Formulation and Development of TLC-Densitometric Analysis of Gallic Acid in Sunscreen Cream Containing *Emblica officinalis* Fruit Extract

การพัฒนาสูตรและวิธีวิเคราะห์ครีมกันแดดที่ผสมสารสกัดผลมะขามป้อม ด้วยวิธี ที แอล ซี เดนซิโตเมตริก

Nathawarot Boonrattana (ณฐวรท บุญรัตนา)* Worawan Saingam (วรวรรณ สายงาม)**
Dr.Prasan Tanguenyongwatana (ดร.ประสาน ตั้งยืนยงวัฒนา) ***

ABSTRACT

The freeze dried extract of *Emblica officinalis* Gaertn. fruit consisted of gallic acid as major active ingredient which has good antioxidant activities. This extract was included in sunscreen creams to enhance the anti-oxidative property. The objectives of this study were to develop formulations and TLC-densitometric analysis method for determination of gallic acid in sunscreen cream. The cream was extracted by using methanol : water (4 : 6) to isolate gallic acid. For TLC-densitometric method validation, linearity was studied by preparing standard stock solution of gallic acid at 7 different concentration levels. A linear relationship (r = 0.9969, y = 9314.038x + 11.458) was obtained in the range of 594.0 - 1485.0 µg/spot and detected at 280 nm. The intra-day precision and inter-day precision of the assay method were evaluated and the percentages of RSD for six assay values were found at 0.37 and 0.76%, respectively. For the recovery studies, they were carried out in triplicate at three concentration levels. The recovery of gallic acid was found in the range of 100.9 - 101.3%. LOD and LOQ were also evaluated which were 10 and 30 ng/mL, respectively.

บทคัดย่อ

สารสกัดของผลแห้งมะขามป้อมที่มีสารกรดกอลลิคซึ่งเป็นสารที่มีฤทธิ์ต้านอนุมูลอิสระที่ดีได้ถูกนำมาเตรียมให้อยู่ในรูปแบบครีมกันแดด วัตถุประสงค์ของการวิจัยนี้คือการพัฒนาสูตรและวิธีวิเคราะห์กรดกอลลิคในครีมกันแดดโดยวิธี ที แอล ซี เดนซิโตเมตริก การสกัดกรดกอลลิคจากเนื้อครีมใช้ตัวทำละลายเมทานอล : น้ำ (4 : 6) เพื่อแยกกรดกอลลิคออกมาก่อนฉีดลงบนแผ่นที แอล ซี จากผลการทดลองพบว่าความเป็นเส้นตรงของการวิเคราะห์มีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.9969 และมีสมการเส้นตรง y = 9314.038x + 11.458 ในช่วงความเข้มข้น 594.0 - 1485.0 ไมโครกรัมต่อจุด โดยวิเคราะห์ที่ความยาวคลื่น 280 นาโนเมตร ค่าความเที่ยงตรงของการวิเคราะห์ในวันเดียวกันและระหว่างวันมีค่าเท่ากับ 0.37 และ 0.76 เปอร์เซนต์ ความถูกต้องของการวิเคราะห์หาโดยการคำนวนเปอร์เซนต์การคืนกลับของสารกอลลิคในระดับสามความเข้มข้น ซึ่งพบอยู่ในช่วง 100.9 - 101.3 เปอร์เซ็นต์ ค่าความเข้มข้นต่ำที่สุดที่ตรวจพบได้และค่าความเข้มข้นต่ำที่สุดที่จะวิเคราะห์ได้มีค่าเท่ากับ 10 และ 30 นาโนกรัมต่อมิลลิลิตร

Key Words: *Emblica officinalis*, Sunscreen, TLC-densitometric method

คำสำคัญ: มะขามป้อม ครีมกันแดด วิธีที แอล ซี-เดนซิโตเมตริก

* *Student, Master of Science Program in Oriental Medicine, Faculty of Oriental Medicine, Rangsit University*

