Effect of solid lipid nanoparticles system on the stability of Green Tea leaves (Camellia sinensis L. Kuntze) extract as sunscreen

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Abstract. Green tea (Camellia sinensis L.) is known to have activity as antioxidant and sunscreen due to its catechin compound. The major catechin-derived compound is epigallocatechin gallate (EGCG). However, EGCG in green tea leaves is unstable due to its rapid degradation and large molecular mass of 458 daltons, making it difficult to penetrate the skin.

The purpose of this research is to formulate solid lipid nanoparticles (SLN) of green tea leaves ethanol extract using high speed homogenization and sonication method, as well as to evaluate SLN ability in improving extract stability as sunscreen using UV-Vis spectrophotometry. SLN formula contained 0.1% green tea leaves ethanol extract, and the most optimal formula was composed of 4% lipid (Precirol ATO 5:Gelucire 44/14) 4:1 and 1% Kolliphor P188 as surfactant. Characterization results showed particle size of 286.7 nm, polydispersity index 0.296, and zeta potential -3.7 mV. Photostability test using 120 minutes irradiation showed decrease in percentage of DPPH free radicals damping at 5.34% extract and 1.77% SLN for UVA, and 7.7% extract and 2.34% SLN for UVB.

1. Introduction
Green tea (Camellia sinensis L.) is known to have several pharmacological activities including antioxidants. Based on some studies, ethanol extract of green tea leaves has IC_{50} value of 3.17 µg/mL [1]. Substances that have high antioxidant activity, will have a low EC_{50} or IC_{50} value. If the IC_{50} value is less than 50 ppm, it is classified as a very strong antioxidant [2]. The antioxidant activity is caused by the chemical compounds in tea named catechins, which molecular structure consisting of two phenol groups (A and B rings) and one dihydropyran group (ring C). Since it has more than one phenol group, catechin compounds are often called polyphenolic compounds. The total content of catechins in fresh dried tea leaves ranged from 13.5 to 31% of its dry weight [3]. Tea leaf also contains caffeine and gallic acid [4].

Catechin derived compounds found in tea leaves are epigallocatechin, epicatechin, epicatechin gallate, and epigallocatechin gallate (EGCG) [5]. EGCG is the major polyphenolic component, which is about 60-70% of total catechins [6]. EGCG in green tea has a very potent antioxidant effect which is 100 times more effective at neutralizing free radicals than vitamin C and 25 times more effective than vitamin E [3]. In addition, a number of studies have described the pharmacological effects of EGCG such as photoprotective/sunscreen, anti-aging, anti-inflammatory, modulation of immunity, anticancer, cardioprotective, neuroprotective, antiviral and antibacterial [7]. Based on the research by Supriadi, et al. as a photoprotection, SPF value of cream containing 8% green tea ethanol extract is 7.48 [8]. And
application of cream containing 20% green tea extract can prevent melanin increase as effective as application of 4% hydroquinone cream on guinea pigs skin exposed to ultraviolet B [9]. However, EGCG compounds are unstable due to its rapid degradation which can cause loss of biological activity. Also, it has a large molecular mass of 458 Dalton, making it difficult to penetrate the skin [10,11]. To improve the stability and effectiveness of green tea ethanol extract in topical applications as photoprotective/sunscreen, it can be developed into Nano carrier formulations, such as solid lipid nanoparticle (SLN).

Solid lipid nanoparticles (SLN) are sub-micron colloidal carriers (50-1000 nm) composed of physiological lipids, dispersed in water or surfactant solutions [12]. SLN is present to overcome the problems that arise due to the use of nanoparticle polymers that have side effects. Polymer nanoparticles are made of monomers and organic solvents that are harmful to the body and cannot be decomposed. The advantage of the SLN system is that it is possible to control drug release and target, improve drug stability, can combine lipophilic and hydrophilic drugs, can avoid toxicity from the carrier, and can avoid the use of organic solvents. SLN is made from a mixture of lipid, water, surfactant, and cosurfactant if needed [13]. In this study, SLN was prepared and their effect in improving the stability of green tea leaf ethanol extract against UVA/UVB radiation was investigated.

