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Research Article

The Development of *Phyllanthus emblica* Extract in Ethosomes for Hair Loss Prevention

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ABSTRACT

Background: Hair loss is not a serious health problem but leads to decreased self- confidence, personality, and psychological problems. According to Thai traditional medical wisdom, *Phyllanthus emblica* has the property to prevent hair loss. Ethosomes are a drug delivery system, which can increase drug delivery to deep skin layers and enhance the effectiveness of the active ingredient. **Objective:** This research aims to develop ethosomes of *Phyllanthus emblica* extract with beneficial properties. **Materials and Methods:** *Phyllanthus emblica* was extracted by a maceration method with 95% ethanol as a solvent. The total phenolic content of the extracts was determined using the Folin–Ciocalteu method. The antioxidation activity was evaluated by DPPH assay. Ethosomes were formulated by a cold method and their properties were observed. **Results:** The results showed that the total phenolic content of *Phyllanthus emblica* extract was 406. 37±2. 39 mg GAE/g extract. The IC_{50} of antioxidant activity was 7. 05±0. 17 µg/ml. Ethosomes with 0. 03% of *Phyllanthus emblica* extract, 2% of soya phosphatidylcholine, and 20% of ethanol had the highest percentage of entrapment efficiency (65. 26%±1. 80%). The ethosomes of *Phyllanthus emblica* extract was the spherical shape and white colloid. The particle size, polydispersity index, zeta potential, and pH of ethosomes were 0.43±0. 00 µm, 0. 44±0. 03, −10. 40±0. 28 mV, and 4. 06±0. 03, respectively. **Conclusion:** The ethosomes of *Phyllanthus emblica* extract had good properties and area possible alternative product for hair loss prevention.

Key words: Entrapment efficiency, Ethosomes, *Phyllanthus emblica.*

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INTRODUCTION

Hair loss is a problem of concern for many people, both male and female. Even though hair loss is not a serious health problem, it can lead in some cases too low self-confidence, psychological problems, and even impaired quality of life. Genes and hormones are major causes of hair loss. Hair loss can be treated with discontinue behavior effect or using medicines such as finasteride and dutasteride which act as 5α- reductase inhibitors. Although, they are effective on hair loss prevention the use of chemical products also causes many side effects as well as altered libido, erectile dysfunction, and ejaculation disorder.[1]

Literature reviews report the use of many Thai traditional herbs for hair loss prevention or treatment including *Phyllanthus emblica* L., *Citrus hystrix* DC, *Acacia concinna* Wall., *Sapindus raruk* DC, *Clitorea ternatea* L, *Averrhoa carambola* L., *Carthamus tinctorius* L., *Zingiber officinale* Roscoe., *Alpinia galangal* Willd., *Trichosanthes cucumerina* L., *Lawsonia inermis* Linn.[2]

Phyllanthus emblica (*P. emblica*) has been used as an ancient Thai traditional medicine by maceration in water overnight before application to the scalp. Moreover, the literature reviews found that extracts of *P. emblica* have antioxidant activities[3-5], stimulating proliferation of hair follicle, and inhibiting 5α-reductase activities.[2] These properties could protect from hair loss. Therefore, *P. emblica* may prevent hair loss but it is inconvenient to use and the exact amount required for use is not known.

Ethosomes is a novel drug delivery system that contains phospholipid, ethanol, and water. They are formed as vesicles containing *P. emblica* extract and can be used to increase the skin delivery to deep layers of skin, improve the systemic circulation, and enhancement of *P. emblica* extract effectiveness. This research aimed to develop ethosomes containing *P. emblica* which have good properties.

MATERIALS AND METHODS

Plant material

Phyllanthus emblica fruits were collected from Na dun, Maha Sarakham, Thailand. All solvents and chemicals used were analytical grade.

Preparation of extract

The 95% ethanol of the maceration method was used as preparation *P. emblica* extract for 7 days at room temperature. The raw material to solvent ratio was 1:6. The extract was filtered with a Whatman No.1 filter and the filtrate was evaporated by rotary evaporator.