** *Researcher, Faculty of Pharmacy, Rangsit University*

*** *Lecturer, Faculty of Oriental Medicine, Rangsit University*

Introduction

Sunlight is now a major cause that is harmful to human health when exposure to UV light from the sun. The radiation can cause sunburn, premature skin aging, DNA damage and skin cancer (Xu et al., 2006). Sunlight composes of three UV regions. UVA (320-400 nm) penetrates deep into skin and its effects are additive to the effects of UVB (280-320 nm) for inducing skin cancer (Lucas et al., 2006). The high incidence rates of melanoma and nonmelanoma skin cancer (NMSC) are probably caused by a combination of increased sun exposure or exposure to ultraviolet (UV) light. One way to avoid this is to use of sunscreens products which can absorb or block UV radiation. This is an effective approach for reducing the skin damage and has the immunosuppressive effects of sunlight (Ferrero et al., 2002). Sunscreen is recognized to be an effective and inexpensive method in preventing the development of skin cancers triggered by UV radiation (Kullavanijaya, 2005).

Indian gooseberry (*Emblica officinalis* Gaertn.) belongs to the family Phyllanthacae. This plant is known to have anti-oxidation, immunomodulatory, antipyretic, analgesic, and cytoprotective activities (Khan, 2009). Gallic acid is a major compound in this herb that has been used in many formulations in Traditional Thai Medicine. Gallic acid is found to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity. The amount of gallic acid in *E. officinalis* is found to be 2.376%w/w (Borde et al., 2011). It is good concept to include *E. officinalis* extract in sunscreen cream to express the anti-oxidation effect.

Thin-layer chromatography (TLC), in general, is still of great interest to chromatographers in the herbal or traditional medicine field and in pharmaceutical analysis for the quality control and stability testing of pharmaceutical active compounds and pharmaceutical formulations. A TLC method could facilitate the investigation of active compound, especially for the analysis of some compounds which don't present any UV chromophores and, therefore, sophisticated detection techniques are required (Reich et al., 2007).

Moreover, another strong argument may exist for application of TLC because of its economic advantages over other chromatographic techniques and save time for huge amount of samples. It is rapid, relatively simple, and samples need not be very pure in comparison with high-performance liquid chromatography (HPLC) (Gabriëls et al., 2003).

Objective of the study

Our objective was to develop sunscreen formulations and a TLC-densitometric method for analysis of gallic acid in sunscreen cream containing Indian gooseberry extract that have never been reported before.

Methodology

Instrument and reagents

Disodium EDTA, glycerin, butylene glycol, Nikkomulese WO, silicone STV-5, Lexfeel D5, cetyllsooctanoate, sodium hyaluronate, Nikomullese-41, Carbopol Ultezc-21, micronized zinc oxide, benzophenone-3, titanium dioxide and DMDM Hydrantoin were purchased from Namsiang, Bangkok, Thailand.

Reagents and solvents were reagent grade and used without further purification. Gallic acid was purchased from Aldrich (USA). Freeze Dryer was performed on EYELA FD-1(Tokyo Rikakikai, Japan). TLC-densitometry was performed on pre-coated TLC silica gel GF_{254} plates cat. No.1.05548.0001 (Merck, Germany). Spotting device was Linomat 5 automatic sample spotter (CAMAG, Muttenz, Switzerland). TLC chamber was glass twin-trough chamber (20 × 10 cm.) (CAMAG, Switzerland). Densitometer was TLC scanner 3 with winCATS software (CAMAG, Switzerland). Syringe was 100 µL size (Hamilton, Bonaduz, Switzerland).

Plant materials

Indian gooseberry (*Emblica officinalis*) was purchased from local market, Pathumthani, Thailand. The material was identified by Dr.Prasan Tangyuenyongwatana, Faculty of Oriental Medicine, Rangsit University, Thailand. The voucher specimen of *E. officinalis* (SRU 052) was deposited at Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand.

Indian gooseberry was dried by hot air oven set at 50 °C for 48 hrs. Dried Indian gooseberry was ground and screened through a 40-mesh sieve.

Preparation of Indian gooseberry crude extracts

Indian gooseberry powder (500 g) was continuously boiled in distilled water 5 L that set at 90 °C for 4 hours. After that, the extract was filtered through a 4 layer cotton muslin cloth. The filtrate was freeze dried by EYELA FD-1 and the dried residue was kept in desiccators for further experiment.

