Journal of the Korean Applied Science and Technology
Vol. 41, No. 1. February, 2024. 9~18
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2024.41.1.9

Skin Permeability Study of Flavonoids Derived from *Smilax china*: Utilizing the Franz Diffusion Cell Assay

Sun-Beom Kwon[1,*] · Ji-Hui Kim[1] · Mi-Su Kim[1] · Su-Hong Kim[1] · Seong-Min Lee[2]
Moo-Sung Kim[2] · Jun-Sub Kim[3] · Gi-Seong Moon[3,†] · Hyang-Yeol Lee[3,†]

[1]*Department of Biotechnology, Korea National University of Transportation, Student*
[2]*Macrocare Co., Ltd, 32, Gakri 1-gil, Ochang-eup, Cheongwon-gu, Cheongju-si, Chungbuk*
[3]*Department of Biotechnology, Korea National University of Transportation, Professor*
(Received December 26, 2023; Revised January 18, 2024; Accepted January 31, 2024)

요 약 : 토복령은 우수한 항균, 항산화, 항염증 효능을 가진 소재로 알려져 있다. 이러한 토복령(*Smilax china*)의 추출물의 기능성을 화장품에 적용하기 위한 기초연구로써 토복령에서 발견되는 플라보노이드인 quercetin, catechin, naringenin의 농도별 경피 투과 특성을 조사할 필요성이 있다. Marzulli의 정의에 적용한 결과 케르세틴의 Kp 값은 0.1 mg/mL에서 "빠름"으로 분류되었고, 0.2 및 0.4 mg/mL에서 "보통"으로 분류되었다. 특히, 농도가 증가함에 따라 투과 속도가 감소하는 경향이 있었다. 나린제닌의 경우 Flux 값은 각각 0.1, 0.2 및 0.4 mg/mL 농도에서 0.69, 1.07 및 1.42 μg/hr/cm^2 이었으며, 해당 Kp 값은 각각 6.95, 5.34 및 3.56이었다. 나린제닌의 Kp 값은 모든 농도에서 "보통" 범주에 속하며, 케르세틴과 관찰된 것과 같이 농도가 높아짐에 따라 투과 속도가 감소하였다. 카테킨의 경우 Flux 값은 각각 0.1, 0.2 및 0.4 mg/mL 농도에서 0.75, 1.09 및 1.66 μg/hr/cm^2 이었으며, 해당 Kp 값은 각각 7.55, 5.46 및 4.16이었다. 카테킨의 Kp 값은 모든 농도에서 일관되게 "보통"으로 분류되었다. 여드름 저해능 및 항염증 효능이 우수한 토복령 추출물의 유효성분인 quercetin, catechin, naringenin의 경피 투과 특성이 보통 이상으로 나타나 기능성 화장품에 사용할 수 있는 우수한 천연물 소재인 것을 확인할 수 있었다.

주제어 : 프란츠 경피투과도 측정, 플라보노이드, 토복령, 케르세틴, 카테킨, 나린제닌

Abstract : *Smilax china* is known for its excellent antimicrobial, antioxidant, and anti-inflammatory properties. As a foundational study for applying the functionality of *Smilax china* extracts to cosmetics, it is necessory to investigate the concentration-dependent skin permation characteristics of the flavonoids in the extract, namely quercetin, catechin, and naringenin. Therefore, it serves as a crucial method for conducting this basic research on the functional aspects fo *Smilax china* extracts for cosmetic applications. This investigation focused on examining the percutaneous permeability characteristics of flavonoids originating from *Smilax china*. Applying Marzulli's definition, the Kp value of quercetin was categorized as "fast" at 0.1 mg/mL and "moderate" at 0.2 and 0.4 mg/mL. Notably, the permeation rate exhibited a decline with increasing concentration. For naringenin, Flux values were 0.69, 1.07, and 1.42 μg/hr/cm^2 at concentrations of 0.1, 0.2, and 0.4 mg/mL,

†Corresponding author
(E-mail: gsmoon@ut.ac.kr, hyl@ut.ac.kr)

respectively, with corresponding Kp values of 6.95, 5.34, and 3.56. Naringenin's Kp value fell into the "moderate" category across all concentrations, and as observed with quercetin, the permeation rate decreased with higher concentrations. Likewise, for catechin, Flux values were 0.75, 1.09, and 1.66 μg/hr/cm^2 , and corresponding Kp values were 7.55, 5.46, and 4.16. Catechin's Kp value was consistently classified as "moderate" across all concentrations. The efficacy of quercetin, catechin, and naringenin, active ingredients in high-performance and anti-inflammatory *Smilax china* extracts, was found to exhibit skin penetration properties above the average. This confirms their suitability as excellent natural materials for use in functional cosmetics, given their outstanding capabilities in preventing acne and reducing inflammation.

