IJPSR (2019), Volume 10, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 December 2018; received in revised form, 23 April 2019; accepted, 13 July 2019; published 01 August 2019

IN-VITRO TRANSPORT EVALUATION OF *GARCINIA MANGOSTANA* L. PERICARP EXTRACT LOADED CREAM AND GEL

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Keywords:

Garcinia mangostana pericarp extract, Mangostin, Radical scavenging, Release, Penetration, Shed snake skin

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ABSTRACT: *Garcinia mangostana* L. pericarp (GMP) has been known as a traditional medicine in Asian countries. The bioactive from GMP has been recognized to be related to complex phenolic compounds linked with free radical scavenging activity. This research aimed to study the effects of topical formulation types (gel, oil in water cream and water in oil cream) of GMP extract on the *in-vitro* release and *in-vitro* shed snake skin penetration. The formulations were evaluated for viscosity and pH. The extent of GMP extracts released and penetrated shed snake skin were evaluated based on stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and expressed by Radical Scavenging activity equivalent GMP Extract concentration (RSGMPE). Mangostin skin penetration was quantified using UV spectrophotometric method. GMP extract exhibited significantly higher DPPH scavenging activity compared to α-mangostin which is known as the major constituent in the GMP extract. RSGMPE released and penetrated through the skin was significantly influenced by the type of formulations. GMP extract loaded gel provided highest RSGMPE amount transported through the skin than that retained in the shed snake skin membrane. On the contrary, total mangostin was found more retained in the shed snake skin membrane. When loaded in the gel, pure α-mangostin showed similar *in-vitro* shed snake skin penetration profile. GMP extract is potential to be developed used for topical applications with suitable formulation strategy for skin targetting.

INTRODUCTION: Topical antioxidant administration has been suggested as a potential approach to alleviate oxidative-stress mediated skin disorders. Prolong solar ultraviolet radiation and other harmful environmental factors may induce the excessive formation of reactive oxygen species (ROS) that may impair natural antioxidant defense system [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(8).3727-34

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(8).3727-34

Products from natural botanical origin contain secondary metabolites that can inhibit or scavenge ROS preventing or slowing down the oxidative stress. There has been a tremendous interest in exploring botanical extracts having antioxidant or radical scavenging activity for topical antioxidant products [2, 3].

Garcinia mangostana L. is famous for its sweet fruit taste in some Asian countries. The pericarp which is the waste part of the fruit has a long history as traditional medicine [4]. During the last few years, *Garcinia mangostana* L. pericarp (GMP) extract has gained increasing interest due to broad biological activities, including antioxidant and radical scavenging activity [3, 4].

These activities have been evaluated based on various methods such as radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibition. The chemical constituents of GMP extract responsible for antioxidant and radical scavenging activity were complex phenolic compounds identified as flavonoids, tannin, and xanthones with the mangostins being the major constituent [5]. Even though GMP extract possesses antioxidant activity, which is potentially useful for topical antioxidant skin treatment, only little is known for GMP extract skin penetration data. Tachaprutinum *et al*., [6] evaluated porcine ear skin penetration of GMP extract in particulate and free form loaded in cream or water vehicle. They reported that GMP extract loaded cream had superior penetration into hair follicle as well as in the stratum corneum compared to that of GMP extract in a water vehicle. The choice of the vehicle was seen to influence GMP extract skin penetration depth. Despite this potential skin penetration ability of GMP extract, to our knowledge, there is only limited data known on skin penetration GMP extract evaluated based on free radical scavenging activity which is important for its topical antioxidant formulation development. This research aimed to study the influence of formulation types on the *in-vitro* skin transport of GMP extract evaluated by stable radical DPPH scavenging activity which was then expressed as radical scavenging activity equivalent GMP extract concentration (RSGMPE).