Formulation development of sunscreen containing Indian gooseberry crude extract

The sunscreen cream containing Indian gooseberry extract was prepared by following procedure; the formulation of the sunscreen cream was shown in Table 1.

Step 1- Water phase preparation (part A) : Disodium EDTA (0.1%w/w) was dissolved in deionized water and followed by addition of glycerin (7%w/w), butylene glycol (4%w/w), DMDM Hydrantoin (1%w/w), sodium hyaluronate (0.4 %w/w) and Indian gooseberry extract (1%w/w). The mixture was then heated up to 80 °C.

Step 2- Oil phase preparation (part B) : Emulsifying agent, silicone STV-5 (5%w/w), carbopol ultezc-21 (0.2%w/w), Lexfeel D5 (6 %w/w), cetyl isooctanoate (5%w/w) and sunscreen agent were added and heated at 75°C

Step 3- Mixing water phase and oil phase: Oil phase was added to aqueous phase with continuous stirring for 20-25 minutes and then it was homogenized to obtain uniform emulsion. The emulsion was then transfered to tube and stored at room temperature not exceeding 30 °C for further experiment.

Total ten formulation of sunscreen cream were prepared using two different emulsifying agent which were Nikomullese-41 (3, 5%w/w) and Nikkomulese WO (5, 8%w/w) and three different sunscreen agent which were titanium dioxide (6, 8, 10 %w/w), micronized zinc oxides (6, 8, 10%w/w) and benzophenone-3 (2, 4, 6%w/w). All the prepared sunscreen cream was then subjected to various evaluate tests and measured SPF value in order to select the best formulation.

Determination of *in vitro* SPF in sunscreen cream

In vitro SPF was determined by measure of sunscreen cream containing Indian gooseberry extract using Optometrics Model SPF-290S (Optometric copration, USA). The condition for measurement the sun protection factor (SPF) of the sunscreen cream was operated from 290-400 nm. Approximately 110 mg of sample was spread on 56 cm^2 area of Transpore tape to obtain a sample film thickness of 2 mg/cm^2. After that the SPF values were evaluated by WIN SPF software. WIN SPF software has used the following equation for calculating SPF value.

$$\mathrm{SPF} = \frac{\int A(\lambda)E(\lambda)d\lambda}{\int A(\lambda)E(\lambda)/\mathrm{MPF}(\lambda)\,d\lambda},$$

Where, $d(\lambda) = 1$ nm, $A(\lambda)$ stands for the Erythema Action Spectrum, $E(\lambda)$ represents the sun's radiation power, and $\mathrm{MPF}(\lambda)$ stands for how much light is given wavelength absorbed and the ability of skin cells to be damaged.

Table 1 Formulation of sunscreen cream

Part	Ingredients (g)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
A	Water	56.2	54.2	47.5	47.5	47.5	44.5	50.5	48.5	44.5	44.5
	Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Glycerin	7	7	7	7	7	7	7	7	7	7
	Butylene glycol	4	4	4	4	4	4	4	4	4	4
	Carbopol Ultezc-21	0.3	0.3	0	0	0	0	0	0	0	0
	Nikomullese-41	3	5	0	0	0	0	0	0	0	0
	Sodium Hyaluronate	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	DMDM Hydrantoin	1	1	1	1	1	1	1	1	1	1
	Indian gooseberry powder	1	1	1	1	1	1	1	1	1	1
B	Nikomullese WO	0	0	8	8	8	8	8	8	8	8
	Silicone STV-5	5	5	5	5	5	5	5	5	5	5
	Lexfeel D5	6	6	6	6	6	6	6	6	6	6
	Cetyl Isooctanoate	5	5	5	5	5	5	5	5	5	5
	Titaium Dioxide	6	6	10	8	5	6	6	6	6	8
	Zinc Oxide	4	4	4	6	8	10	4	4	6	8
	Benzophenone-3	1	1	1	2	2	2	2	4	6	2

Extraction and TLC-densitometric analysis.