Total phenolic content[6]

The total phenolic content was determined by Folin - Ciocalteu reagent method. 20 µl of stock solution

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(0.25 mg/ml) of the *P. emblica* extract, 100 µl of 10 % Folin - Ciocalteu reagent, and 80 µl of 1 M sodium carbonate solution were added to 96 well microplates and mixed well. The mixture was kept at room temperature for 30 min and absorbance of the color developed was recorded at 765 nm with UV Visible spectrophotometer (BMG Labtech, Germany). Total phenolic content estimated from 6 replicates was expressed in mg equivalents of gallic acid per 1 g of crude extract.

Antioxidant activity by DPPH radical scavenging assay[7]

Different 2 fold-dilution of *P. emblica* extract (stock solution 1 mg/ml) were prepared. 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution was prepared by dissolving 6 mg of DPPH in 100 ml of 95% ethanol. Then 100 µl of *P. emblica* extract from each dilution was added in 100 µl of DPPH solution. The mixture was shaken vigorously and left to stand in the dark condition for 30 min. The absorbance of the solution was measured spectrophotometrically at 517 nm with 6 replicate measurements. The % radical scavenging of the extract was calculated using the following formula:

$$\% \text{ radical scavenging} = [(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100 \quad (1)$$

Where Abs_{sample} is the absorbance of the *P. emblica* extract solution and $Abs_{control}$ is the absorbance of the ascorbic acid which was used as standard.

Development of ethosome formulation[8]

Ethosomes were prepared by the cold method. In brief, the *P. emblica* extract was placed in a small round bottom flask and dissolved with 95% ethanol under mixing with the magnetic stirrer at 30°C. The round bottom flask was covered with aluminium foil to avoid ethanol evaporation. Soya phosphatidylcholine (Phospholipon 90G) was added and dissolved. Distilled water was added slowly with a constant rate and continuous stirring to obtain the ethosomal colloidal suspensions. The suspension of ethosomes was continuously stirred for 30 min and the resulting formulations stored at 4°C. The 9 formulations (F1-F9) of ethosomes were prepared with varied concentrations of soya phosphatidylcholine (1-3%) and ethanol (20-40%). The ethosome formulations with the highest percentage of entrapment efficiency was selected. The results are shown in Table 1.

Evaluation of the ethosome preparation

The ethosome formulation which had the highest percentage of entrapment efficiency was evaluated.

Morphology

Surface morphology examined by Scanning Electron Microscopy (SEM) (FEI, Quanta 450, USA). One drop of ethosome formulation was placed on a stub and samples were dried and coated with gold before examination.

pH measurement

The pH of the formulations was monitored by using a digital pH meter (Mettler Toledo, Switzerland) with 6 replicate measurements.

Particle size, size distribution, zeta potential

Particle size, size distribution and zeta potential were measured using Zetasizer (Malvern, UK). The size distribution was reported as the polydispersity index (PDI) with 6 replicate measurements.

Total phenolic contents

The details of total phenolic contents measurement are as mentioned above.

Entrapment efficiency[9]

The percentage of entrapment efficiency (%EE) of ethosomes was determined by using the centrifugation method. 10 ml of ethosome dispersions were centrifuged using a cooling ultracentrifuge (Beckman) at 30,000 rpm. The supernatant was siphoned off carefully to divide the unentrapped *P. emblica* extract. 9 ml of 2% Triton-X 100 was added to the sediment to dissolves the vesicles. The percentage of entrapment efficiency was investigated in terms of % GAE in sediment measured from 6 replicate measurements. The percentage of encapsulated total phenolic content was calculated as follows:

% EE = (amount of GAE in ethosome / amount of GAE added) x 100 (2)

Antioxidants

The antioxidant activity test as mentioned above.

RESULTS AND DISCUSSION

Percentage of yield

P. emblica was extracted with 95% ethanol for 7 days. After evaporation to dryness, the residue was dark brown sticky extract. The percentage of yield was 12.64%.

Total phenolic contents

Total phenolic content of the *P. emblica* extracts was determined with the Folin-Ciocalteu method. Total phenolic contents of *P. emblica* extract were 406.37 ± 2.39 mg GAE/g crude extract (n=6).