GMP extract was evaluated for the antioxidant activity based on scavenging activity towards DPPH radical. GMP extract was loaded into three formulations, *i.e*. gel, oil in water (o/w) cream and water in oil (w/o) cream. *In-vitro* free radical DPPH scavenging equivalent GMP extract concentration release and *in-vitro* skin penetration through shed snake skin were evaluated. GMP extract loaded gel formulation showing highest RSGMPE in vitro skin penetration transport was further evaluated for mangostin in vitro skin transport. Additionally, α-mangostin, known as the main constituent in the GMP extract [7, 8], was also formulated into a gel and evaluated for its *in-vitro* shed snake skin penetration. This report for the first time comparison *in-vitro* skin penetration of mangostin from GMP extract and α-mangostin.

MATERIALS AND METHODS:

Materials: α-mangostin (purity of 98%) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were bought from Sigma Aldrich. Ethanol and methanol were analytical grade & purchased from E Merck. *Garcinia mangostana* pericarp (GMP) dry extract extracted using 70% ethanol was purchased from PT. Borobudur plant. Carboxymethylcellulose sodium (CMC-Na), propilene glikol, methyl-paraben, olive oil, stearic acid, cetyl alcohol, glycerin, propylparaben, triethanolamine (TEA), and cera alba were pharmaceutical grade and purchased from the local store.

Antioxidant Activity Evaluated by Free Radical Scavenging DPPH Assay: The free radical scavenging activity of GMP extract, α-mangostin, and samples obtained from *in-vitro* release studies as well as *in-vitro* skin penetration studies were evaluated by scavenging activity to free radical stable DPPH [9]. For radical scavenging measurements, 100 mM acetate buffer pH 5.5 (1 ml), ethanol (1 ml), and 0.4 mM ethanolic solution of DPPH (0.5 ml) were mixed, sample (0.1 ml) was added then the absorbance was measured after 30 min at 522 nm. The positive control was made from the reaction mixture in the absence of either GMP extract, α-mangostin, or samples obtained from in vitro release studies as well as *in-vitro* skin penetration studies. Blank was prepared from the reaction mixture without DPPH solution. All measurements were performed in triplicate.

Evaluation free radical scavenging activity of GMP extract was prepared by dissolving extract with the aid of propylene glycol and diluted with 20% ethanol in water to make final concentrations in the medium of 2-27 µg/ml. A stock solution of α-mangostin was prepared in methanol (211 µg/ml) and diluted to make final concentrations in the medium of 3.55 - 60 µg/ml.

Formulations: Three GMP extract formulations were prepared, *i.e*., gel, o/w cream, and w/o cream. The gel base was prepared using CMC-Na, methylparaben, and deionized water. The w/o cream base was prepared using cera alba, olive oil, methylparaben, propylparaben, and deionized water. The o/w cream base was prepared using stearic acid, cetyl alcohol, olive oil, glycerin, methyl-paraben, propylparaben, triethanolamine,

and deionized water. GMP extract (10%) was prepared in propylene glycol-water mixture (1:4) and then added to each of the formulation bases. The control formulations did not contain the GMP extract. the α-mangostin loaded gel was prepared using the same gel base formulation (without GMP extract). All formulations were allowed to equilibrate for 24 h before evaluations.

Characterization of the Formulations:

Viscosity and pH Determination: Viscosity was evaluated using Brookfield viscosimeter (spindle no. 6; 100 rpm). pH evaluations of the formulations were done after dilution 1:10 of formulation in water then measured using a calibrated pH meter.

***In-vitro* Release:** *In-vitro* release experiments were conducted using modified franz cells (effective surface area of 0.4 cm^2) using a cellophane membrane (molecular weight cut off 10,000 Da). Receptor phase was filled with 20% ethanol in phosphate buffer saline (1 ml), and then the donor phase was placed with formulation (0.5 g). The donor phase was then covered by parafilm to minimize evaporation. Receptor phase was stirred with a magnetic stirrer (300 rpm) and maintained in room temperature. At a predetermined time during the 24 h experiment, samples (0.5 ml) were taken from the receptor phase, and the same volume of the fresh receptor was placed back to the receptor phase. The obtained samples were assessed for their radical scavenging activity using DPPH method. The formulations without GMP extract were also tested for *in-vitro* release. Three to four replicates were conducted for each of the formulations.

