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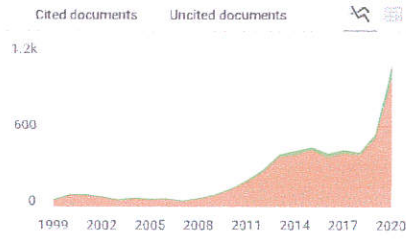
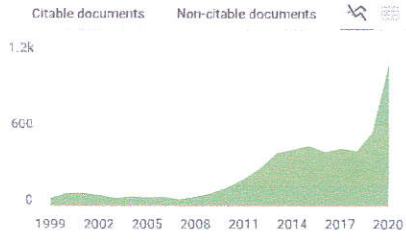
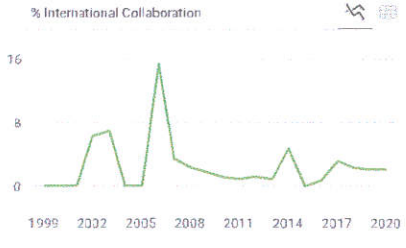
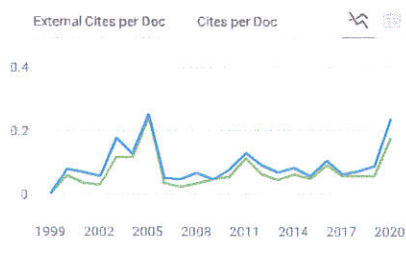
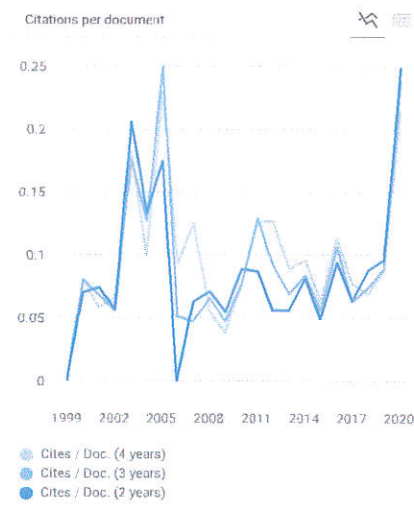
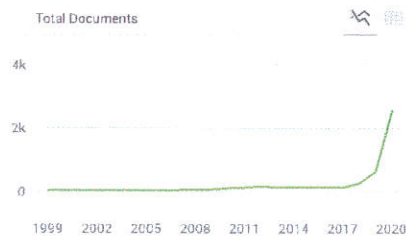
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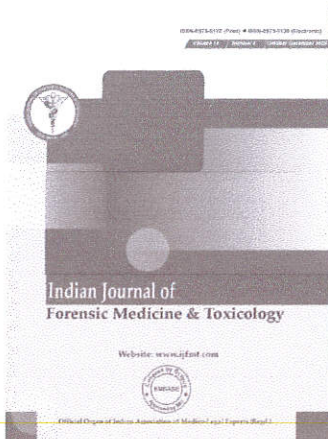
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# Skin Penetration of Topical Epigallocatechin-3-Gallate (EGCG) as an Alternative Agent for Photoaging Prevention

Damayanti<sup>1,2</sup>, Cita Rosita Sigit Prakoeswa<sup>2</sup>, Djoko Agus Purwanto<sup>3</sup>, Anang Endaryanto<sup>4</sup>, Widjiati Widjiati<sup>5</sup>

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## Abstract

Photoaging is skin aging, that especially caused by chronic exposure of ultraviolet radiation. Photoaging impacts to patients' quality of life. Many substances, such as green tea, had been tried to be alternative agents for photoaging prevention. Epigallocatechin-3-gallate (EGCG) is the most abundant component in green tea. The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. The aim of this study was to evaluate the skin penetration of EGCG. The back part of the male Wistar mouse was shaved carefully, then topical EGCG cream 5% and 10% were administered to mouse skin. After 1 week administration, the animal was terminated and the skin biopsy was done. The mouse skin was extracted and high performance liquid chromatography (HPLC) examination was performed to evaluate the skin penetration of topical EGCG. The result of HPLC examination of 2 ppm, 4 ppm, 6 ppm, and 8 ppm EGCG level, showed the curve peak at 3.295, 3.296, 3.295 and 3.293 second. The basic curve showed R<sup>2</sup> of pure EGCG powder used in this study was 0,9999. The result of HPLC examination in this study showed, the curve peak of extracted mouse skin after EGCG cream 5% and 10% administration were between 3.2-3.7 second. The result of HPLC examination in this study showed that EGCG cream could penetrate into Wistar mouse skin after EGCG cream application for 1 week.