Keywords : Franz Diffusion Cell Assay, Flavonoid, Smilax china., Quercetin, Catechin, Naringenin

1. Introduction

The research on the percutaneous permeability of flavonoids, natural compounds derived from plants, is essential in the development of cosmetics. With an increasing consumer demand for natural and botanical ingredients in cosmetic products, studying the skin permeation of flavonoids becomes crucial[1]. Utilizing assays like the Franz diffusion cell allows for a scientific assessment of how these natural compounds penetrate the skin barrier, offering data that guides formulation decisions [2-5]. Investigating the skin permeation characteristics of flavonoids, such as quercetin, naringenin, and catechin, obtained from natural sources such as *Smilax china*, provides valuable insights into their potential efficacy as cosmetic ingredients. These compounds are known for their antioxidant, and anti-inflammatory properties[6-7], making them attractive candidates for inclusion in skincare formulations [8-11]. Additionally, *Smilax china* is known to possess antimicrobial properties, which could be beneficial in targeting the bacteria such as *Cutibacterium acnes* involved in the development of acne[12-13]. From our previous results, the extract of *Smilax china* L. root showed antimicrobial activity against the acne-causing bacterium, *Cutibacterium acnes,* and its active compound, quercetin, exhibited the highest potency among the compounds tested against *Cutibacterium acnes* KCTC 3314, with a minimum inhibitory concentration of 31.25 μg/mL[13]. In the context of cosmetic safety and efficacy, understanding the percutaneous permeability of natural compounds is vital to ensure that active ingredients effectively reach their target layers of the skin[14-16]. This knowledge is essential for optimizing cosmetic formulations to deliver desired benefits while maintaining product safety and compliance with regulatory standards. Conducting research on the skin permeation of flavonoids contributes to the evidence-based development of cosmetics that leverage the potential benefits of natural compounds for skincare. In this study, we investigated the skin permeability characteristics of key components of *Smilax china* extract, known for its acne-inhibiting efficacy. The flavonoids, including quercetin, naringenin, and catechin, were measured using the Franz cell assay system, shedding light on the properties of the skin penetration process associated with these compounds.

2. Experiments

2.1. Materials

The quercetin (95%), (+)-catechin hydrate (98%), and (-)-naringenin (95%) used in the experiments were sourced from Sigma-Aldrich

(St. Louis, MO, USA). The PBS solution was prepared by dissolving one tablet of Sigma-Aldrich's Phosphate Buffered Saline in 200 mL of triple-distilled water. The membranes utilized in the experiment were Cellu Sep T4 dialysis membranes (Seguin, USA) with a molecular weight cut-off (MWCO) ranging from 12,000 to 14,000. Anhydrous ethyl alcohol and methyl alcohol were obtained from Daejung (Siheung, Korea), while Acetonitrile (99.9%), Phosphoric acid (85.0%), and Trifluoroacetic acid (99.0%) were sourced from Samchun (Seoul, Korea). The Franz Diffusion Cell Assay apparatus was supplied by Biense Tech (Daejun, Korea), and High-Performance Liquid Chromatography (HPLC) analysis was performed using Waters' e2695 model.

2.2. Franz Diffusion Cell Assay

To investigate the percutaneous permeation characteristics of each flavonoid at different concentrations, the Franz diffusion cell assay was conducted. The concentrations for all three substances were uniformly set at 0.1, 0.2, and 0.4 mg/mL. Each flavonoid was dissolved in the receptor fluid for the experiment. The water-soluble Catechin used PBS for the receptor fluid, while the lipophilic quercetin and naringenin utilized a 1:1 mixture of PBS and EtOH as the receptor fluid. The receptor fluid completely filled the receptor chamber, and the flavonoid solutions were injected into the Donor chamber at 2 mL each. Pressing the button on the cell part initiated the magnetic stir bar, facilitating the diffusion of flavonoid solutions in the receptor fluid. The moment the button was pressed marked the start of the experiment. Sampling time could be freely adjusted within 24 hours, and in this experiment, samples were taken at 1, 2, 4, 6, 8, and 12 hours after the start of the experiment. The sampling volume was 200 μL, and an equivalent amount of Receptor fluid was replenished after each sampling. The obtained samples were promptly refrigerated, and within 24 hours of storage initiation, quantification analysis was performed using HPLC.