***In-vitro* Skin Penetration:** *In-vitro* skin penetration study used shed snakeskin (*Phyton reticulus*) as the membrane obtained from natural shedding of the snake. The skin was washed using deionized water, dried at room temperature at stored at desiccator before use. For *in-vitro* skin penetration studies, the ventral skin portion was cut into an appropriate size. The cut skin was hydrated overnight, then mounted between donor and receptor compartments of the diffusion cell. The formulation was placed in the donor compartment (0.5 g) and *in-vitro* skin penetration was conducted using similar way described in the *in-vitro* release studies. At 24 h, the experiment was terminated, the receptor phase was removed and analyzed for antioxidant activity using DPPH scavenging method as well as mangostin content

In-vitro skin retention studies were conducted as follows. The remaining formulation in the donor compartment was removed, and the skin was dismantled from the cell, cleaned & washed with distilled water (3 times each with 1.0 ml water), cut into small pieces and placed in a tube. Methanol (1.0 ml) was then added to extract the substances retained in the skin. Samples were then evaluated for DPPH scavenging activity and mangostin quantification. Four replicated were done for each of the formulations studied.

Analytical Assay for Mangostin: Mangostin content in the samples were quantified using an ultraviolet (UV) spectrophotometric method [10]. The α-mangostin was used as the reference standard. The method showed good linearity (R^2 = 0.9996) in the concentration range of 0.37-12 µg/ml.

Data Analysis: The concentration of GMP extract that caused 50% of DPPH scavenging activity was considered as IC_{50}. The DPPH scavenging activity (% inhibition) obtained from *in-vitro* release and skin penetration studies were expressed as DPPH radical scavenging activity GMP extract concentration equivalent (RSGMPE) determined using a calibration curve from GMP extract concentrations against DPPH scavenging activity (% inhibition) of each concentration. All of the data was expressed as mean ± SD (standard deviation). To examine the differences of the data, a statistic analysis was performed by one way ANOVA followed by Tukey test or Kruskal-Wallis followed by Mann-Whitney test using the SPSS software. A significance level was set at $P<0.05$.

RESULTS:

Free Radical Scavenging Activity of *Garcinia mangostana* L. Pericarp (GMP) Extract: GMP extracts antioxidant activity was evaluated *in-vitro* toward stable radical DPPH. Relationship between GMP extract concentration and DPPH scavenging activity is presented in **Fig. 1**. Scavenging DPPH was linearly correlated with GMP extract from 2 to 27 µg/ml showing the antioxidant activity of 6-85, 64%. DPPH radical scavenging activity of α-mangostin is shown in **Fig. 2**.

It shows a lower scavenging activity compared to GMP extract. α-mangostin concentrations from 3.5 - 14 µg/ml produced DPPH scavenging activity of 5 - 14%. DPPH scavenging activity was not increased when α-mangostin concentration was increased from 14 µg/ml up top 60 µg/ml.