**Keywords:** EGCG, skin penetration, HPLC.

## Introduction

Photoaging is skin aging, that especially caused by chronic exposure of ultraviolet radiation. Photoaging impacts to patients' quality of life.<sup>1,2</sup> Ultraviolet radiation promotes inhibition of transforming growth factor  $\beta$  receptor II (TGF $\beta$  RII) and causes increasing of matrix metalloproteinase-1 (MMP-1), that play role in photoaging pathogenesis by degrading the collagen and inhibiting the collagen synthesis.<sup>2,3,4</sup>

Many substances, such as green tea, had been tried to be alternative agents for photoaging prevention. Topical green tea extract prevent photoaging by preventing the MMP-1 elevation and dermal collagen reduction in photoaging mouse model.<sup>5,6,7</sup> EGCG is the most abundant component and the most polyphenolic catechin in green tea (approximately 59%). EGCG is assumed as the main source of biological activity of green tea.<sup>8,9,10</sup>

The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. Topical substance should be able to penetrate through stratum corneum as the skin barrier. Topical substance applied on the skin would be transferred based on active substance concentration gradient (passive diffusion). Concentration gradient is the different between active substance concentration applied on the skin with active

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substance concentration in the skin layers.<sup>11,12</sup> Because of the high importance of topical substance' skin penetration that play role in its activity, this study was aimed to evaluate the skin penetration of EGCG as an alternative agent in photoaging prevention.

**Material and Methods**

**Materials**

Pure EGCG powder was purchased from Xi' An Rongsheng Biotechnology Co., LTD, China (batch number 190702). Male Wistar rats aged 10-12 weeks with average weight 100-250 grams was provided by Faculty of Veterinary, Airlangga University, Surabaya, Indonesia.

**Preparation of Topical EGCG**

Preparation of topical EGCG cream was started from base cream preparation. The base cream consist of virgin coconut oil (VCO), cetacium, cera alba, olive oil, and aqua destilata. Cera alba and cetacium were boiled in the porcelain bowl above the water bath until the mixture was melting. Aqua destilata was added to the mixture and stir. VCO and olive oil were added to the mixture and stir until it became the base cream. Topical EGCG cream 5% was prepared by adding the pure EGCG powder to the base cream (1:19). Topical EGCG cream 10% was prepared by adding the pure EGCG powder to the base cream (1:9).<sup>13</sup>

**Intervention**

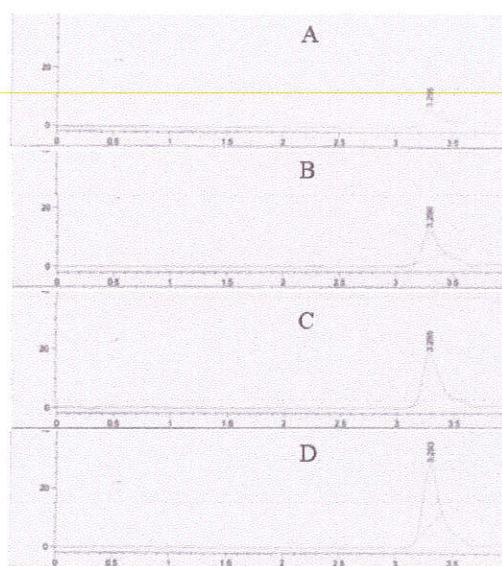
The back part of the male Wistar mouse was shaved carefully in 3x3 cm2 size. Topical EGCG cream 5% and 10% was administered to mouse skin twice a day for a week (7 days). The size of topical EGCG cream applied to the mouse skin was 4 mg/cm2 body surface area.<sup>14</sup> After 1 week administration, the animal was terminated and the skin biopsy was done. After that, the mouse skin was extracted. The mouse skin was pounded until became smooth. Chloroform was added into the refined skin and the mixture was filtered. Ethyl acetate was added into the refined skin and the mixture was filtered, put into the Becker glass. The whole ethyl acetate in the mixture was evaporated and dissolved into methanol solution.

The first step of HPLC examination was making the mobile phase and creating the component solution.