2. Materials and methods

2.1. Material
Green tea leaves (Camellia sinensis), obtained from Kertamanah tea plantation in Pengalengan, West Java, and determined at Herbarium in Department of Biology, Padjadjaran University. Ethanol 96%, Gliceryl Monostearate (GMS), Cetyl alcohol, Cetyl palmitate obtained from CV. Sumber Rezeki, Gelucire 44/14® (Lauroyl macrogol-32 glycerides), Precirol ATO 5® (Glycerol distearate) obtained from PT. Menjangan Sakti, Kolliphor® P 188, DPPH (sigma aldrich).

2.2. Method

2.2.1. Sample preparation and extraction. Green tea leaves sample that have been determined at Herbarium in Department of Biology, Padjadjaran University and macerated with 96% ethanol. Ethanol filtrate was evaporated, and the yield, extract parameters was calculated [14]. Phytochemical screening of extracts was carried out to determine the content of secondary metabolites found in the extract.

2.2.2. Solid Lipid Nanoparticles formulation. Formulation of green tea leaves ethanol extract SLN was performed using high speed homogenization method combined with sonication. Formula optimization was initiated by screening several lipids: Gliceryl Monostearate (GMS), cetyl alcohol, cetyl palmitate, Gelucire 44/14, Precirol ATO 5, and surfactant screening, which are tween 80 and Kolliphor P 188. The formulation was conducted by selecting lipids which best for dissolving tea leaves ethanol extract. 25 mg of extract was dissolved in 1 g of melted lipid. The solubility of tea leaves ethanol extract in lipids will affect the encapsulation efficiency or capacity of extracts loading in SLN system. Lipids are then combined with surfactants. The combination of lipids and surfactants was carried out in various concentrations. Screening of surfactants toward lipids was monitored by measuring particle size and polydispersity index, as well as centrifugation test. Selected formula was then mixed with tea leaves ethanol extract in SLN system. Tea leaves ethanol extract was first dissolved in the selected solid lipid which had been melted, then the surfactant which had been dissolved in water was heated until reach the same temperature, then they were mixed slowly. The mixing of the oil phase and the water phase was performed initially by light stirring and then stirred using ultra-turrax at 15,000 rpm for 15 minutes which then continued with cooling for 10 minutes and sonication for 50 minutes.
2.2.3. **Physical evaluation of Solid Lipid Nanoparticles (SLN).** Physical evaluation of SLN conducted includes particle size, particle size distribution (polydispersity index), and zeta potential using dynamic light scattering device [15].

2.2.4. **Entrapment efficiency.** Green tea leaf extract SLN was dried by freeze drying method. The amount of entrapped extracts can be calculated by DPPH free radical damping method using UV-Vis spectrophotometer [16].

\[
\% \text{EE} = 100\% - \left( \frac{W_t}{W_i} \times 100\% \right)
\]

Where : Wi: Percentage of initial damping extract added to SLN system

Wt: Percentage of trapped damping extract in SLN system

2.2.5. **SLN photostability using UVA/UVB irradiation.** SLN ethanol extract of green tea leaves was irradiated using UVA/UVB radiation simulator, then the level of antioxidant degradation of ethanol extract in SLN was measured using UV-Vis spectrophotometer. Data analysis was performed using the SPSS-Anava program [17].

3. **Results and discussion**

3.1. **Solid Lipid Nanoparticle formulation**

3.1.1. **Lipid and surfactant screening.** Lipids are the main component in the formation of SLN because it plays a role in extract loading capacity, entrapment efficiency, and active substances stability. The choice of solid lipids with the condition of being able to condense at room temperature, have “Generally-Recognized-As Safe” (GRAS) status, and physiologically can be tolerated/degraded by the body. Precirol ATO 5, Gelucire 44/14 dan combination of both were selected lipids because they can perfectly dissolve the ethanol extract of green tea leaves by not leaving the extract particles and does not form extract sediment when the lipid resolidified.