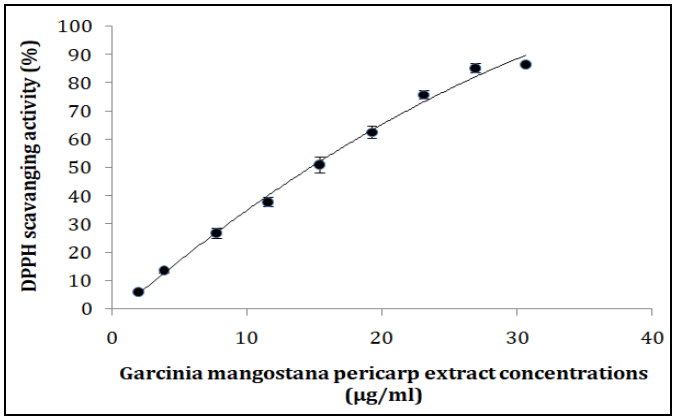

FIG. 1: DPPH SCAVANGING ACTIVITY AS A FUNCTION OF *GARCINIA MANGOSTANA* L. PERICARP EXTRACT CONCENTRATIONS

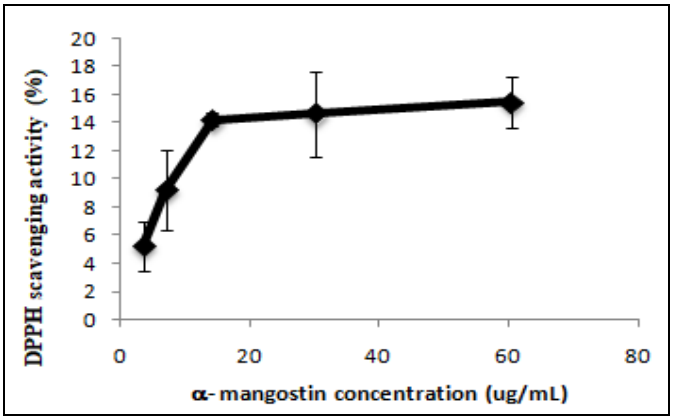

FIG. 2: DPPH SCAVANGING ACTIVITY AS A FUNCTION α-MANGOSTIN CONCENTRATIONS

Physical Characterization of *G. mangostana* L. Pericarp (GMP) Extract Formulations: GMP extract loaded gel or cream was characterized for the pH and viscosity as presented in **Table 1**. The viscosity of various GMP extract formulations was varied from 3291-6157.5 mPas. The gel had the lowest (P <0.05) viscosity, whereas w/o cream had the highest (p<0.05) viscosity value. As for pH, gel and w/o cream showed a pH of 4.80 and 4.10, respectively, while o/w cream had the highest (P<0.05) pH of 6.27.

TABLE 1: VISCOSITY AND PH CHARACTERISTICS OF *GARCINIA MANGOSTANA* L. PERICARP (GMP) EXTRACT LOADED GEL OR CREAM

Formulations	Characteristic formulation	
	Viscosity (mPas ± SD)	pH ± SD
GMP extract loaded gel	3291.0 ± 97.7	4.80 ± 0.01
GMP extract loaded o/w cream	4487.5 ± 332.2	6.27 ± 0.03
GMP extract loaded w/o cream	6157.5 ± 532.4	4.10 ± 0.04

***In-vitro Garcinia mangostana* Pericarp (GMP) Extract Release:** Antioxidant substance(s) released from the GMP extract loaded formulations into the receptor compartment was quantified using the DPPH method. The free radical scavenging activity was expressed as DPPH radical scavenging activity equivalent *Garcinia mangostana* L. pericarp extract (RSGMPE). Cumulative *in-vitro* RSGMPE released from various formulations loaded GMP extract is presented in **Fig. 3**. Both gel and o/w cream released the same amount of RSGMPE at the first hour (P>0.05). Much different antioxidant released from these formulations were seen during 3 h which was maintained up to 24 h (P<0.05). In contrast, w/o cream showed much less RSGMPE released in all-time points during 24 h observation.

