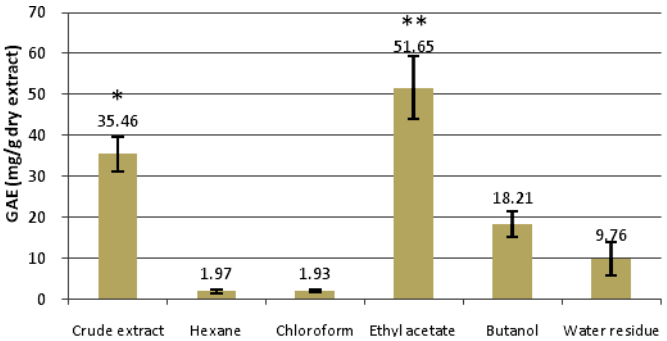
*p<0.05 and **p<0.01 (ANOVA and Multiple comparison with LSD)

***Total phenolic contents of P. emblica extracts***

The amounts of the total phenolic contents in *P. emblica* crude and fractional extracts were shown in Figure 2. The ethyl acetate fraction had the highest total phenolic content with GAE of 51.65±7.69 mg/g dry extract.

***Correlations between antioxidant activity and total phenolic content***

The correlations between antioxidant activity and the total phenolic content were evaluated and the results are shown in Table 2. The correlation coefficients (r) of all relationships were greater than 0.7 or less than -0.7, indicating good correlations between these paramters. From the result, it was highly possible that polyphenolic compounds were responsible for the antioxidation activity via two mechanisms i.e., radical sacvenging and reducing ability.




*p<0.05 and **p<0.01 (ANOVA and Multiple comparison with LSD)

**Figure 2 : GAE value of *P. emblica* fractions**

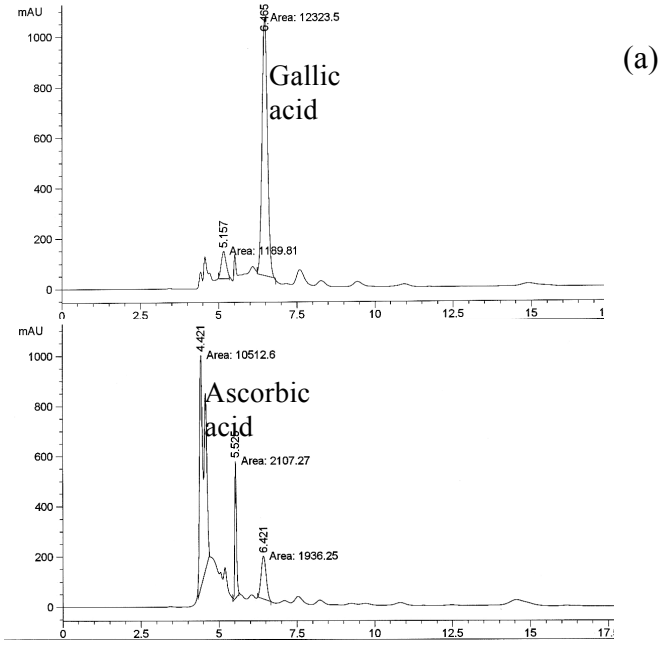
***HPLC analysis of the fractional Extracts***

HPLC chromatograms of *P. emblica* fractions are shown in Figure 3. The chromatogram of the crude extract (data not shown) demonstrated 2 major peaks at retention times of 4.421 min and 6.465 min, corresponding to ascorbic acid and gallic acid, respectively. The HPLC chromatograms of ethyl acetate and water fractions showed that polyphenols, particularly gallic acid were substantially extracted by ethyl acetate, while ascorbic acid remained in the water residue due to its high polarity.

**Table 2 The correlation coefficients of antioxidant activity and total phenolic content**

| Relationship | Correlation coefficients (r) |
|---|---|
| IC50 and EC1 | -0.704** |
| GAE and IC50 | -0.750** |
| GAE and EC1 | 0.981** |

**p<0.01




(a)

**Figure 3 : HPLC chromatograms of ethyl acetat and water fractions (b)**

## CONCLUSIONS

Ethyl acetate fractional extract is a good candidate for further product formulation because it has higher polyphenolic compound content and antioxidation activity and better appearance than the crude extract. However, the lack of benefit from vitamin C should be considered for using this fraction. In addition, the stability study of this fraction should be performed and encapsulation may be used to improve the stability in case of necessary.

## REFERENCES

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