Ethosomes properties

The ethosomes of *Phyllanthus emblica* extract had the spherical shapes and were a white colloidal suspension. The particle size, polydispersity index, zeta potential, and pH of *P. emblica* extracts were 1.49 ± 0.38 µm, 0.91 ± 0.01, -34.10±0.99 mV, and 3.75 ± 0.01, respectively. The properties of F1-F9 ethosome formulations were as reported in Table 2. From the results, F1 ethosome formulation is the highest of a percentage of entrapment efficiency (65.26 ± 1.80 %EE). The particle size, polydispersity index, zeta potential, and pH of the F1 ethosome formulation were 0.43 ± 0.00 µm, 0.44 ± 0.03, and -10.40±0.28 mV, and 4.06 ± 0.03, respectively.

Morphology

SEM photographs showed the surface morphology of ethosomes. The ethosomes were revealed to be spherical vesicles with a smooth surface as shown in Figure 1.

pH measurement

The pH of the ethosomes formulations was between in ranges of 3.83 ± 0.01 to 4.19 ± 0.02.

Table 1: Formulation of *P. emblica* extract in ethosomes.

Components	F1	F2	F3	F4	F5	F6	F7	F8	F9
P. emblica extract (mg)	10	10	10	10	10	10	10	10	10
Soya phosphatidyl choline (mg)	600	600	600	900	900	900	1,200	1,200	1,200
95% Ethanol (ml)	6	9	12	6	9	12	6	9	12
Distilled water q.s. to (ml)	30	30	30	30	30	30	30	30	30

Table 2: The properties of *P. emblica* extracts and various ethosome formulations (n=6).

Formulation	pH	Particle size (µm)	PDI	Zeta potential (mV)	% EE
P. emblica extracts	3.75 ± 0.01	1.49 ± 0.38	0.91 ± 0.01	-34.10±0.99	-
F1	4.06 ± 0.03	0.43 ± 0.00	0.44 ± 0.03	-10.40 ± 0.28	65.26 ± 1.80
F2	3.94 ± 0.03	0.36 ± 0.00	0.37 ± 0.02	-2.17 ± 0.10	65.15 ± 2.08
F3	4.19 ± 0.02	0.37 ± 0.00	0.37 ± 0.02	-2.99 ± 0.11	53.48 ± 2.06
F4	4.03 ± 0.01	0.64 ± 0.01	0.79 ± 0.04	-3.33 ± 0.12	63.79 ± 0.79
F5	3.94 ± 0.01	1.06 ± 0.01	0.30 ± 0.01	-1.51 ± 0.18	58.97 ± 2.17
F6	3.96 ± 0.02	2.34 ± 0.08	0.97 ± 0.02	-0.26 ± 0.12	49.12 ± 0.97
F7	3.83 ± 0.01	1.49 ± 0.06	0.97 ± 0.03	-0.34 ± 0.22	58.20 ± 1.84
F8	4.02 ± 0.03	1.03 ± 0.03	0.45 ± 0.02	-0.24 ± 0.33	57.92 ± 2.16
F9	3.94 ± 0.02	1.23 ± 0.02	0.33 ± 0.04	-8.58 ± 0.26	43.34 ± 1.69

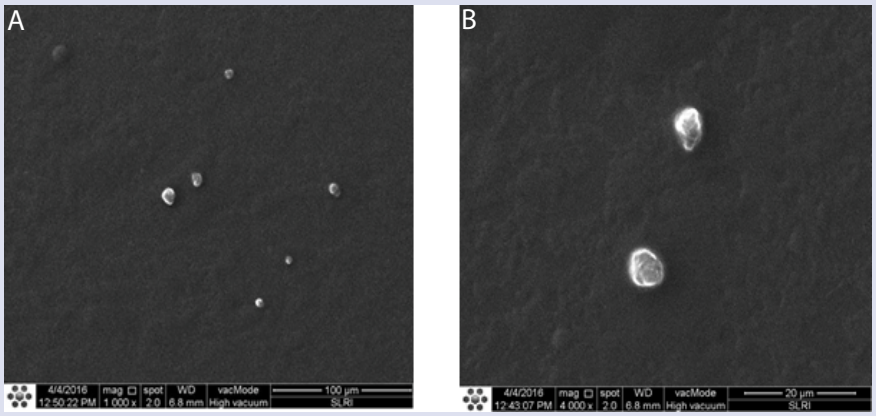

Figure 1: SEM image of ethosomes （A）（1000X）and（B）（4000X）.

