Indonesian Journal of Pharmacy

VOL 31 (3) 2020: 187–192 | RESEARCH ARTICLE

The influence of Propylene glycol/Water and Ethanol/Water Binary Solvents on the *in vitro* Permeation of *Garcinia mangostana* L. Pericarp Extract across Shed Snakeskin

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Info Article

Submitted: 24-10-2019
Revised: 15-08-2020
Accepted: 28-09-2020

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ABSTRACT

Garcinia mangostana L. pericarp (GMP) has antioxidant activity thus may be developed into a topical antioxidant formulation. This research aimed to study the topical delivery of GMP extract. Some commonly studied topical solvents, i.e., water and binary solvents of propylene glycol (PG)/water and ethanol/water (0; 10; 20 and 40%), were used to dissolve GMP extract and further tested on GMP extract *in vitro* skin permeation using shed snakeskin. The influence of solvents was evaluated based on radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and expressed as radical scavenging activity equivalent GMP Extract (RSGMPE). The results showed that RSGMPE could be delivered into shed snakeskin. The extent of RSGMPE shed snakeskin retention was similar among water and all PG/water or ethanol/water binary solvents. RSGMPE was also found in the receptor phase from all PG/water binary solvents, whereas that from ethanol/water solvents only shown from 20% ethanol/water solvent even though all ethanol-water solvents produced significantly higher RSGMPE compared to water and PG/water binary solvents.

Key words: *Garcinia mangostana* pericarp extract, solvent, skin permeation, antioxidant

INTRODUCTION

Researches on the topical product with antioxidant activity have continued enormously. Natural sources with antioxidants activity have been explored, characterized, and established in line with the "back to nature trend" (Nagula *et al.*, 2019; Nunes *et al.*, 2018; Ribeiro *et al.*, 2015). The high antioxidant activity of plant extracts has been associated with total phenolic and flavonoid content (Campanini *et al.*, 2014; Martorana *et al.*, 2013; Nunes *et al.*, 2018). *Garcinia mangostana* L. pericarp (GMP) extract, sourced from the pericarp of *Garcinia mangostana* L. fruit, is one of the interesting natural sources of antioxidant (Suttirak *et al.*, 2014). The extract contains bioactive substances such as phenolic acids and flavonoids, which are partly responsible for the antioxidant activity (Sukatta *et al.*, 2013). Various methods have been used to evaluate GMP extract antioxidant activity including free radical scavenging activities based on radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), chelating ability, reducing power, and lipid oxidation inhibitory. A review of the GMP extract antioxidant activity has been summarized and published (Suttirak *et al.*, 2014).

Vehicle plays a significant role in the extent of chemical delivery to the skin (Censi *et al.*, 2012). Propylene glycol (PG) and ethanol are one of the most widely investigated vehicles in topical and transdermal delivery. Fonseca *et al.* (2011) showed the incorporation of these vehicles into topical formulation containing botanical extract resulting in high actives skin retention with minimal permeation. The application of this formulation to hairless mice *in vivo* showed an effective UVB photoprotection. However, it is also known that the vehicle may not significantly affect the extent of permeation (Censi *et al.*, 2012).

The development of topical GMP extract formulation has been an attractive topic. Despite many reports on the potential antioxidant activity of GMP extract, there is still insufficient information on GMP extract skin permeation. Therefore, this research studied *in vitro* GMP extract skin delivery using water and commonly studied topical vehicles as, i.e., water,

binary solvents mixtures of PG/water, and ethanol/water, i.e., 10, 20, and 40%. The degree of GMP extract dissolved in those solvents as well as permeated into/through shed snakeskin were evaluated in terms of functional antioxidant activity based on the radical DPPH scavenging method.

MATERIALS AND METHODS

Dried extract of *Garcinia mangostana* pericarp fruit (extracted using 70% ethanol, local supplier), radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), ethanol, methanol (E-Merck), propylene glycol (PG), and phosphate buffer saline (PBS) pH 7.4.