2.3. HPLC analysis

The analysis conditions for quercetin, catechin and naringenin involved using a solvent A of 0.05% TFA in distilled water and solvent B as ACN. The solvents were delivered at an A 87: B 13 ratio isocratically. The flow rate was set at 1 mL/min, and the column utilized was the Phenomenex Gemini 5 μm C18 110Å 150 × 4.6mm. The column temperature was maintained at 30℃, and detection was carried out at a wavelength of 210 nm using a PDA detector (Table 1).

Quantitative analysis of each sample was conducted using HPLC, and based on the obtained data, Flux values and Kp values were determined. The average Kp value was then plugged into Marzulli's definition to indicate the extent of percutaneous permeation.

2.4. Statistical analysis

Calculations were made using Microsoft® Excel® Office 365 and statistical analysis were

Table 1. HPLC analysis conditions for quercetin, catechin and naringenin

Mobile Phase	A : 0.05% TFA in Water, B : ACN
Flow rate	1mL/min
Column	Phenomenex Gemini 5 μm C18 110Å 150 × 4.6mm
Column Temperature	30℃
Wave Length	210nm
Method	Isocratic 87%(A) : 13%(B)

performed using GraphPad Prism 8.03. All data sets are shown as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Skin Permeation Test of Flavonoids, Quercetin, Catechin, and Naringenin

Due to ethical concerns related to animal experimentation, non-clinical test methods that can replace animals are actively being utilized. In the development of cosmetics, substance skin permeability assessments using dermal absorption cells, following OECD guidelines, are widely employed, eliminating the need for animal testing. In this study, following the skin permeation guidelines, we utilized dermal absorption cells, using acetaminophen as the control group and quercetin, catechin, and naringenin as the test substances. The units representing the skin permeation characteristics can be observed and confirmed through the skin permeation rate, Flux, and penetration rate, Kp values. Flux, representing the permeation rate, can be calculated as the amount of the sample passing through a constant skin area per unit time. All three substances exhibit an increasing trend in the amount of permeated solute over time. The results are depicted in
Figure 1.

3.2. Permeability rates (Flux) and coefficients (Kp) of quercetin, catechin and naringenin

Permeability rates (Flux) are measurements indicating the amount of substance passing through a unit area per unit time. All three substances show an increase in Flux values with higher concentrations, and there is a tendency for permeability rates to decrease over time. The results are illustrated in Figure 2 and Table 2.

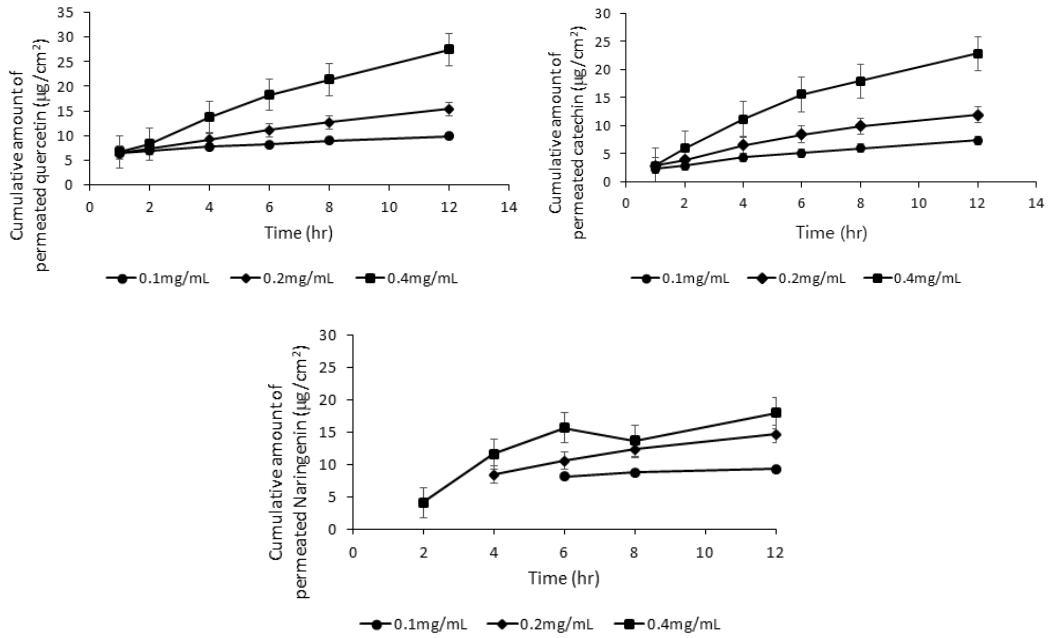

Fig. 1. Cumulative amount of permeated quercetin, catechin and naringein through the membrane. Values are presented as mean ± SD.

