DEVELOPMENT OF *PHYSALIS PERUVIANA* L. FRUIT EXTRACT AS ANTIOXIDANT AND UVABSORPTION AGENT AND ITS APPLICATION IN SUNSCREENING PRODUCT

Thitiporn Jeamsrichai

E-mail: harajuku_forever@hotmail.com

Master of Science Programme in Cosmetic Science

School of Cosmetic Science, Mae Fah Luang University

Dr. Mayuramas Sang-ngern, Ph. D.

E-mail: mayuramas@mfu.ac.th

School of Cosmetic Science, Mae Fah Luang University

Abstract

The present study aimed to determine the suitable ratio between dried plant material and solvent using as procedure for extraction of dried fruits of *P. peruviana* L. The suitable ratio for extraction process was 1:1.25 due to the solvent absorption capacity of dried fruit. The percentage yield of crude ethanolic extract of *P. peruviana* L. was 18.01. The determination of total ethanolic content (TPC) and antioxidant capacity (AOC) was evaluated with 2, 2'-azino-bis (3ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. The TPC of crude ethanolic extract of *P. peruviana* L. was found in the range of 76.159 to 858.51 mg GAE/g of sample. The maximum ABTS value found was 48.541 µM Trolox/g of crude ethanolic extract of *P. peruviana* L. The maximum DPPH value found was 206.572 mg/ml of crude ethanolic extract of *P. peruviana* L. cream based containing crude ethanolic extract of *P. peruviana* exhibited sun prorection factor(SPF) values within the range of 30-60 and UVA/UVB ratio 0.4-1.0, therefore, the cream based containing *P. peruviana* extract exhibited sunscreen protection property with PA^{+++} according to Thai FDA standard of sunscreen and ISO 24443.

Keywords : *P. peruviana* L., Total Phenolic Content, ABTS, DPPH, SPF value

Introduction

Ultraviolet (UV) radiation is part of the electromagnetic spectrum that reaches the surface of the earth were originated from the sun. The exposure to UV light is considered as a major risk factor of skin proven human carcinogen (Kim & He, 2014). Among of them, UVA spectrum reaches to the earth's surface with 96.5% while UVB spectrum is 3.5% (Korac and Khambholja, 2011; Tuchinda et al., 2006). The penetration of UVA through the skin is deeper than UVB with significant adverse effects such as immunosuppression, photo-aging, and skin cancer. UVB is preliminarily associated with erythema and sunburn including a cause of immunosuppression and photocarcinogenesis (Korac & Khambholja, 2011; Tuchinda et al., 2006). Therefore, to reduce harmfulness from UV radiation by using the cosmetic product containing UV protection or suppression ingredients becomes a major function of more types of cosmetic formulations. Consequently, the natural compounds rapidly growing demand due to their lack of side effects on the human body instead enriches the body with nutrients and other useful minerals (Gediya et al., 2011). In this study used natural ingredients to add in the sunscreen formulation for test efficacy to compare sunscreen in the market.

Objectives

1.2.1 To study the ratio between dried *P. peruviana* L. (PP) fruit and organic solvent (95% ethanol) to using for extraction process.

1.2.2 To determine the total phenolic contents and antioxidant capacity of *P. peruviana* L. fruit crude extract.

1.2.3 To study the stability and photo-protective effects of crude ethanolic extracts of *P. peruviana* L. fruit in sunscreen formulation.

Materials and methods

Chemical substances; Butylene glycol, C12-15 alkylbenzoate, cyclopentasiloxane, deionized water, disodium EDTA, ethylhexyl methoxycinnamate, bis-ethylhexyloxyphenolmethoxyphenyltriazine, Parsol TX® (titanium dioxide, silica, dimethicone, C12-15 alkyl benzoate), PEG-240/HDI copolymer bis-decyltetradeceth-20 Ether, phenyltrimethicone, phenoxyethanol, polyethylene glycol, and silica.

Reagents; 2, 2 azinobis-(3-3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 95% Ethanol, Dithiothreitol; 2, 2-diphenyl-1-picrylhydrazyl (DPPH), butanol, ethyl acetate, folin ciocalteu's (FC) reagents, gallic acid, hexane, and potassium per sulfate.

Plant material preparation: The ten kilograms ripeness stage of *P. peruviana* L. fruits was collected from Thai Royal Project, Chiang Mai, Thailand. The fruits were separated from their calyces were removed carefully by hand. The fruit was washed with water. Fresh fruit was sliced by knife on chopping block, placed on trays, and air-dried under the shade. The dried fruits material was stored under -20 °C until used.