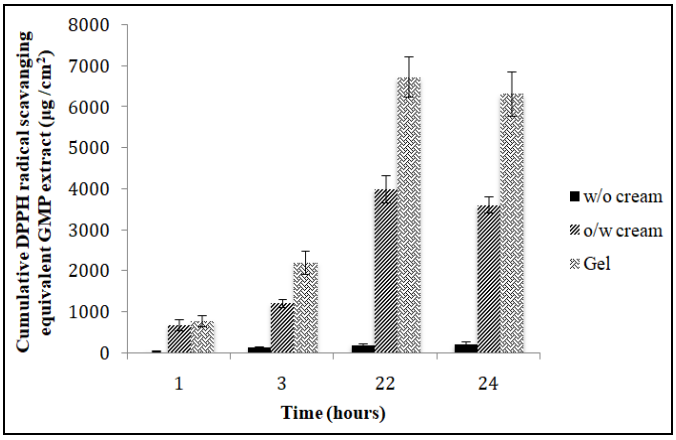

FIG. 3: *IN-VITRO* RELEASE OF *GARCINIA MANGOSTANA* L. PERICARP (GMP) EXTRACT LOADED WATER IN OIL (W/O) CREAM, OIL IN WATER (O/W) CREAM, AND GEL EVALUATED BASED ON DPPH RADICAL SCAVENGING ACTIVITY EQUIVALENT *GARCINIA MANGOSTANA* L. PERICARP EXTRACT CONCENTRATION (RSGMPE)

***In-vitro Garcinia mangostana* L. Pericarp (GMP) Extract Skin Penetration:** GMP extract skin penetration evaluations were conducted using DPPH similar to the method used in the release studies. Amount of RSGMPE found in receptor phase and retained in the membrane following in vitro skin penetration studies from GMP extract loaded formulations were shown in **Fig. 4**. Highest RSGMPE amount (P<0.05) penetrated into receptor phase was resulted from gel (64 µg/cm^2), while o/w and w/o cream gave similar values (P > 0.05) *i.e.* 2.7-fold (23.4 µg/cm^2) and 3.5x-fold (18.09

µg/cm^2) less RSGMPE penetration than that of gel, respectively.

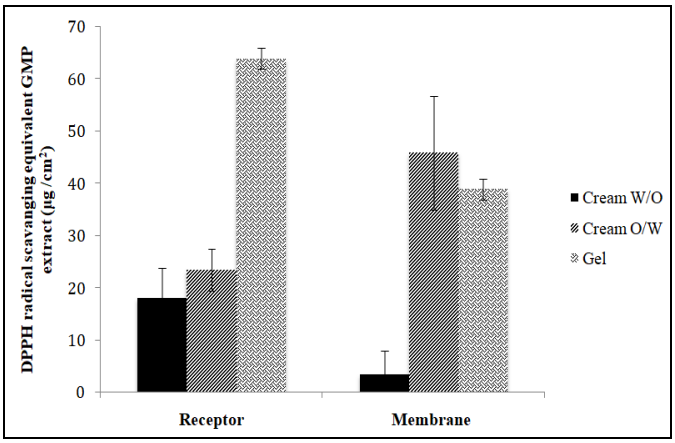

FIG 4: IN-VITRO SHED SNAKE SKIN PENETRATION OF *GARCINIA MANGOSTANA* L. PERICARP (GMP) EXTRACT LOADED WATER IN OIL (W/O) CREAM, OIL IN WATER (O/W) CREAM, AND GEL EVALUATED BASED ON DPPH RADICAL SCAVENGING ACTIVITY EQUIVALENT *GARCINIA MANGOSTANA* L. PERICARP EXTRACT CONCENTRATION (RSGMPE)

RSGMPE retained in the skin given by the various type of formulations of GMP extract showed different profile compared to that found in the receptor phase. While gel exhibited highest RSGMPE in receptor phase, the amount retained in the skin was comparable to that of o/w cream (P>0.05). w/o cream had the lowest RSGMPE amount in the skin. The amount RSGMPE retained in the skin after 24h was 3.5-45.84 µg/cm^2.

As GMP extract loaded gel showed highest RSGMPE skin penetration, this formulation was also assessed for mangostin skin penetration shown in **Table 2**. The data showed that mangostin was more retained in the skin membrane (13.45 µg/cm^2) than transported into the receptor phase (5.37 µg /cm^2). Pure α-mangostin loaded gel *in-vitro* skin penetration evaluation also showed similar skin distribution profile to that of mangostin from GMP extract. The amount α-mangostin retained in the skin membrane was higher (33.36 µg/cm^2) than that found in the receptor phase (7.11 µg/cm^2).