Surfactants have an important role in solid lipid nanoparticles (SLN) formation as stabilizing agents that help prevent SLN particles from forming aggregates so that the formed size can be maintained. Surfactants can also minimize free energy using the interfacial tension between lipid globules and dispersing fluids. In that case, determining the amount of surfactant that is compatible with each lipid is very important to get optimal results. From screening result (Table 1), that 4% Tween 80 and 1% Kolliphor P 188 was the selected surfactant that produced inseparable colloidal system and did not produce deposits when mixed with lipids.

| Table 1. Surfactants screening result. |
|---------------------------------------|
| **Selected Lipids** | **Lipid Amount (%)** | **Surfactants** | **Surfactant amount (%)** | **Result** |
|----------------------|----------------------|----------------|--------------------------|------------|
|                       |                      |                | Physical Appearance      | Centrifugation Result |
|                       |                      |                |                          | (3500 rpm, 3 hours)   |
| Precirol ATO 5 :      | 4%                   | Kolliphor P 188| 0.25                     | Colloidal system     |
| Gelucire 44/14 (1:1)  |                      |                |                          | Few sediments       |
|                      | 0.5                  |                |                          | Colloidal system     |
|                      | 0.75                 |                |                          | Few sediments       |
|                      | 1                    |                |                          | Not separated        |
| Precirol ATO 5 :      | 4%                   | Kolliphor P 188| 0.25                     | Colloidal system     |
| Gelucire 44/14 (4:1)  |                      |                |                          | Sediments            |
|                      | 0.5                  |                |                          | Sediments            |
|                      | 0.75                 |                |                          | Sediments            |
|                      | 1                    |                |                          | Not separated        |
Table 1. cont.

| Precirol ATO 5 : Gelucire 44/14 (1:1) dan (4:1) | 4% | Tween 80 | 4 | Colloidal system | Not separated |
|-----------------------------------------------|----|----------|---|------------------|---------------|
| 6 Colloidal system                            | Not separated |           |
| 8 Colloidal system                            | Not separated |           |
| 10 Colloidal system                           | Not separated |           |

3.1.2. Solid Lipid Nanoparticles (SLN) formulation

Table 2. Result of solid lipid nanoparticles (SLN) formula optimization.

| Formula | Lipid | Lipid Concentration (%) | Surfactant | Surfactant Concentration (%) | Particle Size (nm) |
|---------|-------|-------------------------|------------|------------------------------|--------------------|
| F1      | Precirol ATO 5 : Gelucire 44/14 (1:1) | 4 | Tween 80 | 4 | 1273 |
| F2      | Precirol ATO 5 : Gelucire 44/14 (4:1) | 4 | Tween 80 | 4 | 940 |
| F3      | Precirol ATO 5 : Gelucire 44/14 (1:1) | 4 | Kolliphor® P 188 | 1 | 262 |
| F4      | Precirol ATO 5 : Gelucire 44/14 (4:1) | 4 | Kolliphor® P 188 | 1 | 250 |

The optimal particle size for SLN has a range of 100-300 nm and the range of acceptable polydispersity index is 0 (monodispersed) to 0.5 [18]. Polydispersity index provides information about the physical stability of a dispersion system. Low polydispersity index indicates more stable dispersion system for long term and higher polydispersity index indicates low homogeneity [19-21]. Based on this argument, formula F4 with the particle size of 250 nm and polydispersity index 0.275 was the most optimal formula and was selected as a carrier for green tea leaves ethanol extract in SLN.

3.2. Physical evaluation of Solid Lipid Nanoparticles (SLN)

Table 3. Characterization result of green tea leaves ethanol extract SLN.

| Components                   | Concentration (%) | Particle size (nm) | PDI  | Potensial Zeta (mV) |
|------------------------------|-------------------|--------------------|------|---------------------|
| Green tea leaves ethanol extract | 0.1               |                    |      |                     |
| Precirol ATO 5 : Gelucire 44/14 (4:1) | 4               | 286.7              | 0.296| -3.7                |
| Kolliphor® P 188             | 1                 |                    |      |                     |
| DI Water                     | Ad 100            |                    |      |                     |