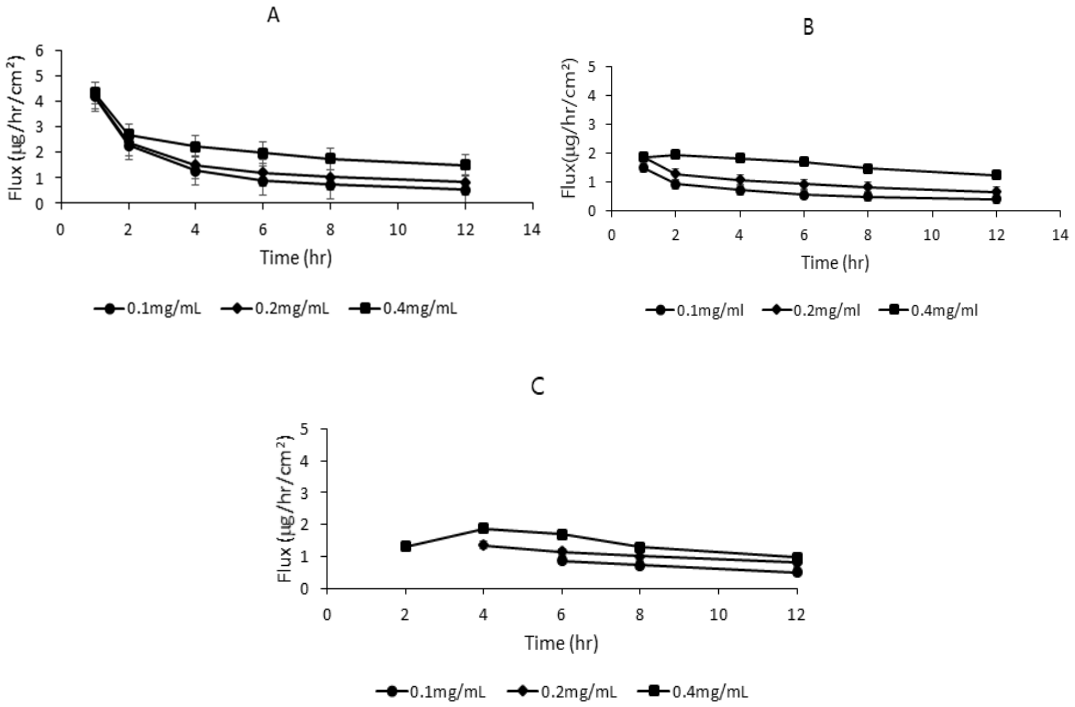

Fig. 2. Flux of permeated quercetine, catechin and naringenin.
Values are presented as mean ± SD.

Table 2. Permeability rates (Flux) of each tested substances

	Flux (Control) (μg/hr/cm^2)	Flux (A) (μg/hr/cm^2)	Flux (B) (μg/hr/cm^2)	Flux (C) (μg/hr/cm^2)
0.1mg/mL	0.97	1.64 ± 0.03	0.75 ± 0.06	0.69 ± 0.04
0.2mg/mL	1.44	1.87 ± 0.14	1.09 ± 0.23	1.07 ± 0.06
0.4mg/mL	2.58	2.41 ± 0.10	1.54 ± 0.08	1.42 ± 0.44

Control: acetoaminophen, A: quercetin, B: catechin, C: naringenin
Values represent the mean ± SD of three independent measurements.

The permeability coefficient (Kp) is measurement indicators of the dermal permeability of a substance, representing the ability of a substance to be absorbed into the body through the skin. Kp is used as an index reflecting the capability of a substance to pass through the skin and be absorbed into the body. For all three substances, there is a tendency for Kp values to decrease with increasing concentration and over time. The results are depicted in Figure 3.