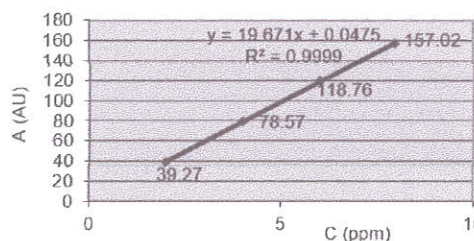
Then, the standard solution was made. The initial setting of HPLC system was checked before the examination, and continue to manually inject the sample and data collection. The result of HPLC examination was showed as curve peak in HPLC chromatograms.<sup>15</sup>

**Result**

The purity of pure EGCG powder used in this study was evaluated with high performance liquid chromatography (HPLC) examination. The result of HPLC examination of 2 ppm, 4 ppm, 6 ppm, and 8 ppm EGCG level, showed the curve peak at 3.295, 3.296, 3.295 and 3.293 second (Picture 1). The basic curve showed R2 of pure EGCG powder used in this study was 0,9999 (Picture 2).



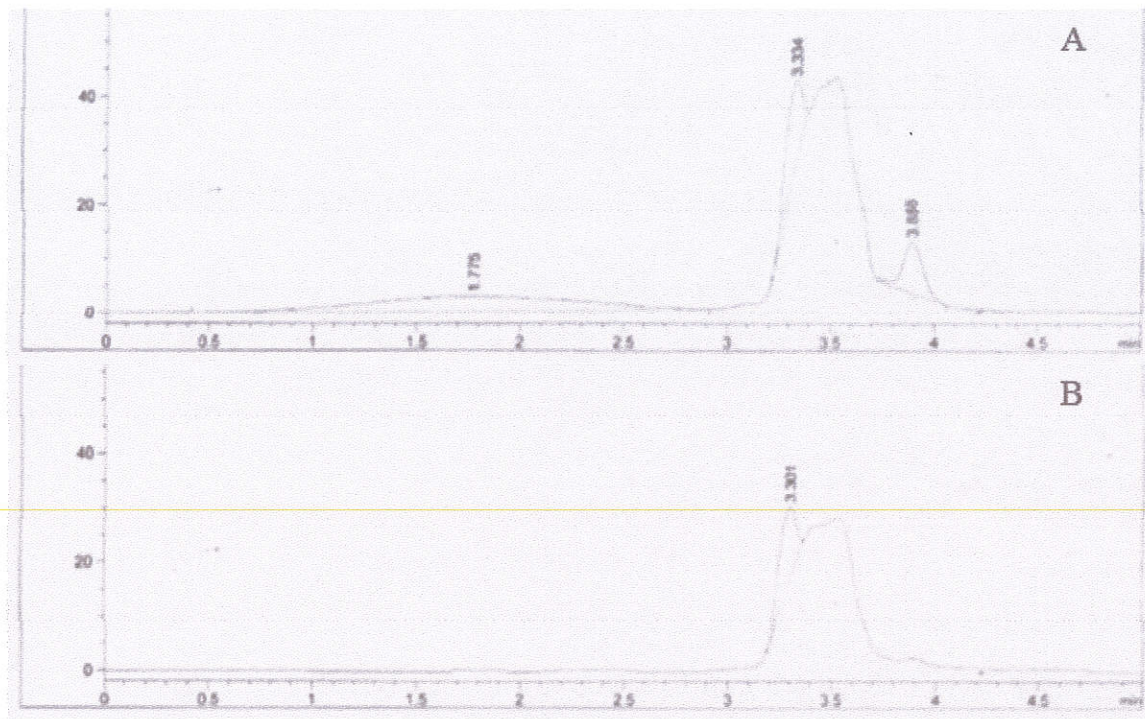
**Picture 1. The result of HPLC examination of 2 ppm (Picture 1A), 4 ppm (Picture 1B), 6 ppm (Picture 1C), and 8 ppm (Picture 1D) EGCG level.**



**Picture 2. The basic curve of pure EGCG powder.**

Topical EGCG cream 5% and 10% were administered to mouse skin twice a day for 1 week. The result of HPLC examination from mouse skin extraction

in this study showed, the curve peak after EGCG cream 5% administration was at 3.334 second (between 3.2-3.7 second), and the curve peak of mouse skin extracted after EGCG cream 10% administration was 3.301 second (between 3.2-3.7 second) (Picture 3). This result showed that topical EGCG cream 5% and 10% could penetrate into the Wistar mouse skin after EGCG cream administration for 1 week. .