Sunscreen cream (1.0 g) was accurately weighed and transferred to a 50 mL beaker and methanol : water (4 : 6) (8 mL) was added. The mixture was stirred with magnetic stirrer until the cream was dissolved. Then the mixture was added to 10 mL volumetric flask and the flask was placed in an ultrasonic bath and sonicated for 15 min. After that the solvent was added to volume and mixed well. Five milliliters of the mixture was transfer to a test tube and centrifuges at 3,000 rpm for 15 min. The upper layer was collected and and 3 µL of the filtrate was injected into the TLC plate and analysis with TLC-densitometric method.

Method validation

For TLC-densitometric validation method, stock standard solution was prepared by dissolving 2.97 mg of gallic acid in methanol in a 10 mL volumetric flask and adjusted to volume with methanol. Standard and sample were applied on precoated aluminium-backed TLC silica gel 60 GF_{254} plate using Linomat 5. Each sample solution (3 µL) was applied in triplicate as narrow bands of 6 mm length using a nitrogen aspirator. The constant application rate of 150 nL/s, 5.0 x 0.45 mm densitometer slit dimension, and 20 mm/s scanning speed were used. The mobile phase consisted of dichloromethane : ethyl acetate : formic acid (40 : 56 : 4, v/v/v). Linear ascending development was performed in a twin-trough glass chamber saturated with 50 mL of the mobile phase for a plate. The optimized chamber saturation time for the mobile phase was 25 min at room temperature (25 ± 2 ºC). The distance covered by the solvent font was 8 cm, which took about 20 min for each run. The spots were scanned using the TLC scanner 3 in the reflectance-absorbance mode at 280 nm, operated by winCATS software. The amount of gallic acid in the samples was calculated using the calibration graph.

Various amounts of the standard solution were prepared by transferring 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 µL of stock solution into TLC plate (10 x 20 cm.) by using Linomat 5. Linearity relationship between peak area and concentration of the standard solutions was evaluated at 7 concentration levels over the range of 594.0 - 1485.0 µg/spot. Each concentration was done in triplicate. Repeatability and intermediate precision of the developed method were expressed in term of relative standard deviation (RSD) of peak area. Intraday precision was studied by repeat analyzing on the same day of sunscreen cream containing Indian gooseberry extract (n=6). Interday precision was determined on three different days at the same concentration by the proposed method. The accuracy of the method was evaluated by performing recovery study by adding known amount of the reference compound at three levels to the sunscreen cream containing Indian gooseberry extract. Then the solutions were analysis by the proposed method. Three determinations were performed for each level of concentration and the recovery results were calculated. The limit of detection (LOD) and limit of quantitation (LOQ) were performed by determination a series of concentrations of gallic acid standard solution (10-200 ng/mL). Signal-to-noise ratios of 3:1 and 10: 1 were considered as LOD and LOQ, respectively.

Results

The sunscreen cream formulation of Indian gooseberry extracted was studied for physical properties and SPF value determination. The sample results of sunscreen cream formula 1, 3, and 5 were showed in Figure 1. The SPF results was demonstrated in Table 2.

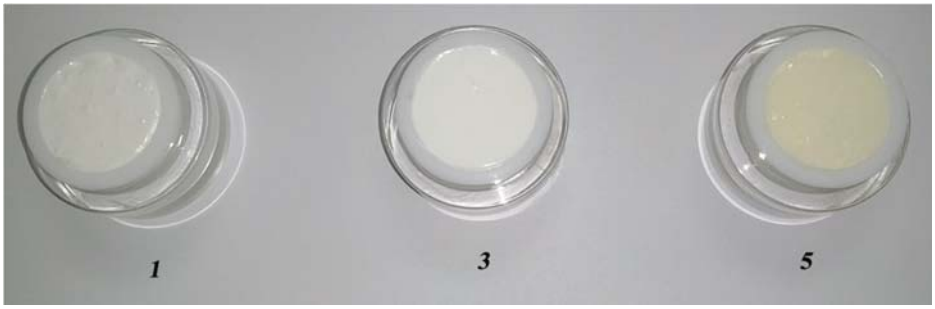

Figure 1 sunscreen cream containing Indian gooseberry extract formula 1, 3, and 5