TABLE 2: *IN-VITRO* TRANSPORT THROUGH SHED SNAKE SKIN OF GMP EXTRACT LOADED GEL AND α-MANGOSTIN LOADED GEL

Formulations	Mangostin (µg/cm^2 ± SD)	
	Receptor	Membrane
10% GMP extract loaded gel	5.37±0.74	13.45 ± 1.06
3.3%α-mangostin loaded gel	7.11 ± 3.06	33.36 ± 5.65

DISCUSSION: Antioxidant activity of GMP extract attracts its potential application as a topical formulation. This study evaluated free radical scavenging activity of GMP extract and α-mangostin which is recognized as the main constituent in GMP extract [7, 8, 11], based on DPPH scavenging activity. Further, GMP extract was loaded into some types of formulation, *i.e.* o/w cream, w/o cream, and gel and evaluated in terms of *in-vitro* GMP extract radical scavenging release and *in-vitro* skin penetration using shed snake skin as the membrane.

Antioxidant activity of GMP ethanolic extracts and various fractions (hexane, ethyl acetate, butanol, and water) have been evaluated by different methods including superoxide dismutase (SOD) activity, total antioxidant assay (TAS) and DPPH radical scavenging activity [5, 12]. All extracting solvents exhibited antioxidant activity, however, the highest SOD and TAS activity was shown by GMP water fraction and GMP ethyl acetate fraction, respectively, reflecting variation in the antioxidant constituents in each of the solvents tested. GMP extract concentration that inhibited 50% DPPH activity (IC_{50}) was also dependent on the type of solvents used being between 3.62 to 13.29 µg/ml.

The present study also confirms free radical DPPH scavenging activity of ethanolic GMP extract with an IC_{50} of 15 µg/ml. This IC_{50} value is lower than the same extracting solvent reported by Tjahyani *et al.*, [5] *i.e.* IC_{50} of 6.56 µg/ml. GMP antioxidant activity was affected by several factors such as place of growth & plant maturity stage. Mature GMP plant had lower DPPH scavenging activty than that of young GMP plant. Quantification of the bioactive content in young GMP maturity stage revealed that young GMP contained more phenolics and tannins with less total flavonoids and α-mangostin content [13].

Being the major xanthone derivative, α-mangostin has been reported to have antioxidant activity evaluated based on DPPH scavenging activity, superoxide dismutase activity and total antioxidant status assay [5]. In this study, it was shown that DPPH radical scavenging of α-mangostin was much weaker than that of GMP extract. The IC_{50} of α-mangostin could not be determined in the

concentrations tested (the highest tested α-mangostin concentration of 60 µg/ml only showed 14% DPPH scavenging activity). This data supports previous α-mangostin DPPH scavenging activity from Pothitirat *et al*., [13] and Tjahjani *et al*., [5] reporting IC_{50} α-mangostin of >150 µg/ml and 66.63 µg/ml, respectively. Stronger radical DPPH scavenging activity of GMP extract compared to α-mangostin suggests that other constituents in GMP extract in addition to α-mangostin such as phenolic compounds, tannin, and flavonoids may act synergistically. In this case, for the development of topical antioxidant formulation containing GMP extract, evaluation of the total scavenging activity of the extract would be more appropriate rather than quantification of the major constituent, *i.e.,* α-mangostin.

Supplementation of topical antioxidant is an attractive approach for prevention and treatment oxidative stress-mediated skin diseases [14]. For example, using *in-vivo* animal model, Cuelho *et al.,* 2018 [15] provided evidence that topical Yerba mate extracts rich in polyphenols, flavonoids, and tannins formulation having antioxidant activity was protective against acute UV-B exposure as shown by a decreased in myeloperoxidase and metalloproteinase-2 activities. Recently, beneficial effects of topical antioxidant have been shown by a non-invasive method on the volar forearm of the 25 Japanese volunteers [14].