To apply Marzulli's definition to the experimental results, the average K_p values for each flavonoid concentration were calculated. The results are presented in Table 3.

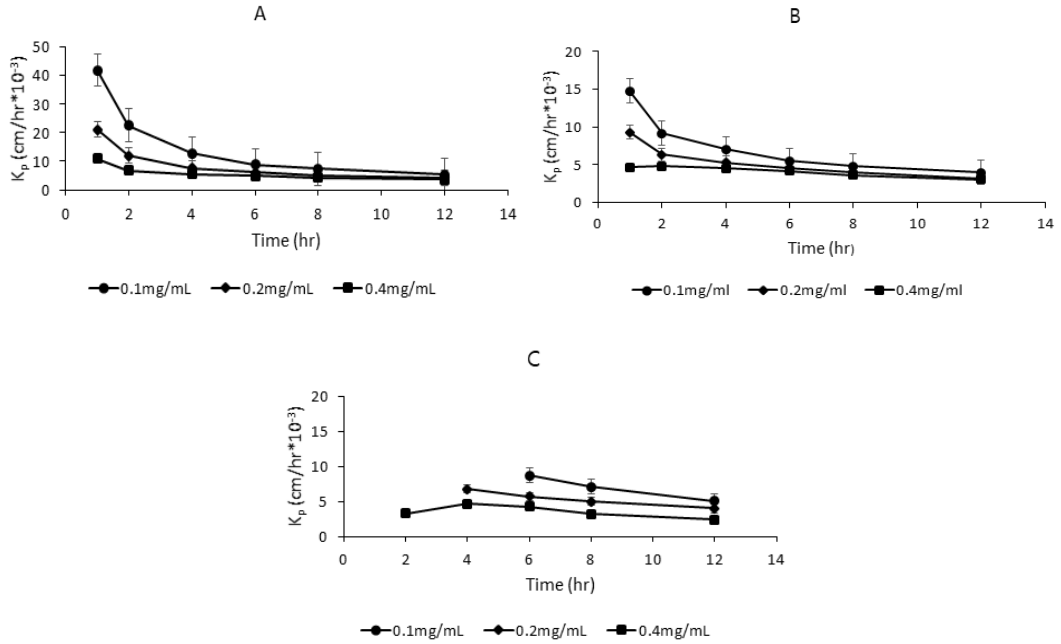

Fig. 3. K_p value of qurcetine (A), Catechin (B), and Naringenin (C).
Values are presented as mean ± SD.

Table 3. Permeability coefficient (K_p) of test substances

	Control K_p value (cm/hr × 10^{-3})	A K_p value (cm/hr × 10^{-3})	B K_p value (cm/hr × 10^{-3})	C K_p value (cm/hr × 10^{-3})
0.1mg/mL	9.73	16.43 ± 0.03	7.55 ± 0.13	6.95 ± 0.44
0.2mg/mL	7.18	9.33 ± 0.07	5.46 ± 1.16	5.34 ± 0.28
0.4mg/mL	6.45	6.03 ± 0.24	3.84 ± 0.21	3.56 ± 1.10

Control: acetoaminophen, A: quercetin, B: catechin, C: naringenin
Values represent the mean ± SD of three independent measurements.

Upon applying Marzulli's definition to the experimental results, the permeation rate of quercetin appeared as 'Fast' at a concentration of 0.1 mg/mL and as 'Moderate' at concentrations of 0.2 and 0.4 mg/mL. Both catechin and naringenin exhibited a 'Moderate' permeation rate at all concentrations. All three substances showed a tendency for the permeation rate to decrease with increasing concentration. The results are illustrated in Figure 4.