Extraction method: The maceration technique was used in the first step for this study which is simple, widely, and suitable for the initial and bulky extractions (Sarker et al., 2006). The dried *P. peruviana* L. fruits were extracted using 95% ethanol at room temperature for three days. Occasional of the preparation (by using stirring rod to homogenous mixing). The *P. peruviana* L. ethanolic extracts were concentrated under vacuum using a rotary evaporator.

The liquid-liquid extraction technique (Partition) this classical separation technique uses solvent partitioning. In this step, the crude ethanolic extract were initially separate into various discrete fractions containing compounds of similar polarities or molecular sizes. The crude ethanolic extracts from *P. peruviana* L. was separately suspended in and then extracted with hexane, ethyl acetate, butanol, and deionized water, successively. Each partition had concentrated under reduced pressure to yield a residue. This residue was subjected to bioassay and screening procedures was used to partition the group of compounds according to its polarity property (Sarker et al., 2006).

1. Determination of total phenolic content

Preparation of sample: The *P. peruviana* L. fruit was modified from the studies of Narvaez-Cuenca et al. (2014). Stock solution (sample) was prepared by the crude ethanolic extract 5 mg were mixed, the volume was graduated to 10 mL in a volumetric flask with 95% ethanol, and is a stock solution used for the analysis of the total phenolic content (TPC), and antioxidant capacities (AOC).

Total phenolic content (TPC) protocol: The TPC was determined using the Folin-Ciocalteu (FC) method as adapted from Re *et al., (1999)*. A calibration curve was prepared by using a standard solution of gallic acid at concentrations ranging from 0.01 to 0.30 mg/mL The results were expressed as gallic acid equivalents (mg GAE/g of crude ethanolic extract). All measurements were done in triplicate.

2. Determination of antioxidant capacities (ABTS, and DPPH)

The ABTS assay modified from Re et al. (1999) and Narvaez-Cuenca *et al.* (2014). A calibration curve used to be generated with Trolox in concentrations ranging from 100 to 800 µM. The results were expressed as Trolox equivalent (µmol TEAC/g of crude ethanolic extract). All measurements were done in triplicate.

The DPPH scavenging capacity assay was performed Sanchez-Moreno et al. (1998) & Narvaez-Cuenca et al. (2014) with some modifications. A calibration curve was prepared with a troloxequivalent at 100 to 1,000 µM (five data points, r^2= 0.99x is acceptable). A stock solution was prepared by dissolving 0.0025 g DPPH in 100 mL 95% ethanol. An aliquot of 100 µL of sample was mixed with DPPH solution and kept in dark for 30 min. The absorbance was measured at 517 nm using 96 well plate by microplate reader. A blank was 200 µL 95% ethanol. As a positive control, 100 µL DPPH solution was added to 100 µL 95% ethanol. As a negative control 200 µL 95% ethanol. The results were calculated as mg/100g of Trolox equivalent (µmol TEAC/g of crude ethanolic extract). All measurements were done in triplicate.

The radical scavenging capacities (in percentage) calculated as;

$$(A_1 - A_2 / A_0)*100$$

Where;

A_0 = the absorbance of the control (without extract)

A_1 = the absorbance in the presence of the extract once the steady state plateau was reached

A_2 = the absorbance without DPPH.

Formulation of cream base emulsions

The formulation of emulsions was performed according to the components as shown in table 1.

Table 1. Components and ratios of cream base emulsion

Ingredients	Master formulation (g)							
	F1	**F2**	**F3**	**F4**	**F4A**	**F4B**	**F4C**	**F4D**
Oil phase								
- Ethylhexylmethoxy cinnamate	6	6	6	6	6	6	6	6
- C12-15 alkyl benzoate	3	3	3	3	3	3	3	3
- Bis-ethylhexyloxy phenol methoxyphenyl triazine	3	3	3	3	3	3	3	3
- Phenyltrimethicone	1	1	1	1	1	1	1	1
- Cyclopentasiloxane	1	1	1	1	1	1	1	1
- Silica	1	1	1	0.8	1	0.8	0.8	0.8
- PEG-240/HDI copolymer bis-decyltetradeceth-20 ether	2	1	1.3	1.5	1.5	1.5	1.5	1.5

Table 1. (continued)