In vitro antioxidant evaluation by radical DPPH scavenging

The antioxidant activity of the GMP extract was measured using the radical DPPH scavenging method (Campanini *et al.*, 2014; Kuswahyuning *et al.*, 2019). GMP extract was dissolved in PG to make a GMP extract concentration of 20 mg/mL with the aid of sonication (15min). From this stock solution, a 10 mg/mL GMP extract concentration was made which then further diluted to make GMP concentrations of 0.05-0.70 mg/mL. Each of the GMP extract concentration (0.1mL) was added with 0.1M acetate buffer pH 5.5 (1.0 mL), ethanol (1.0 mL) and 0.4M DPPH in ethanol (0.5 mL). The reaction mixture was kept dark for 30 min at room temperature. The absorbance was measured at the maximum absorption wavelength previously determined for the reaction mixture (522 nm) against a corresponding blank. The concentration of GMP extract that caused 50% of scavenging activity was considered as IC_{50}. The obtained radical DPPH scavenging activity (%) of each GMP extract concentration was plotted against corresponding radical DPPH scavenging activity (%) to estimate radical scavenging activity equivalent GMP extract/RSGMPE (μg/mL) of the GMP extract dissolved in the solvent and from *in vitro* skin permeation.

Solvent mixtures

Binary solvents PG/water and ethanol/water were each prepared in various concentrations i.e. 10; 20 and 40% w/v. GMP dry extract was added in excess in each of the solvent mixtures and sonicated for 15 min. The mixture was centrifuged for 5000 rpm 10 min. The obtained supernatant was carefully taken, diluted to the same degree (1000-times) and tested for the extent of GMP extract dissolved in the vehicle. The results were compared with that obtained using water as GMP extract solvent. The obtained radical DPPH scavenging activity (%) was transformed into radical scavenging activity equivalent to GMP extract (RSGMPE).

In vitro skin permeation study

In vitro skin permeation studies were conducted using diffusion cells. The shed snakeskin, obtained from *Phyton morulus sp*, was used as a skin membrane. Previously hydrated shed snakeskin (effective surface area of 1.4 cm^2) was mounted between the donor and receptor compartment. The receptor phase (1.0 mL) was 20% ethanol in phosphate buffer saline and stirred with a magnetic stirrer at 300 rpm. The donor compartment was filled with tested solvent (2.0 mL) then covered by parafilm. *In vitro* skin permeation study was conducted for 24h at room temperature (28±2°C). After 24h, the receptor phase was taken (0.5 mL) and tested for DPPH scavenging activity. For *in-vitro* skin retention studies, the remaining formulation in the donor compartment was removed, and the skin was dismantled from the cell, cleaned & washed with distilled water. The skin then cut into small pieces and placed in a tube. Methanol (1.0 mL) was added to extract the substances retained in the skin as previously published method (Kuswahyuning *et al.*, 2019). Samples (0.1 mL) were then evaluated for DPPH scavenging activity. Four replicates were done for each of the formulations studied. The GMP extract-free formulation was served as blank. The obtained radical DPPH scavenging activity (%) was transformed into radical scavenging activity equivalent to GMP extract (RSGMPE).

Data analysis

All of the data was expressed as mean ±SD (standard deviation). One-way ANOVA followed by the Tukey test, was performed for the statistic analysis. A significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

This study showed that GMP extract has antioxidant activity against DPPH radical with IC_{50} of 15.30 μg/mL confirming antioxidant activity from previous studies (Kuswahyuning *et al.*, 2019; Pothitirat *et al.*, 2009; Tjahjani *et al.*, 2014). The identification of the chemical constituents in the GMP reported some chemicals with antioxidant activity. In terms of DPPH scavenging activity, the