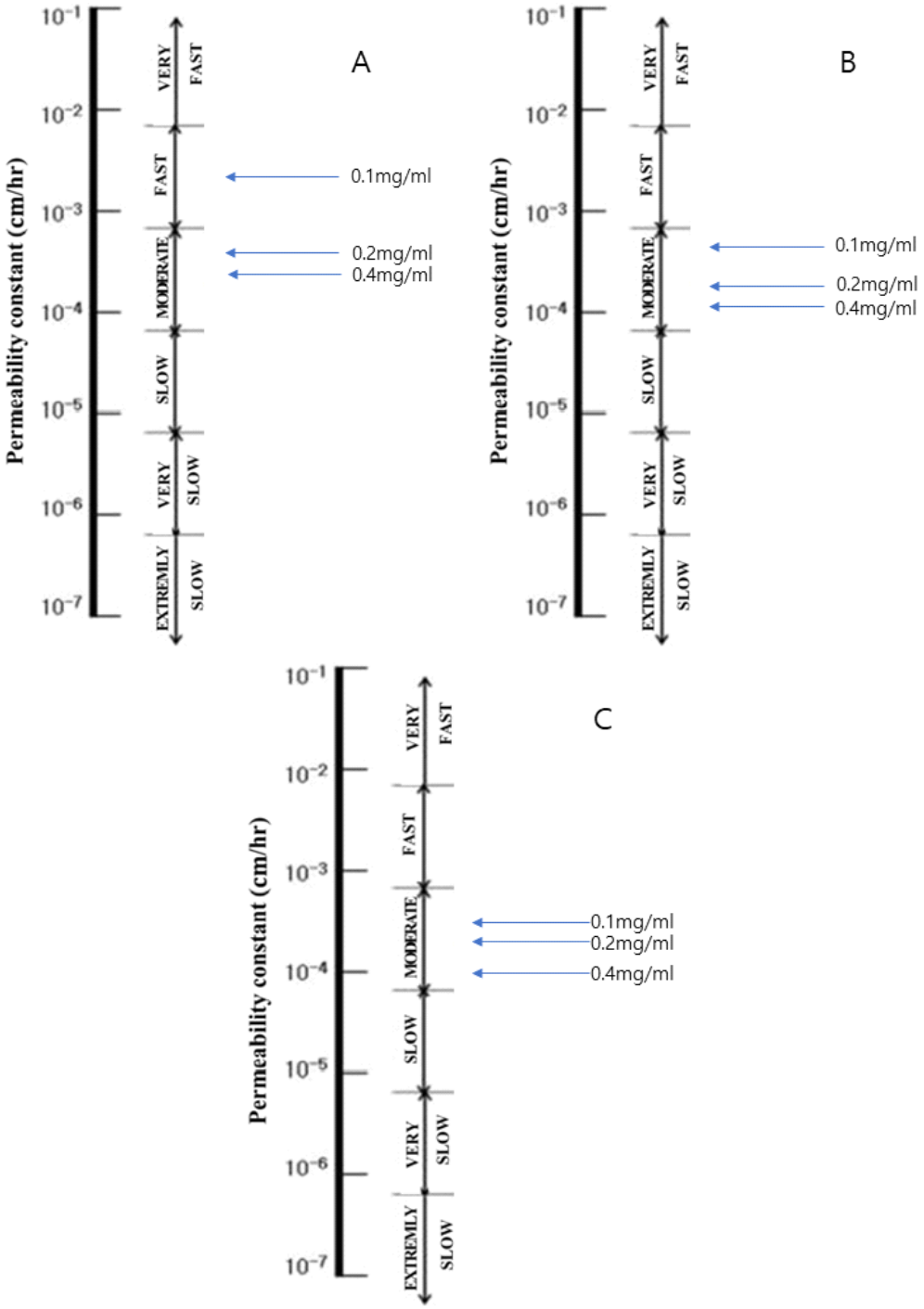

Fig. 4. The Average Kp values of quercetin, catechin, and naringenine.
A: quercetin, B: catechin, C: naringenin.

Applying the calculated Kp values and judging based on Marzulli's definition, the permeability of catechin was found to be in the range of 'moderate,' which is most similar to the control group using acetaminophen.

Franz diffusion cells are widely used as a research tool to evaluate skin permeability and the interactions between skin and tested products. These cells usually contain excised human or animal skin, but synthetic membranes are a more convenient option. Synthetic membranes mimic skin properties and vary in forms. They are useful for studying drug diffusion or active compounds in products[17]. This study used a polysulfone membrane to measure the *in vitro* release of flavonoids (quercetin, catechin, and naringenin) from *Smilax china*.

To investigate the dermal permeation characteristics of the flavonoids, experiments were conducted using the Franz Diffusion Cell Assay and HPLC. For quercetin, Flux values of 1.64, 1.86, and 2.41 $\mu g/hr/cm^2$ at concentrations of 0.1, 0.2, and 0.4 mg/mL, respectively, were observed, with corresponding Kp values of 16.42, 9.32, and 6.03. Applying Marzulli's definition, quercetin's Kp was categorized as 'fast' at 0.1 mg/mL and 'moderate' at 0.2 and 0.4 mg/mL. The permeation rate declined with increasing concentration. Naringenin exhibited Flux values of 0.69, 1.07, and 1.42 $\mu g/hr/cm^2$ at concentrations of 0.1, 0.2, and 0.4 mg/mL, respectively, with corresponding Kp values of 6.95, 5.34, and 3.56, consistently falling into the 'moderate' category. Similar to quercetin, the permeation rate decreased with higher concentrations. Catechin displayed Flux values of 0.75, 1.09, and 1.66 $\mu g/hr/cm^2$, and corresponding Kp values of 7.55, 5.46, and 4.16, consistently classified as 'moderate' across all concentrations.