Ingredients	Master formulation (g)							
	F1	**F2**	**F3**	**F4**	**F4A**	**F4B**	**F4C**	**F4D**
Aqueous phase (2)								
- Deionized water	65.5	65.5	65.5	65.5	65.5	64.5	63.5	62.5
- Phenoxyethanol	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
- Ethyhexylglycerin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
- *P. peruviana* L. fruits extracts	-	-	-	-	-	1	2	3

Preparation of cream base emulsion:

1. Oil phase were heated up to 75 ± 0.5 °C.
2. Aqueous phase (1) was heated up to 75 ± 0.5 °C until homogenize.
3. Aqueous phase (2) was heated up to 75 ± 0.5 °C until homogenize.
4. Aqueous phase (1) added into aqueous phase (2) to aqueous phase.
5. Oil phase added into aqueous phase using a homogenizer at 6,000 rpm until the emulsion cooled at room temperature.

Physical stability studies

Heating - cooling cycling: modified from Shukla & Patel (2010)**:** For one cycle of the heating – cooling cycle, the emulsion was stored in the refrigerator at 4 °C for 24 hrs and in the hot air oven at 45 °C for 24 hrs. After the performig for 6 cycles, the centrifugation, pH value, and viscosity were determined.

Physical analysis: The obtained emulsions were submitted to a set of organoleptic (color, look, feel, thickness), and physical (phase separation, and creaming) analysis according to Akhtar et al. (2011) & Smaoui et al. (2013).

Stability test: was achieved at different conditions for emulsions to explore the effect of these conditions on the storage of emulsions. These tests were performed under accelerated condition by placing the formulations at 4 °C ± 2 °C (in refrigerator), 25 °C ± 2 °C (at room temperature), and 45 °C ± 2 °C (in incubator).

Color, phase separation, and liquefaction of emulsions were observed at various time intervals during 28 days.

Centrifugation test: Centrifugal tests performed for emulsions directly after preparation. Repeated after 1, 7, 14, 21, and 28 days of storage. They were performed at 6,000 rpm for 15 min by placing 1 g of each sample in centrifugal tubes.

pH determination: The pH value of various emulsions which stored at different conditions were determined by using a digital pH meter. The pH value was repeated for multiple emulsions after 1, 7, 14, 21, and 28 days of storage.

Viscosity value determination: The viscosity of emulsion was determined by using viscometer (Nasirideen et al., 1998). The viscosity value was repeated for multiple emulsions after 1, 7, 14, 21, and 28 days of storage.

3. *In vitro* method for SPF Determination

The SPF of the sunscreen products were determined using an *in vitro* method modified from the procedure of Couteau et al., 2007 & Couteau et al., 2012. About 25 mg of product exactly weighed spread on PMMA plates over the whole surface (25 cm^2) using a cot-coated finger. Three plates were prepared for each product to be tested and 9 measures were performed on each plate. Transmission measurements between 290 and 400 nm were UV Transmittance. The standard used the 17 mg of vaseline (mandated by Thai FDA standard of sunscreen and ISO 24443).

Literature review

Sunscreens and photoprotection have been proven to be an essential tool for skin cancer prevention since the late 1920s (Lim & Draelos, 2009). The formulation of sunscreen contains physical and chemical UV-filters which absorb and reflect UV-light or other additives such as antioxidants which play an important role in protecting the skin from the effects of exposure of UV-light (Kockler et al., 2012). Physical or inorganic sunscreen can be reflected and scatter ultraviolet and visible radiation of inert metal particles such as Zinc oxide (Zn), Titanium dioxide (TiO_2) (Rai & Srinivas, 2007) which forms an opaque barrier. Depend on the particle size, which that protect against ultraviolet radiation by both reflection and absorption.

Natural antioxidant from herbs has been used as UV protection in cosmetics preparation due to their highly potential in antioxidant activities (Korac & Khambholja, 2011). A large number of antioxidants have been marketed as additives for UV-protections such as ascorbic acid (vitamin C), tocopherol (vitamin E), and β-carotene exhibit protective effects in preventing free radical formation by scavenging them or promoting their decomposition (Gediya et al., 2011). Moreover, naturally occurring herbal compound extracts from green tea such as polyphenol exhibited potential on a photoprotective property by against sunburn (erythema), non-melanoma skin cancers, UV-induced immunosuppression and photo-aging (Yusuf et al., 2007). Consequently, the natural compounds rapidly growing demand due to their lack of side effects on human body, instead enrich the body with nutrients and other useful minerals (Gediya et al., 2011).