Despite the prospective application of topical antioxidant against oxidative stress-mediated skin diseases, creating an effective topical formulation is challenging [16]. The skin as the site of topical formulation application provides the main barrier for the effective antioxidant delivery. Dosage forms such as cream and gel are popularly used as the topical vehicle. Characteristics topically applied formulation such as pH and viscosity are of importance [17]. Gel and cream w/o loaded extract had relatively acidic pH, *i.e.* 4.80 and 4.10, respectively, which was relatively close with the pH of the extract itself (pH 4). The higher pH of cream o/w (pH 6.80) might be caused by the addition of triethanolamine in the formulation. Viscosity is a relevant parameter to both technical manufacturing, applications, and customer acceptance. The difference viscosity value obtained in the formula caused by the composition of the formulation and type of thickening agent, *i.e.* CMC-Na, beeswax, and cetyl alcohol. Probably due to the highest oil content combined with the present of cera alba in the formulation, w/o cream exhibited the highest viscosity. The more aqueous formulation, *i.e.* gel, on the other hand, showed the lowest viscosity.

In-vitro release study served as a valuable screening test to evaluate the suitability of the developed GMP extract formulations. The extent of the GMP extract antioxidant released from formulations to the receptor phase was quantified using the scavenging activity towards stable radical DPPH. Since all GMP extract formulations had the same amount of GMP extract, differences in RSGMPE release may vary due to thermodynamic activity difference. Cumulative RSGMPE *in-vitro* release showed that gel formulation had the highest release to the receptor phase ($P<0.05$) followed by o/w cream, whereas cream w/o exhibited the lowest RSGMPE released. RSGMPE compound(s) from GMP extract were significantly easier to be released from a relatively more hydrophilic gel than o/w cream. The lipophilic nature of w/o cream may produce higher solubility of antioxidant compounds, reducing its release from the formulation. This result is in agreement with Casagrande *et al.,* [9] who reported that o/w emulsion with less lipidic component released faster lipophilic quercetin from the emulsion. Another reason for the high release antioxidant from gel may also due to its low viscosity. It is generally known that drug release is inversely correlated with viscosity [18]. The low gel viscosity facilitated antioxidant compound(s) to diffuse out easier from the gel matrix system.

Topical antioxidant skin delivery was evaluated using shed snake skin as the skin membrane. The amount RSGMPE found in the receptor phase from various GMP extract formulation showed the same order to that found from release evaluations, *i.e.* gel > o/w cream > w/o cream. It appears that RSGMPE delivery through the skin was governed by its release from formulation. However, it was also noted that relative comparison among gel, o/w and w/o cream on the degree RSGMPE released *vs.* penetrated through skin existed. Even though cumulative released at 24 h from o/w cream and w/o cream were 2-fold and 50-fold lower than that

of gel, respectively, the RSGMPE amount penetrated across shed snake skin into the receptor phase from o/w and w/o cream were not statistically different (P>0.05) accounted for about 2-fold lower that of gel. These results indicate that RSGMPE delivery through the skin was not only dictated by the effect of the significant formulation on the active(s) release. It is possible that either difference in the structural type of the formulation or type of excipients affects active(s) partition into skin and or diffusion through the skin.

The possible alteration GMP extract formulation types and/or excipients on antioxidant skin penetration was also reflected by the higher amount RSGMPE retained in the skin from o/w cream than other formulations. Its value was 1.2-fold higher than gel which produced the highest antioxidant penetrated receptor phase. The existence of excipient in the o/w cream might increase RSGMPE solubility in the skin or decrease its diffusion through the skin. On the other hand, the least amount RSGMPE retained in the skin from w/o cream might explain the relative increase RSGMPE delivery through the skin. Excipient in the formulation may affect skin penetration results. Both creams tested had much different olive oil content (6% in o/w cream *vs.* 45% in w/o cream). Olive oil is rich in oleic acid [19,] which is usually regarded as a skin penetration enhancer. When used in a low amount in the formulation, GMP extract constituents solubility in the skin might be increased whereas their diffusivity in the skin might be increased by incorporating olive oil in high amount in the formulation. Jantharaprapap and Stagni [20] reported that penetration enhancers activity of oleic acid was concentration dependent.