SLN of green tea leaves ethanol extract has particle size of 286.7 nm which is not very different from SLN carrier system without extract in formula 4 with the size of 246.8 nm. It is predicted that green tea leaves ethanol extract can be entrapped in lipids in SLN system. Also, polydispersity index of 0.296 describes homogeneous particle size distribution. Zeta potential shows electrostatic repulsion between particles in colloidal dispersions which is related to colloidal stability and aggregation or flocculation of colloidal particles. A colloid is said to be stable if the zeta potential approaches + 30/-30 mV [16]. The zeta potential in this study was -3.7 mV. Negative zeta potential value in this study showed that SLN has an electrode potential at zero charge, so the system is said to be stable because charges and flocculation on the globules did not occurred [22].

3.3. Entrapment efficiency

500 mL SLN (F4) containing 500 mg of green tea leaves extract was dried using freeze dryer, and the yield obtained was 22.6102 g. The amount of entrapped extracts was calculated by comparing the results of DPPH free radicals damping between 0.01% green tea leaves ethanol extract and SLN proportioned
to 0.01% green tea leaves ethanol extract using UV-Vis spectrophotometer [16]. The results showed entrapment percentage of green tea leaves ethanol extract was 86.33%.

3.4. SLN Photostability using UVA/UVB Irradiation

Extrasts and green tea leaves ethanol extract SLN (F4) were irradiated with UVA/UVB radiation simulator, then the antioxidant degradation level (DPPH radical damping) of ethanol extract in SLN was measured using UV-Vis spectrophotometer [17].

| Table 4. Photostability Result of UVA Radiation towards Extract and SLN. |
|--------------------------|--------------------------|
| Irradiation | Duration | Damping Percentage (%) | Extract | SLN |
| UVA | 0 | 80.99 | 79.43 |
| | 15 | 80.43 | 78.44 |
| | 30 | 78.09 | 78.23 |
| | 60 | 77.66 | 78.16 |
| | 120 | 75.60 | 77.66 |
| Total decrease of DPPH radical damping | 5.39 | 1.77 |

| Table 5. Photostability Result of UVB Radiation towards Extract and SLN. |
|--------------------------|--------------------------|
| Irradiation | Duration | Damping Percentage (%) | Extract | SLN |
| UVB | 0 | 81.80 | 77.92 |
| | 15 | 79.24 | 77.84 |
| | 30 | 78.07 | 77.45 |
| | 60 | 76.21 | 76.52 |
| | 120 | 74.03 | 75.58 |
| Total decrease of DPPH radical damping | 5.39 | 1.77 |

Longer irradiation time increased the damaged ethanol extract of green tea leaves. It is showed by the decreasing percentage of DPPH free radical damping, which indicated weaker antioxidant ability. SLN system can reduce the decrease in percentage of DPPH free radical damping in green tea leaves ethanol extract. It means SLN system can improve the photostability of green tea leaves ethanol extract against UVA and UVB radiation. Statistical analysis using unpaired t method tests showed no significant mean score difference between extract group and SLN group with p=0.167 after UVA irradiation, while they differ significantly after UVB irradiation with p=0.01 [23].

4. Conclusion

SLN for 0.1% green tea leaves extract was successfully formulated, with 4% lipid carrier (Precirol ATO 5 : Gelucire 44/14) 4:1 and 1% Kolliphor P188 as surfactant. Results showed particle size of 286.7 nm, polydispersity index 0.296, and zeta potential -3.7 mV. Photostability test of extract and SLN using UVA and UVB irradiation for 120 minutes showed decreased in percentage of DPPH free radicals damping at 5.34% extract and 1.77% SLN for UVA, and 7.7% extract and 2.34% SLN for UVB. Statistical analysis using independent t-test (α=0.05) showed no significant difference between extract and SLN with p=0.167 for UVA, while significant difference was shown for UVB with p=0.01.

Acknowledgement

The Authors are thankful to LPPM-UNISBA, Faculty of Mathematics and Natural Sciences-Department of Pharmacy, laboratory assistants of Research Laboratory, PT. Menjangan Sakti, as well as students and fellow lecturers for providing help.
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