antioxidant activity of GMP extract has been associated with its polyphenolic content including α-mangostin, flavonoids, and tannin (Suttirak *et al.*, 2014). The composition of these polyphenolics constituents in the extract was dependent on the type of extracting solvents, which will strongly influence its antioxidant activity. High flavonoids (epicatechin) and tannin content without α-mangostin were found in GMP water extract. GMP water extract had higher DPPH scavenging activity compared with methanol or hexane GMP extract (Ngawhirunpat *et al.*, 2010). Less polar GMP extracts, i.e., ethanol, methanol, and hexane extract, also showed DPPH scavenging activity, partly due to flavonoids, tannin, and α-mangostin. α-mangostin is a non-polar compound and has been identified as the major constituent in GMP extract (Jung *et al.*, 2006; Suttirak *et al.*, 2014). Since GMP extract used in this study was extracted using 70% ethanol, based on those published studies, we speculated that present GMP extract might contain α-mangostin, flavonoids (such as epicatechin), and tannin, which may be partly responsible for its DPPH scavenging activity.

As this research interest was on the GMP antioxidant activity and considering that crude GMP extract consists of a variety of complex polyphenolic substances, the effects of the vehicle on the extent of the GMP extract dissolved in the vehicle as well as skin permeation ability were evaluated based on the DPPH scavenging activity method. The method has been suggested as one of the simple antioxidant evaluation methods. For this purpose, various concentrations of GMP extract was made and quantified for their DPPH scavenging activities. Observed DPPH scavenging activities of these GMP extract concentrations showed linearity (r^2=0.9966) from 2 to 27 μg/mL corresponding to 6-84% DPPH scavenging activity. The assay had a detection limit of 1.1 μg/mL corresponding to 4.76% DPPH scavenging activity and a quantification limit of 3.95 μg/mL corresponding to 13.68% DPPH scavenging activity, respectively.

Solvents effects on the extent of GMP extract dissolved in the vehicles

GMP extract needs to be investigated for its potential topical skin delivery. PG and ethanol are widely investigated as vehicles and commonly used as topical formulation components (Megrab *et al.*, 1995; Panchagnula *et al.*, 2001), thus chosen as the tested GMP extract vehicles.

The effect of PG or ethanol concentrations in the binary solvent mixtures on the extent of GMP extract dissolved in the solvents as measured by DPPH scavenging activities is shown in Figure 1. DPPH scavenging activity of GMP extract in various solvents was expressed as their corresponding GMP extract equivalent concentration (RSGMPE). Using only water as the vehicle, GMP extract showed a 27.08% DPPH scavenging activity corresponding to 210.68±5.96 mg/mL RSGMPE. PG/water or ethanol/water binary solvents significantly increased ($p<0.05$) GMP extract DPPH scavenging activity compared to water solvent. The α-mangostin, the major constituent in GMP extract (Jung *et al.*, 2006), was shown to have DPPH scavenging activity (Kuswahyuning *et al.*, 2019; Ngawhirunpat *et al.*, 2010; Pothitirat *et al.*, 2009; Tjahjani *et al.*, 2014) and low water solubility (Ngawhirunpat *et al.*, 2010; Pan-In *et al.*, 2015; Xu *et al.*, 2017). The solubility of α-mangostin in saline was reported 0.21 mg/mL. Its solubility was markly increased in ethanol (218.01 mg/mL) and polyethylene glycol 400 (247.59 mg/mL)(Xu *et al.*, 2017). In this study, the addition of PG or ethanol in water solvent might better dissolve α-mangostin as well as other non-polar antioxidant constituents in the GMP extract, resulting in an increased DPPH scavenging activity.