All the prepared sunscreen creams (F1-F10) were silky white in color and good homogeneous except for F1. When applied sunscreen cream on the skin, the results displayed a good spreadability. The pH of sunscreen cream was found to be 5.50 - 5.90. Viscosity values were in range of 2241.80 - 5465.00 cps. The property of sunscreen cream complied with official acceptance criteria and SPF of these creams were found between 3.48 and 20.14. F4 which composed of 6%w/w micronized zinc oxides shown the lowest SPF at 3.48 ± 0.61 while F10 which composed of three different sunscreen agents shown the highest SPF value at 20.14 ± 4.05 (Table 2). For F3, the SPF value was 14.71± 3.7; this high value should come from titanium dioxide 10% while F6 showed SPF at 13.85 ± 2.73 which zinc oxide was also added in 10%. For the best formulation F10, this sunscreen was mixed with three sunscreen agents in a good combination. The UVA/UVB ratios of F3, F6, and F10 were in the range of 0.73-0.75 while Boots Star Rating of three formula were 3 which mean they are good sunscreen for protection for UVA.

Table 2 Data of SPF and other parameters of sunscreen cream formulation

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
SPF	4.27	8.35	14.71	3.48	5.44	13.85	6.32	9.29	9.76	20.14
STDV(±)	.51	.65	3.7	.61	1.2	2.73	1	1.23	1.02	4.05
UVA/UVB ratio	.679	.771	.737	.871	.883	.731	.513	.735	.799	.751
Boots Star Rating	3	3	3	4	4	3	2	3	3	3

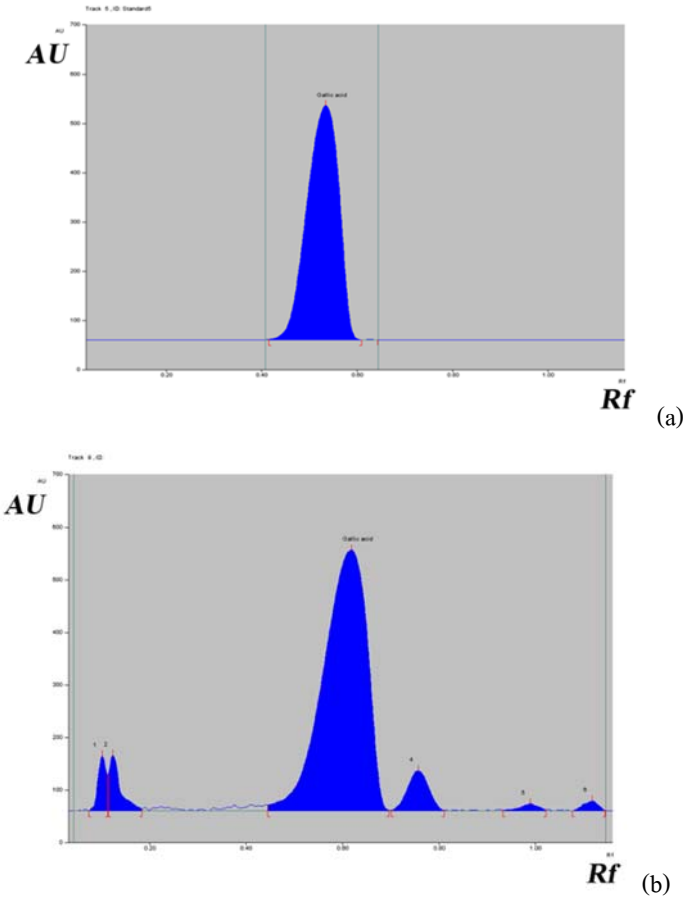

Figure 2 TLC-densitometric chromatograms (AU = absorbance unit, Rf = retention factor) (a) standard gallic acid (b) sunscreen containing Indian gooseberry extract