Physalis peruviana L. (Cape gooseberry or golden berry) is a genus of the Solanaceae family, containing more than 120 species (Sharma et al., 2015). *P. peruviana* L. originates in tropical South America and is native to the Amazonian and Andean areas of Peru and Chile (Puente et al., 2011; Morton, 1987). *P. peruviana* L. can be found worldwide, distributed across Africa, Europe, Asia, and the Pacific. *P. peruviana* L. has 1.0-2.50 cm of diameter, 4-10 g weight of fresh, orange skin, juicy pulp contains numerous small yellowish seeds and calyces is the cover. The fruits of *P. peruviana* L. can be eaten fresh or used as an ingredient in salads, desserts or jam making. *P. peruviana* L. is a medicinal plant widely used in folk medicine in tropical countries and is used for the treatment of a variety of ailments including asthma, dermatitis, diuretic, hepatitis, rheumatism, and malaria (Puente et al., 2011).

Research results and discussion

Physalis peruviana L. extraction: The maceration method was used for the extraction process in erlenmeyer flask. In this study, there are show the percent yield of crude extract in five ratios between dried fruit sample (mg), and 95% ethanol (mL) were used in the extraction processes.. The results shown that the effective ratios of A, B, and C with percentage yields were27.26, 26.12, and 25.33, respectively. By the way, the suitable ratio for extraction depends on the nature of materials as well. In this research, the ratio of B (1:1.25) was appropriated and selected for using in the

extraction process according to the solvent absorptivity of dried plant material. Hence, the 1,045.66 grams of dried PP fruit were extracted with approximate 1,250 mL of 95% ethanol to yield 188.3234 grams (18.01% yield) of crude ethanolic extract. Furthermore, partitioning process with hexane, ethyl acetate, butanol, and deionized water (DI) to yield 8.347, 6.47, 5.628, and 5.014 grams, successively.

The amount of total phenolic in the extracts was determined with Folin-Ciocalteu reagent. The maximum value were the first time of crude ethanolic extract : ethanol = 1:1.25 had 112.479. The minimum value were the deionized water in the part of partition crude ethanolic extract : ethanol = 1:1.5 had 60.714.

ABTS assay: The amount of ABTS in the extracts was determined with 2, 2 azinobis-(3-3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagent. The maximum value were the first time of crude ethanolic extract : ethanol = 1:2 had 46.952. The minimum value were the third time crude ethanolic extract : ethanol = 1:1.5 had 43.432.

DPPH assay: The amount of DPPH in the extracts was determined with DPPH reagent. DPPH was used as a trolox, and the DPPH were expressed as μg/g Trolox equivalents antioxidant capacity (TEAC). The maximum value were the first time of crude ethanolic extract : ethanol = 1:2 had 206.572. The minimum value were deionized water in the part of partition crude ethanolic extract : ethanol = 1:1.5 had 107.878.

In vitro method for stability determination of formulation containing crude ethanolic extract of *P. peruviana* L: The four formulations (F1-F4) cosmetic bases were prepared without the addition of *P. peruviana* L. extract. The result shown that F4 was the most formula selected by volunteer's due to non-separation, non-sticky, easy rubbing and did not leave the white residue on the skin. Therefore, formula F4 was selected as the best cosmetic base performance for further study.

The cosmetic base (F4A) and three formulations (F4B-F4D) containing crude ethanolic extract of *P. peruviana* L. were prepared following the components in Table 1. All formulas were submitted to centrifugation at 6,000 rpm for 15 min including accelerated stability testing. The physical and chemical properties of products were observed.

Physical stability studies

After accelerated the kinetic stability of test formulas (F4A – F4D) by force (centrifugation at 6,000 rpm for 15 minutes), the cosmetic base (F4A) and the cosmetic containing 1% of *P. peruviana* L. extract (F4B) were not showed phase separation after centrifugation, on the other hand, the cosmetic containing 2 and 3% of *P. peruviana* L. extract, F4C and F4D, respectively, exhibited phase separation. The color, odor, pH, and viscosity of F4A and F4B were not changed while the color, odor, pH and viscosity of F4C and F4D did not determine due to phase separation. Therefore, the formulas F4A and F4B were selected for further stability testing.

Stability testing: The formulas F4A–F4B were conducted in an interval of 28 days under three distinct storage temperature conditions. The color, odor, phase separation, pH, and viscosity were observed.