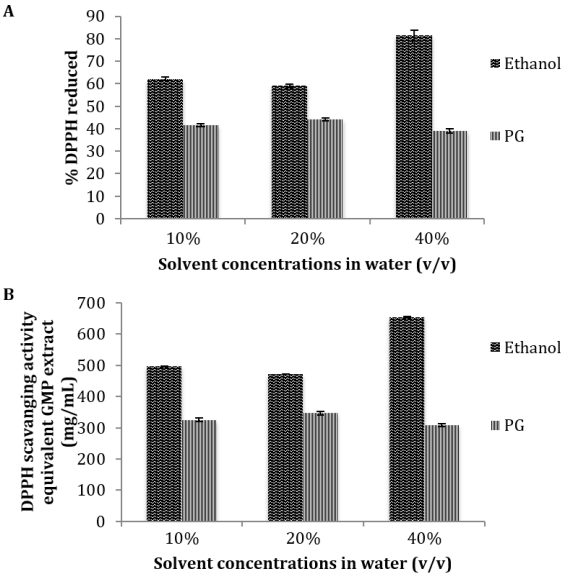

Figure 1. DPPH scavenging activity (% DPPH reduction) (**A**) and its radical scavenging activity equivalent GMP extract (RSGMPE) (**B**) in various ethanol/water or PG/water binary solvent mixtures. Each bar represents the mean ± SD of three replicates.

Table I. Percentage of radical DPPH scavenging activity and corresponding RSGMPE concentration estimated to the permeation

Solvent	DPPH scavenging activity (%) ± SD	RSGMPE (μg/cm²) ± SD
Water	11.67 ± 1.39	- *
10% Ethanol	12.12 ± 16.67	- *
20% Ethanol	28.97 ± 14.29	25.63 ± 12.08
40% Ethanol	10.14 ± 7.06	_*
10% PG	18.97 ± 1.93	16.38 ± 1.77
20% PG	46.39 ± 1.83	41.74 ± 1.70
40% PG	17.79 ± 0.95	15.26 ± 0.89

* The mean value of DPPH scavenging activity is under the quantification limit, so the corresponding RSGMPE could not be estimated.

Table II. Percentage of radical DPPH scavenging activity and corresponding RSGMPE concentration estimated to the skin retention

Solvent	DPPH scavenging activity (%) ± SD	RSGMPE (μg/cm²) ± SD
Water	75.07 ± 0.24	51.86 ± 0.45
10% Ethanol	71.50 ± 3.55	49.13 ± 2.48
20% Ethanol	68.67 ± 1.05	47.15 ± 0.73
40% Ethanol	75.47 ± 1.84	51.90 ± 1.29
10% PG	73.39 ± 3.10	50.45 ± 2.16
20% PG	71.54 ± 0.60	49.15 ± 0.42
40% PG	71.99 ± 0.43	49.47 ± 0.29

All of the PG/water binary mixtures as GMP extract solvents resulted in statistically increased DPPH scavenging activity ($p<0.05$), i.e., 39-41% corresponding to 308.35-347.53mg/mL RSGMPE. Ethanol/water binary solvents showed 62-81% DPPH scavenging activity corresponding to 471-654mg/mL RSGMPE. Only 20% ethanol and 40% ethanol showed a statistical difference in radical DPPH scavenging activity. The 40% ethanol/water binary solvent produced the highest GMP extract DPPH scavenging activity. Relatively polar GMP extract constituents having DPPH scavenging activity, such as epicatechin and tannin, as well as non-polar antioxidant constituent such as α-mangostin (Ngawhirunpat *et al.*, 2010) might dissolve in a much higher amount in 40% ethanol/water mixture.

Solvents effects on *in vitro* GMP extract skin permeation

In vitro skin permeation of GMP extract in various solvent systems were evaluated using shed snakeskin as the membrane. The ability of GMP extracts to penetrate into and through shed snakeskin was also quantified by the radical DPPH scavenging method. Table I and Table II present *in vitro* skin permeation study results.

GMP extract in water solvent showed antioxidant activity in the receptor phase of 11.67 ±1.39%, suggesting that its corresponding RSGMPE could not be estimated (lower than quantification limit), while that retained in the membrane was 75.07±0.24% corresponding to 51.86±0.45 μg/cm² RSGMPE. GMP extract in water solvent might contain polar DPPH scavenging compounds such as epicatechin and tannin. Due to big molecular weight, their diffusion through the skin could be limited. Furthermore, the existence of polyphenolic groups in flavonoids and tannin could pose more barrier for skin penetration, resulting in more skin retention.