Particle size, polydispersity index, zeta potential

The particle size of ethosomes was in the range of 0.36 ± 0.00 to 2.34 ± 0.08 µm. The polydispersity index (PDI) was 0.30 ± 0.01 to 0.97± 0.03 and zeta potential was -10.40 ± 0.28 to 0.26 ± 0.12 mV.

Entrapment efficiency

The percentage of entrapment efficiency (%EE) of ethosomes formulations ranged from 43.34± 1.69 to 65.26± 1.80%. The F9 formulation showed minimum entrapment whereas F1 showed maximum entrapment of extract.

Antioxidants

The IC_{50} of *P. emblica* extracts was 7.05±0.17 µg/ml whereas IC_{50} of F1 was 1.06±0.10 µg/ml. The IC_{50} of ascorbic acid which was used as standard reference was 6.42 ± 0.20 µg/ml.

According to the like dissolves like rule in reference to solubility of polar and non-polar substances, 95% ethanol is a very polar substance and tends to extract a high percentage of yield. This result corresponds to the result of Kornthip *et al.*[10] who found for *P. emblica* bark that there was a greater percentage of yield in 95% ethanol (15.60%) when compared with 50% ethanol (14.18%). Moreover, a study using *P. emblica* from Chiang Mai produced 21.63% of crude extract when macerated with 95% ethanol.[2] The different percentage of yields may be due to different sources of *P. emblica.*

Total phenolics contents could have been inhibitory to a 5α-reductase enzyme which is responsible for changing androgen testosterone into the more potent androgen dihydrotestosterone (DHT). Overexpression of DHT causes androgenic alopecia in males. Therefore, we have determined average total phenolic contents of 406.37 ± 2.39 mg GAE/g crude extract which macerated in 95% ethanol for 7 days, and this corresponds to the results of Jantima *et al.*[11] who determined total phenolic contents in *P. emblica* from 4 sources in Thailand extracted with ethyl acetate, and found that *P. emblica* from Maha Sarakham had total phenolic contents 494.00 ± 19.50 mg GAE/g crude extract. Although, the total phenolic contents extracted with ethyl acetate was more than that from 95% ethanol extraction, the ethyl acetate is toxic to the skin. Therefore, the research team used 95% ethanol extract in order to reduce toxicity so that the preparation can be used in dermal cosmetics.

As humans get older they produce more free radicals, while the endogenous defense mechanisms decrease. This imbalance leads to progressive damage to cellular structures. Thus, free radicals might lead to pattern baldness by damaging hair follicles. The researchers were interested in determining the antioxidant effect from *P. emblica* crude extract by the DPPH method. The IC_{50} was 7.05 ± 0.17 µg/ml whereas the ascorbic acid standard solution had IC_{50} at 6.42 ± 0.20 µg/ml, corresponding with a result from Pientaweeratch *et al.*[4] who found the IC_{50} of *P. emblica* crude extract from Chaopraya Abhaiphubejhr hospital, Prachin Buri, Thailand at 1.70 ± 0.07 µg/ml. The different sources of *P. emblica* may cause different results.

The ethosome was proper with 30,000 rpm at 4 °C for 90 min to separated sediment and supernatant parts. Then Triton X-100, a non-ionic surfactant that had no effect with total phenolic compounds analysis used as a marker in this study, was used as vesicle lysing agent for determining the percentage of entrapment efficacy. Based on the results of the study. The researchers found that increasing soya phosphatidylcholine and 95 % ethanol contents in ethosome formulations decreased the percentage of entrapment efficiency. Thus, the researchers choose F1 which had minimum soya phosphatidylcholine and 95 % ethanol contents but had the highest percentage of entrapment efficiency to apply as a hair tonic. These findings were opposite to Chen *et al.*[12] and Sivakranth *et al.*[13]