Creaming leads to phase separation and is often attributed to density differences between the two phases under the influence of gravity (Rousseau, 2000). The color and odor of cream base and emulsion containing crude extract were stable and the phase separation of both emulsion formulas was not observed during the 28 days observation period of time which indicating good stability, mainly in high temperature conditions.

pH value determination: The changing of pH value of emulsion during stability testing may temperature affecting. The pH changes indicate the take of chemical reactions that can affect the quality of the final product (Issa et al., 2000). The observation of pH values of formulas F4A and F4B under the temperatures of 4, 25, and 40 °C for interval 28 days have been shown in Tables 4.9-4.10 and Figures 4.7-4.8, respectively. The pH values of F4A at 3 different temperatures were 6.36, 6.39, 6.64, while the pH values of F4B were 3.65, 3.56, and 4.47, respectively. The pH values of formulas F4A and F4B were continuing increase from the first day and up to the last day (28 days). The results shown that the pH value of F4B is far from the neutral pH may cause of chemical interaction of adding of crude *P. peruviana* L. ethanolic extract (pH 4.5). The high temperature contributes to the destabilization of the emulsions by hydrolysis. However, it did not affect the overall quality of emulsions because the pH values remained around 6.64 which nearby the pH of

normal human skin and acceptable for non-skin irritating pH value (Smaoui et al., 2013).

Viscosity determination: The viscosity of the emulsions studied for 28 days under 3 differences temperature conditions. This reported for present study shown that there was no significant change in viscosity for cream base formula (F4A). On the other hand, the formulation containing crude extracts at concentration of 1% exhibited increasing of viscosity especially at room temperature. This phenomenon may affect by variation of temperature during experiment because the viscosity is very sensitive to the temperature hence: the increment temperature caused reduction of emulsion viscosity (Khan et al., 2013; Lim et al., 2011).

Evaluation of the sun protection factor: The sun protection factor (SPF) was compared with Thai FDA standard (Dutra et al., 2004). The variation of SPF of the sunscreen emulsion determined upon exposure to different temperature conditions (4, 25, and 45°C) during period of study times (28 days). An initial SPF determination was performed in sunscreen emulsion with (F4B) and without (F4A) crude ethanolic *P. peruviana* L. extract. The SPF values remained stable throughout the whole period of study. The formulations F4A (cream base emulsion) and F4B (containing extract) exhibited SPF values within the range of 30-60 and UVA/UVB ratio 0.4-1.0, therefore, the cream base itself and cream based containing extract exhibited sunscreen protection property with protection grade of UVA $(PA)^{+++}$ according to Thai FDA standard of sunscreen and ISO 24443. Even though they did not show significantly difference SPF value between F4A and F4B, however, the interesting point was focused on the UVA/UVB ration at temperature of 25 °C of F4B. In comparison between F4B with F4A and commercial sunscreen (Biore), the UVA/UVB ration of F4B was in the range of 0.684-0.842 while F4A and commercial sunscreen were in the range of 0.571-0.754, and 0.35-0.74, respectively. It's may affect by additional of crude *P. peruviana* L. extract.

Conclusion

In this study, the fifteen conditions of extraction method were determined. The suitable ratio between dried *P. peruviana* L. and 95% ethanol was 1:1.25. Therefore,

the 1,045.66 grams of dried PP fruit were extracted with approximate 1,250 ml of 95% ethanol to yield 188.3234 grams (18.01% yield) of crude ethanolic extracts. The partitioning process with hexane, ethyl acetate, butanol, and deionized water (DI) to yield 8.347, 6.47, 5.628, and 5.014 g, successively.

The total phenolic content and antioxidant capacities from the extraction exhibited in the ranged from 76.159 to 858.51 mg GAE/g of sample. The maximum ABTS value found was 48.541 µM Trolox/g of crude ethanolic extract of *P. peruviana* L. The maximum DPPH value found was 206.572 mg/ml of crude ethanolic extract of *P. peruviana* L.

The formulations F4A (cream base emulsion), and F4B (containing extracts) exhibited SPF values within the range of 30-60 and UVA/UVB ratio 0.4-1.0, therefore, the cream base itself and cream based containing extract exhibited sunscreen protection property with PA^{+++} according to Thai FDA standard of sunscreen and ISO 24443. Even though they did not show significantly difference SPF value between F4A and F4B, however, the interesting point was focused on the UVA/UVB ration at temperature of 25°C of F4B. In comparison of F4B with F4A and commercial sunscreen (Biore), the UVA/UVB ration of F4B was in the range of 0.684-0.842 while F4A and commercial sunscreen were in the range of 0.571-0.754 and 0.35-0.74, respectively. It may affect by additional of crude *P. peruviana* L. extract.