The extent of RSGMPE retention in the shed snakeskin membrane (Table II) was not influenced by the concentration of PG/water or ethanol/water binary solvent mixtures. Compared with water as the solvent, all the solvents showed relatively the same degree of skin DPPH scavenging activity and thus RSGMPE retained in the shed snakeskin ($p>0.05$). Even though skin DPPH scavenging

activity appeared similar, the type and amount of each antioxidant chemicals delivered into the skin could not be concluded to be the same in the present study. The different polarity of binary solvent mixtures might induce different antioxidant chemicals dissolved in the vehicles. Therefore, GMP extract thermodynamic activity in the vehicle as well as their effects on the skin, which might further influence chemicals partition to the skin, could not be ruled out. The α-mangostin, which has DPPH scavenging activity, might be dissolved to a certain degree in PG/water or ethanol/water solvent mixtures and partition from the vehicle to the skin. The α-mangostin is a lipophilic compound with an estimated log P of 4.64 (Chin *et al.*, 2016) and completely insoluble in water (Xu *et al.*, 2017). It has a molecular weight of 410.5g/mol and 3 H-bond donor. These physicochemical properties may favor α-mangostin to be retained in the skin. Chin *et al.* (2016) reported that α-mangostin in water suspension resulted α-mangostin viable epidermis/dermis skin retention of 18.85±2.41 μg/g after 48h *in vitro* skin permeation experiment; whereas the flux across the skin was only 0.81±0.23 ng/cm^2/h.

Some binary solvent vehicles promoted RSGMPE extract penetration through shed snakeskin into the receptor. Increasing PG concentrations in water i.e. 10% and 20% significantly increased RSGMPE in the receptor phase compared with water as solvent ($p<0.05$). For ethanol/water solvent systems, only 20% ethanol/water resulted in RSGMPE penetration into the receptor phase. As a penetration enhancer, PG may enhance chemical penetration through various mechanisms such as increasing chemical solubility as well as carrier mechanism. Ethanol may act as a penetration enhancer by thermodynamic activity effects and/or reducing skin barrier resistance, such as by increasing lipid bilayer fluidity (Lane, 2013). In this study, it was not possible to know which polyphenolic GMP extract constituents delivered into the receptor phase based on the DPPH scavenging method. DPPH scavenging chemicals of the GMP extracts that had been delivered into the skin but had low diffusion ability might penetrate the receptor phase if PG or ethanol could interact with the skin facilitating their diffusion. Additionally, other small phenolic compounds in the GMP extract i.e. phenolic acids, such as protocatechuic acid, p-coumaric acid, caffeic acid, and ferulic acid (Suttirak *et al.*, 2014), might also present in the GMP extract vehicle. If these phenolic acids could also be delivered into the skin, they might potentially penetrate into the receptor phase due to their small size.

The use of 40% PG or 40% ethanol concentration in water solvent mixtures resulted in a decrease in the RSGMPE permeated into the receptor phase. This PG or ethanol concentration in the water solution might cause an increase in GMP extract solubility and hence reducing chemical thermodynamic activity. This could be indicated by the increase of RSGMPE dissolved in 40% ethanol/water vehicle. Other studies reported that the enhancement result was not necessarily linear to PG or ethanol concentrations since there may be drug-vehicle-skin interactions (Megrab *et al.*, 1995; Panchagnula *et al.*, 2001). Concerning these present results, more works need to be done to probe the mechanism on GMP extract penetration.

CONCLUSION

This study showed that GMP extract, having antioxidant activity measured by radical DPPH scavenging method, could permeate into shed snakeskin. The extent of GMP extract retained in the shed snakeskin appeared to be more than that transported through the skin regardless of the vehicles tested, i.e., water, binary PG/water, or ethanol/water. More works have to be done to get a better understanding of GMP extract topical delivery.

ACKNOWLEDGMENT

This work was funded by the grant from the Faculty of Pharmacy, Universitas Gajah Mada "Hibah Penelitian Pengembangan Laboratorium 2016". The data in this study were used by Galuh Wahyu Ananingrum and Rias Reno Andini to obtain their academic degree.

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