For all three substances, both Flux and Kp values decrease over time, suggesting that the permeation characteristics involve an initial rapid release followed by a slower release in the later stages. Furthermore, the increase in concentration is associated with higher Flux values, while Kp values decrease with higher concentrations. This is interpreted as an increase in the amount of permeating solute with higher concentrations, although the proportion of permeated solute relative to the total solute decreases. Therefore, all three substances exhibit similar permeation characteristics, and as concentration increases, the permeation efficiency decreases. The dermal permeation rates are fastest for quercetin, followed by catechin and naringenin.

Especially, flavonoids exert potent anti-inflammatory effects on the skin, showing positive effects on some skin diseases, such as photoaging, psoriasis, and atopic dermatitis [18]. However, the cutaneous absorption of flavonoids is influenced by many factors, such as their chemical structure, solubility, concentration, formulation, and skin barrier function. Therefore, it is not clear how much flavonoids can penetrate the skin and exert their bioactivity. Moreover, the systemic exposure, metabolism, and toxicity of flavonoids after topical application are also poorly understood. Thus, more research is needed to evaluate the safety and efficacy of flavonoids as functional cosmetic ingredients.

4. Conclusion

In summary, the discussed data reveals that naringenin and catechin, active components in extract, *Smilax china* exhibits a “moderate” skin penetration profile, with their Kp values consistently falling into this category across various concentrations. Overall, the empasized point in the results is the that quercetin, catechin, and naringenin surpass the average in terms of skin penetration, indicationg their effective ability to penetrate the skin. These properties render them promising candidates for incorporation into functional cosmetics, bolstered by their proven efficacy in mitigating

acne and inflammation. The moderate skin penetration classification further implies their potential for optimal skin absorption, an attribute highly sought after in cosmetic formulation. This data-driven insight could pave the way for the development of efficacious, natural ingredient-based cosmetics.

Acknowledgement

This research was supported by "Regional Innovation Strategy (RIS)" through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(MOE) (2021RIS-001).