who found that increasing soya phosphatidylcholine and 95 % ethanol contents had a greater percentage of entrapment efficiency. However, if the ethanol content was more than 45%, it caused leakage of ethosome vesicles. Iizhar *et al.*[14] found that greater entrapment efficiency was found when the ethosomes were resized by sonication. Similarly Shirwaikar *et al.*[15] found that a sonication effect on ethosome vesicle arrangement which increases the stability and resized the vesicle. Also, due to the vesicle size being decreased, greater permeability and deeper penetration into the target was found. While the primary study of this research shows the percentage of entrapment efficiency was decreased when the ethosomes were resized by sonication. Base on this study, the researchers found the correlation that when the concentration of phospholipid was constant and varied the concentration of ethanol. The formulations which had more ethanol contents were larger. This correlation is opposite to that reported by Touitou *et al.*[16] who found when determining the concentration of phospholipid was constant and varied the concentration of ethanol. The formulations which had more ethanol contents were smaller. The polydispersity index of F1 formulation was 0.44 ± 0.03 which not more than 1. These results showed the particles of ethosomal vesicles had distributed regularly.[17] The zeta potential of F1 formulation had a more negative charge on the surface of ethosomal vesicle. F1 formulation was aggregated loosely when left overnight. However, the particles of ethosome could be quickly dispersed and suspend when shaking with a little force.

The antioxidant activity of ethosomes was greater than *P. emblica* extract solution. This study showed the development of *P. emblica* in ethosomes may improve the antioxidant activity of *P. emblica*. Koli and Lin[18] reported the development of ethosomes could protect active ingredients from oxidation reactions that may promote antioxidant activity.

CONCLUSION

The combination of 20% ethanol, 2% soya phosphatidylcholine, and *P. emblica* extract (10 mg) could be used to prepare ethosomes with good properties. The ethosomes of *P. emblica* extract can be used for hair loss prevention products.

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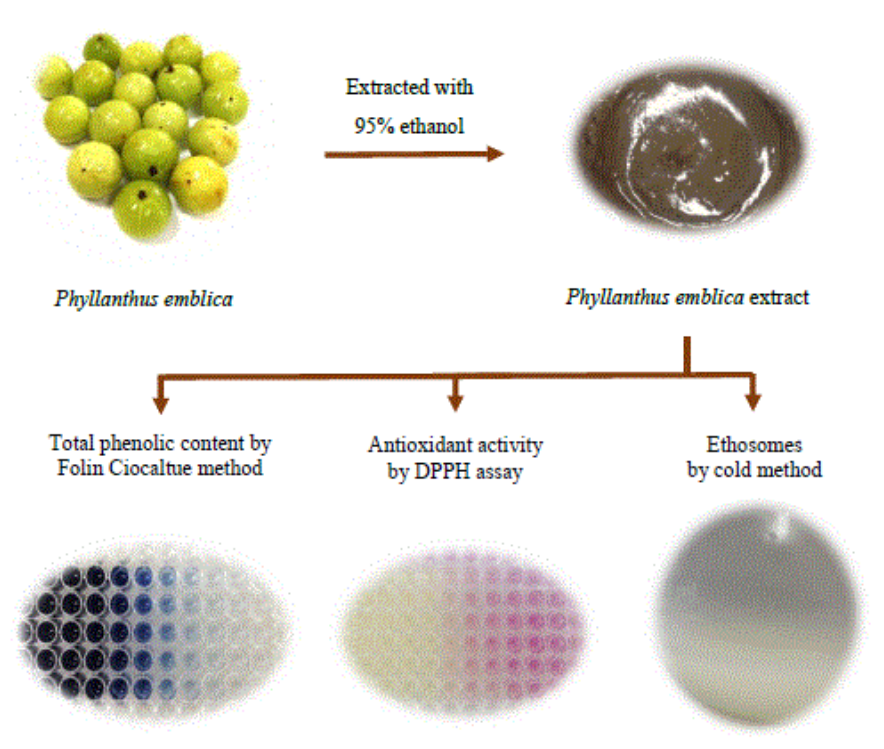
GRAPHICAL ABSTRACT



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