For TLC-densitometric method validation, the TLC-densitometric chromatograms of standard gallic acid and sunscreen containing Indian gooseberry extract sample were demonstrated in Figure 2. The linear relationship (r = 0.9969, y = 9314.038x + 11.458) between peak area and amount of gallic acid spot was observed in the range of 594.0 - 1485.0 µg/spot (Figure 3). The intra-day precision

and inter-day precision of the assay method were evaluated and the percentages of RSD of six assay values were found at 0.37 and 0.76, respectively. For the accuracy of the method, recovery studies were carried out in triplicate at three concentration levels. The recovery of gallic acid was found to be in the

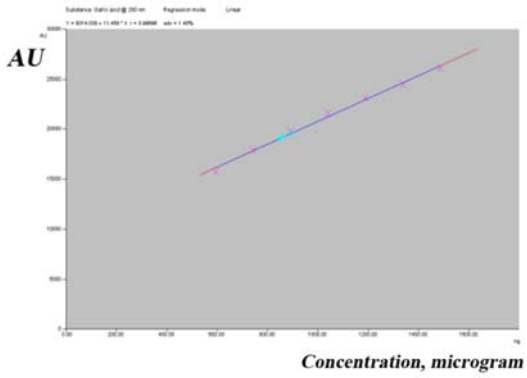

Figure 3 Calibration curve of gallic acid

range of 100.9 – 101.3%. LOD and LOQ were also evaluated which were 10 and 30 ng/mL, respectively. For peak purity of gallic acid between standard and sunscreen sample peaks, the correlation coefficient (r) was equal to 0.9998 (Figure 4). When samples were subjected to analysis with this method, the concentrations of gallic acid were 0.31 ± 0.01 %w/w

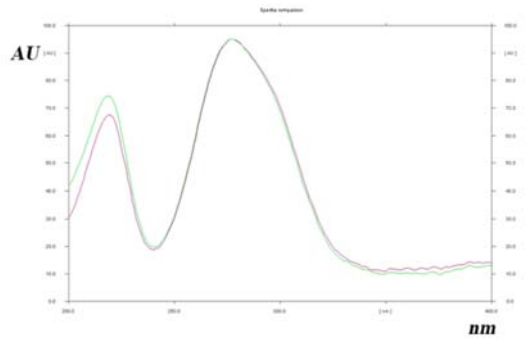

Figure 4 Peak purity of gallic acid between standard and sunscreen sample

Discussion and Conclusions

The TLC-densitometric method showed specificity and good analysis results in each parameter when compared to other method such as HPLC. The HPLC method is a time consuming analysis method when compared to TLC-densitometric method. However HPLC gives a good high resolution than TLC-densitometric method. The TLC-densitometric method can improve to HPTLC if we use HPTLC plate which gives us a higher performance of qualitative and quantitative analysis. However HPTLC plate is more expensive than standard TLC plate. In our country which is still a developing country, we choose to use standard TLC plate with densitometer detector for cost saving but still obtains a good result.

The extraction processes can get rid of insoluble materials in sunscreen sample by using appropriate solvent which composed of water to dissolves gallic acid and precipitate other ingredients. The ultrasonic extraction made the preparation procedure of sample far more easy and accurate. The detector wavelength at 280 nm that was used in this analysis showed specificity for gallic acid. Gallic acid retention factor (Rf) showed at 0.55-0.57. The validation of the assay for linearity gave a good linear range of 594.0 - 1485.0 µg/spot with correlation coefficient r = 0.9969 for gallic acid. The intraday precision of injection results demonstrated the excellent relative standard deviation (RSD) of precision of less than 0.37% (n=6) and the interday precision of less than 0.76% (n=6). The recovery study was performed by spiking the standard compound in three different concentrations to a control sunscreen cream. After the analysis, the recovery results of gallic acid at three different

concentrations were 100.9, 101.3 and 101.2% (n=3), with an average of 101.1 ± 0.21%. The LOD and LOQ were 10 and 30 ng/mL, respectively.

For the formulation, the combination of three sunscreens in the precise ratio made the SPF value high together with UVA/UVB ratio and Boots Star Rating. For the sample analysis, the analysis method showed an appropriate procedure and obtained the concentration of gallic acid in the samples range from 0.30 to 0.31%w/w. This TLC-densitometric method demonstrated a reasonable linear regression, together with high precision and accuracy. The low cost and time of analysis make this method suitable for sunscreen quality control.

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