GMP extract has been identified to have mangostin as the main compound [7, 8, 11]. Therefore it is also of interest to evaluate *in-vitro* skin penetration of mangostin from GMP extract loaded formulation. Being able to provide highest RSGMPE penetration through the skin, gel loaded GMP extract was also further characterized for mangostin skin delivery. Quantification of mangostin *in-vitro* skin transport showed that mangostin was more retained in the shed snake skin membrane than transported into the receptor phase. Combining this result with RSGMPE skin penetration data of GMP extract loaded gel indicated that much higher RSGMPE delivery into receptor phase than that of retained in the skin might occur due to RSGMPE compound(s) other than mangostin which was able to penetrate through the skin.

Similar to mangostin profile skin transport result from GMP extract loaded gel, skin penetration -mangostin loaded gel also showed greater retention in the shed snake skin than amount transported through the skin. Mangostin may have a reservoir depot in the skin. Direct skin transport comparison for mangostin data obtained from GMP extract *vs.* pure α-mangostin loaded gel in this present result is difficult since another mangostin form *e.g.* β-mangostin, γ-mangostin presents in GMP extract may also be quantified in addition to α-mangostin using UV spectrophotometric method used in this study. Current findings reported that the amount of α-mangostin, β-mangostin, and γ-mangostin in GMP extracts were varied between 7-72%, 0.4-5%, and 1.8-16.9%, respectively, dependent on the extracting solvents [21]. The dry GMP extract used in this study was obtained using 70% ethanol as extracting solvent and contained 17% of total mangostin (equal to 1.7% total mangostin in 10% dry GMP extract loaded gel). When using 75% ethanol as extracting solvent, Abdalrahim *et al.* [21], found that α-mangostin, β-mangostin, and γ- mangostin in GMP ethanolic extract were 53%, 1.4%, and 11%, respectively.

CONCLUSION: This study confirmed the DPPH radical scavenging activity of GMP extract and α-mangostin. Type of formulation as well as the ingredients used in the formulation could modulate free radical DPPH scavenging equivalent GMP extract release profile from topical formulations. Highest free radical scavenging equivalent GMP extract release was obtained from GMP extract loaded gel, which might reflect the highest thermodynamic activity in the gel.

Also, this effect partly resulted in high *in-vitro* free radical DPPH scavenging equivalent GMP extract penetration through shed snake skin. While the free radical DPPH scavenging equivalent GMP extract compound was transported through shed snake skin in a much higher amount than that retained in the skin, mangostin which is usually known as the major constituent in the GMP extract was shown to be more retained in the shed snake skin.

The current finding is the first report comparing *in-vitro* shed snake skin penetration of GMP extract evaluated based on radical DPPH scavenging activity and mangostin. The results are useful information for the formulation scientists for optimizing GMP extract formulation as well as a better designing strategy for antioxidant or mangostin skin targeting.

ACKNOWLEDGEMENT: This research was funded by the Indonesian Ministry of Research, Technology and Higher Education Research Grant, 2017.

CONFLICT OF INTEREST: The authors declare there are no conflicts of interest regarding this study.

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How to cite this article:
Kuswahyuning R, Yuniarti N, Lesmana I and Fadhilah N: *In-vitro* transport evaluation of *Garcinia mangostana* L. pericarp extract loaded cream and gel. Int J Pharm Sci & Res 2019; 10(8): 3727-34. doi: 10.13040/IJPSR.0975-8232.10(8).3727-34.