**Picture 3. The result of HPLC examination from extracted mouse skin after EGCG cream 5% (Picture 3A) and EGCG cream 10% (Picture 3B) administration.**

### Discussion

EGCG is the most abundant component and the most polyphenolic catechin in green tea. Cellular uptake of catechin in EGCG is higher than other catechins. EGCG in this study was formulated as topically substance. Scalia et al evaluated percutaneous permeation of catechin in 1% oil in water emulsion and 1% hydrophilic gel on human skin using non-invasive tape-stripping technique. Scalia's study showed that EGCG could penetrate into the skin layer and there was no significant difference of the EGCG dose diffused in the skin layer in oil in water emulsion and hydrophilic gel formulation.<sup>10,14</sup>

The result of HPLC examination from mouse skin extraction in this study showed, the curve peak after EGCG cream 5% and 10% administration were at 3.334 second and 3.301 second (between 3.2-3.7 second).

This result showed that topical EGCG cream 5% and 10% could penetrate into the Wistar mouse skin after EGCG cream administration for 1 week.

The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. Skin penetration of a substance is influenced by the substance factor and the host factor. The substance factors are substance concentration, partition coefficient and molecule size.<sup>16,17</sup>

There are three interaction after the topical substance was applied on the skin. The first interaction is the interaction of active substance soluted in the vehiculum. The second interaction is the interaction of vehiculum and the skin. The third interaction is the interaction of the soluted substance with the skin layer.<sup>11</sup>

Topical substance applied on the skin would be transferred based on active substance concentration gradient (passive diffusion). The effect of topical substance depends on the substance concentration that reach therapeutic target area. Topical substance diffuse to the inner layer of the skin based on diffusion law Fick I and Fick II. Diffusion law Fick I stated steady state flux of substance ( $J = \text{moles/cm/second}$ ) per unit path length ( $\delta$ , cm) in accordance to concentration gradient ( $\Delta C$ ) and diffusion coefficient ( $D$ ,  $\text{cm}^2/\text{second}$ ), or symbolized as  $J = -D(\Delta C/\Delta \delta)$ . Diffusion law Fick II predicted the drug flow on nonsteady state condition, and stated that diffusion is the effective transport mechanism in short distance or symbolized as  $\Delta t = x^2/2D$  ( $\Delta t = \text{time}$ ,  $x = \text{path length}$ ).<sup>11,12,18</sup>

Vehiculum or base cream of the substance also plays role in the skin penetration. Vehiculum is inactive substance plays as a carrier of active substance into the skin. The preparation form of vehiculum used in this study was cream. Cream is semisolid preparation. The cream preparation used in this study was oil in water emulsion preparation, with water ingredient more than 31 %. Oil in water emulsion is more often to use because it is easy to be applied on the skin, easy to be washed, not too oily and the skin penetration is higher.<sup>16,17,19</sup>

VCO used in this study as the ingredient of the base cream plays role as an emollient and moisturizer. VCO makes the skin moister and plays role in the reduction of skin diffusion resistance. Oleic acid and lauric acid in VCO increase the penetration rate of active substance in VCO based cream used in this study. VCO based cream has good adhesion capacity, that makes bigger possibility for the active substance to penetrate into the skin. Good adhesion capacity also plays role in the skin hydration elevation.<sup>13,19</sup>

VCO also acts as penetration enhancer, that could increase the active substance penetration into the skin. Lauric acid in VCO increase lipophilic and hidrophilic active substance by ruining the bond of intercellular lipid lamellar in the stratum corneum. It caused decreasing of membrane viscoscity, increasing of skin permeability, and increasing of skin penetration of substance.<sup>13</sup>

Host factor that plays role in topical substance penetration is skin barrier function. The skin barrier function depends on stratum corneum function and skin

hydration. The decreasing of stratum corneum function and skin hydration could decrease the penetration of topical substance.<sup>12,16,19</sup>

## Conclusion

The result of HPLC examination in this study showed that topical EGCG could penetrate into Wistar mouse skin after EGCG cream application for 1 week. It is therefore conceivable that topical EGCG may be beneficial for preventing photoaging. Further study about the role and the mechanism of topical EGCG in photoaging prevention was needed.

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