References

1. X. Hu, H. He, "A Review of Cosmetic Skin Delivery", *J. Cosm. Derm.,* Vol.20, No.7 pp. 2020-2030, (2021). doi:10.1111/jocd.14037.
2. J. E. Seo, J. Lee, B. H. Kim, "A Permeation Characteristics Study of Water- or Oil-soluble Substances through Condition for the In Vitro Skin Absorption Method", *J. Environ. Health Sci.*, Vol.43, No.1 pp. 77-86, (2017).
3. T. J. Franz, "Percutaneous Absorption in the Relevance of in Vitro Data", *J. Invest. Dermatol.,* Vol.64, No.3 pp. 190-195, (1975). doi:10.1111/1523-1747.ep12533356.
4. OECD. "OECD Guideline for the Testing of Chemicals", No.428 (2004).
5. S. Zsikó, E. Csányi, A. Kovács, M. Budai-Szűcs, A. Gácsi, S. Berkó, "Methods to Evaluate Skin Penetration *In Vitro*", *Sci. Pharm.*, Vol.87, No.3 pp. 19, (2019)
6. S. Jiang, Q. Wei, X. Ye, D. Luo, X. Zhang, Z. Li, P. You, X. Huang, Y. Liu, "The Anti-Inflammatory Effect of *Smilax china* L. Extract on LPS-Stimulated THP-1 via Downregulation of MAPK and NF-kappaB Signaling Pathway", *Evid. Based Complement Alternat. Med, Vol. 2021*, pp. 9958808, (2021). doi:10.1155/2021/9958808.
7. H. Feng, Y. He, L. La, C. Hou, L. Song, Q. Yang, F. Wu, W. Liu, L. Hou, Y. Li, "The Flavonoid-Enriched Extract from the Root of *Smilax china* L. Inhibits Inflammatory Responses via the TLR-4-mediated Signaling Pathway", *J. Ethnopharmacol.,* Vol.256, pp. 112785, (2020). doi:10.1016/j.jep.2020.112785.
8. C. M. Kawakami, L. R. Gaspar, "Mangiferin and Naringenin Affect the Photostability and Phototoxicity of Sunscreens Containing Avobenzone", *J. Photochem. and Photobiol. B: Biology,* Vol.151, pp. 239-247, (2015). doi:https://doi.org/10.1016/j.jphotobiol.2015.08.014.
9. J. H. Kim, T. S. Park, S. H. Yang, J. W. Suh, S. M. Shim, "Microbial Bioconversion and Processing Methods Enhance the Phenolic Acid and Flavonoids and the Radical Scavenging Capacity of *Smilax china* L. Leaf", *J. Sci. Food. Agric.,* Vol.96, No.3 pp. 878-885, (2016). doi:10.1002/jsfa.7160.
10. L. Kong, W. Zhang, S. Liu, Z. Zhong, G. Zheng, "Quercetin, Engelitin and Caffeic Acid of *Smilax china* L. Polyphenols, Stimulate 3T3-L1 Adipocytes to Brown-like Adipocytes Via beta3-AR/AMPK Signaling Pathway", *Plant Foods Hum. Nutr.,* Vol.77, No.4 pp. 529-537, (2022). doi:10.1007/s11130-022-00996-x.
11. Y. H. Kuo, Y. W. Hsu, C. C. Liaw, J. K. Lee, H. C. Huang, L. M. Kuo, "Cytotoxic Phenylpropanoid Glycosides from the Stems of *Smilax china*", *J. Nat. Prod.,* Vol.68, No.10 pp. 1475-1478, (2005). doi:10.1021/np050109q.
12. H. E. Lee, J. A. Kim, W. K. Whang, "Chemical Constituents of *Smilax china* L. Stems and Their Inhibitory Activities against Glycation, Aldose Reductase, alpha-Glucosidase, and Lipase", *Molecules,* Vol.22, No.3, pp. 451, (2017). doi:10.3390/

molecules22030451.

13. J. H. Joo, M. H. Han, J. I. Kim, J. E. Kim, K. H. Jung, H. S. Oh, Y. S. Chung, H. J. An, J. D. Lee, G. S. Moon, "Antimicrobial Activity of *Smilax china* L. Root Extracts against the Acne-Causing Bacterium, *Cutibacterium acnes*, and Its Active Compounds", *Molecules,* Vol.27, No.23 pp. 8331, (2022). doi:10.3390/molecules27238331.
14. B. Kim, H. -E. Cho, S. H. Moon, H. -J. Ahn, S. Bae, H. -D. Cho, S. An, "Transdermal Delivery Systems in Cosmetics", *Biomed. Dermat.,* Vol.4, No.10 (2020). doi:10.1186/s41702-020-0058-7.
15. S. F. Ng, J. J. Rouse, F. D. Sanderson, V. Meidan, G. M. Eccleston, "Validation of a Static Franz Diffusion Cell System for *In Vitro* Permeation Studies", *AAPS PharmSciTech,* Vol.11, No.3 pp. 1432-1441, (2010). doi:10.1208/s12249-010-9522-9.
16. I. Pulsoni, M. Lubda, M. Aiello, A. Fedi, M. Marzagalli, J. von Hagen, S. Scaglione, "Comparison Between Franz Diffusion Cell and a Novel Micro-physiological System for *In Vitro* Penetration Assay Using Different Skin Models", *SLAS Tech.,* Vol.27, No.3 pp. 161-171, (2022). doi: https://doi.org/10.1016/j.slast.2021.12.006.
17. S.-F. Ng, J. Rouse, D. Sanderson, G. Eccleston, "A Comparative Study of Transmembrane Diffusion and Permeation of Ibuprofen across Synthetic Membranes Using Franz Diffusion Cells". *Pharmaceutics,* Vol.2, No.2 pp. 209-223, (2010).
18. R. Sawamura, H. Sakurai, N. Wada, Y. Nishiya, T. Honda, M. Kazui M., A. Kurihara, A, Shinagawa., T. Izumi. "Bioactivation of Loxoprofen to a Pharmacologically Active Metabolite and Its Disposition Kinetics in Human Skin", *Biopharm. Drug Dispos.* Vol.36, No.6 pp. 352-363. (2015). doi: 10.1002/bdd.1945.