In conclusion, the *P. peruviana* L. extract can be exhibited interesting antioxidant activities including may potential as photo-protection booster and then *P. peruviana* L. mixed with cream base and test of activity of UV protection which that in *P. peruviana* L. have characteristics to protect from UV, which can be develop in sunscreening product in pharmaceutical. Further study need to be study.

References

Akhtar, N., Zaman, S., Khan, B.A., M. H., Khan, S., Ahmad M., Rasool, F., Mahmood, T., & Rasul, A. (2011). Evaluation of various functional skin parameters using a topical cream of *Calendula officinalis* extract. *African Journal of Pharmacy and Pharmacology*, *5*(2), 199-206.

Couteau, C., Pommier, M., Paparis, E., & Coiffard, L.J.M. (2007). Study of the efficacy of 18 sun filters authorized in European Union tested in *vitro*. *Journal of Die Pharmazie*, *62*(6), 449-452.

Couteau, C., Chauvet, C., Paparis, E., & Coiffard, L. (2012). UV Filters, Ingredients with a recognized Anti-Inflammatory effect. Retrieved from http://www.plosone.org, *7*(12) (e46187), 1-6.

Dutra, E.A., Oliveira, D.A.G.C., Kedor-Hackmann, E.R.M., & Santoro, M.I.R.M. (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Brazilian Journal of Pharmaceutical Sciences, 40*(3), 381-385.

Gediya, S.K., Mistry, R.B., Patel, U.K., Blessy, M., & Jain, H.N. (2011). Herbal Plants: Used as a cosmetics. *Journal of Natural Product and Plant Resources, 1*, 24-32.

International Organization for Standardization (2012). ISO 24443 : Determination of sunscreen UVA photoprotection *in vitro*, 27.

Khan, B.A., Akhtar, N., Khan, H., & Braga, V.A. (2013). Development, characterization and antioxidant activity of polysorbate based O/W emulaion containing poluphenols derived from *Hippophae rhamnoides* and *Cassia fistula*. Brazillian Journal of Pharmaceutical Sciences. *49*(4): 763-773.

Kockler, J., Oelgemoller, M., Robertson, S., & Glass, B.D. (2012). Photostability of sunscreens. *Journal of Photochemistry and Photobiology C*: *Photochemistry Reviews*, *13*, 91-110.

Korac, R.K., & Khambholja, K.M. (2011). Potential of herbs in skin protection from ultraviolet radiation. *Journal of Pharmacognosy Reviews, 5*(10), 164-173.

Lim, H.W., & Draelos, Z.D. (2009). Clinical Guide to Sunscreens and Photoprotection. *Informa Healthcare USA inc. New York*.

Narvaez-Cuenca, C.E., Mateus-Gomez, A., & Restrepo-Sanchez, L.P. (2014). Antioxidant capacity and total phenolic content of air-dried cape gooseberry (*Physalis peruviana* L.) at different ripeness stages. *Journal of Agronomia Columbiana*., *32*(2), 232-237.

Nasirideen, S., Kas, H.S., Onert, F., Alpart, R., & Hincal A.A. (1998). Naproxen incorporated lipid emulsions. I. Formulation and stability studies. *Journal of Clinical Pharmacy and Therapeutics*, *23*, 57-65.

Rai, R., & Srinivas, C.R., (2007) Photoprotection. *Indian Journal of Dermatology, Venereology and Leprology*, *73*(2), 73-79.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C., (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Journal of Free Radical Biology and Medicine*, *26*(9-10), 1231-1237.

Sanchez-Moreno, C., Larrauri, J.A., & Saura-Calixto, F., (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food Agriculture*, *76*, 270-276.

Sarker, S.D., Latif, Z., & Gray, A.I. (2006). Natural Products Isolation. 2nd edition. *Journal of Natural Products, 70*(4), 32.

Shukla, J.B., & Patel, S.J. (2010). Formulation and evaluation of self micro emulsifying system of *Candesartan cilexetil*. International Journal of Pharmacy and Pharmaceutical Sciences, *2*(4), 143-146.

Smaoui, S., Hlima, H.B., Chobba, I.B., & Kadri, A. (2017). Development and stability studies of sunscreen cream formulations containing three photo-protective filters. *Arabian Journal of Chemistry*, *10*, 1216-1222.

Tuchinda, C., Srivannaboon, S., & Lim, H.W. (2006). Photoprotection by window glass, automobile glass, and sunglasses. *Journal of the American Academy of Dermatology*, *54*(5), 845-854.