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ORIGINAL ARTICLE

Characterization of Biocompatible Lipid-Based Vesicles Contained with Medicinal Herb Extracts

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Abstract

In order to increase the medicinal herbs efficiency of drug delivery, vesicles contained with medicinal herbs were prepared by phosphatidylcholines and surface active agent. Vesicles loaded with medicinal herbs were characterized by UV-spectroscopy, Zetasizer. The antioxidant activity of vesicles was measured by DPPH assay and ABTS radical scavenging assays. Also, an analysis was conducted to determine the effects of anti-inflammatory of vesicles contained medicinal herbs. In addition, the whitening effects of vesicles contained medicinal herbs extract were studied via tyrosinase inhibition assay. The results of vesicles were as follows. Vesicles appeared an average diameter of approximatively 164-599 nm. All studied vesicles contained with medicinal herbs showed antioxidant, anti-inflammatory and whitening effects in a dose-dependent manner. Therefore, this experiment achieves its purpose of synthesizing of vesicles. In conclusion, we recommended that the vesicles loaded with medicinal herbs have ability for anti-aging materials. Specifically, it will apply to cosmetic ingredients.

Key words : Antioxidant activity, Anti-inflammatory drug delivery system, Biocompatible Vesicles, Eco-friendly

1. Introduction

As the level of living improves, interest in quality of life is increasing. One of these concerns is the desire for health, which is confronted with the problem of aging. Although it is difficult to determine the definition of aging, it can be seen as a phase in which the body undergoes a irreversible reaction in accordance with the passage of time since human maturity. There are many types and numbers of diseases that cause aging, but many have been reported to be associated with reactive oxygen species (Ames et al., 1993).

Reactive Oxygen Species (ROS) generated during the metabolic process of the body causes the oxidation reaction in all parts of the body. When ROS is elevated in the cell, are high, cellular structures as protein, nucleic acid, cell membrane are destroyed or inflammatory reaction is caused. If ROS accumulates continuously, abnormalities in the signaling system of cells may cause serious diseases such as diabetes, cancer, heart disease, and hypertension, etc. (Kiyoshima et al. 2012).

Eventually, degeneration of the cells, which originated from ROS, induces disease and promotes aging (Alok et al., 2014). A number of studies have

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been carried out to use natural materials to control oxidation and inflammation caused by these free radicals (Matouskova et al., 2016).

Golden-and-silver honeysuckle (*Lonicera japonica*) is known to inhibit the inflammatory response by influencing various factors involved in the inflammatory response mechanism. In vivo and in vitro studies have been carried out, and further research is underway for the treatment of skin, muscle and skeletal disorders(Lu et al. 2015).

Fish mint (*Houttuynia cordata*) has a number of flavonoid derivatives, and in vitro tests have shown that the extracts are effective against *Streptococcus pneumoniae*, Staphylococcus aureus, and Escherichia coli, including decanoyl acetaldehyde (Kumar et al., 2014). It is known that it has a strong antimicrobial activity because it contains antibiotic substances.

Lianas (*Uncaria sinensis* (Oliv.) Havil.) has been studied as an antioxidant activity of hyperin present in the stem as a vine bract from the madder. In addition, 1-methoxyoctadecan-1-ol, one of the substances present in the extract, has a pharmacological effect that can protect against neurotoxins (Jang et al., 2014).

Gardenia (*Gardenia jasminoides*) lives in Korea, China, Japan, and fruit is mainly used for medicine. Gardenia extracts have been shown to be involved in immune responses that affect Cytokines and antioxidants that can remove Reactive Oxygen Species (ROS) from the body (Park et al., 2014).

Baikal skullcap (*Scutellaria baicalensis*), was used as the root of the deciduous grass. It is a perennial plant which grows in various fields of Korea and is cultivated mainly in field. Traditionally, gold has been used as a remedy for the treatment and prevention of inflammation, and has a medicinal effect on brain blood flow, such as stroke (Zhang et al., 2013)

Recently, there have been many studies on drug delivery systems. Even if it possesses excellent pharmacological effect, the possibility of utilization can not be significantly lowered unless it is delivered to a target tissue cell. Therefore, we use carriers such as nanoparticles for more effective pharmacological effects (Zamani et al., 2013).

In order to improve efficiency by using the absorption transmission system of active materials as one of the methods for enhancing the biological effectiveness of these naturally occurring active materials, studies are continuing to produce multi-layer or single-walled vesicle structures (Fang et al., 2008). Recently, the most commonly used method utilizes the principle that the film is formed spontaneously by exposure to an aqueous environment using the amphiphilic property of phosphatidylcholine. The produced vesicles have the characteristics of cell membranes and are non-antigenic and non-toxic. Another type of phospholipid or amphipathic substance can be incorporated into the membrane of the vesicle being formed, and hydrophilic materials can form hydrated vesicles (Brunetti et al., 2016). It can also be used as an environmentally friendly and biocompatible safe material using an environmentally friendly manufacturing process.

Therefore, it was intended to develop a biocompatible delivery system that would enable more effective application of functional activity, including the antioxidant capabilities of herbal medicines. In particular, cosmetics containing medicinal herbs, which have functionality such as antioxidants, are widely available and popular in domestic and abroad. it has many benefits for efficacy and safety, research and development is still needed to increase efficiency in the area of absorption in cells and a number of studies are under way. Thus, some improvements are made to the method used in the drug delivery system to enhance the activity of functional materials in these cosmetics and apply it to the development of cosmetic products.

This study prepared vesicles such as trans ethosomes using phosphatidylcholine melted in ethanol and omija oil surfactant. Trans ethosomes have the advantage of being able to pass through the stratum cornea better than the universal ethosomes and can penetrate active substances better into the skin (Ascenso et al., 2015). The development of vesicle, which can have effective skin absorption and transfer properties within cells, is the best formulation for the recent trend of environmentally friendly and biocompatible products. Therefore, the purpose of this study was to develop an efficient formulation of medicinal herbs that could best meet the needs of the increasing anti-aging market.

2. Materials and Methods

2.1. Preparation of herbal medicine extracts

Golden-and-silver honeysuckle, fish mint, lianas, gardenia, and Baikal skullcap used in this study were purchased from Kwangmyung Pharmaceutical Co., Ltd..

Each 100 g of medicinal herbs were hydrothermal extracted with 2 L of tertiary distilled water for 150 minutes. The extracts were filtered with a 0.45 μm filter and lyophilized. The lyophilized extract was dissolved in tertiary distilled water before use and filtered with a 0.22 μm filter (Huie, 2002).

2.2. Reagent

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-sulfonic acid (ABTS), potassium persulfate, N-1-naphthylethylenediamine, kojic acid, sulfanilamide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Welgene (Welgene, Daegu, Korea) for cell culture.

2.3. Cell culture

RAW 264.7 macrophage cells were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and penicillin (100 U/mL)- streptomycin (100 μg/mL) at 37℃ in a humidified incubator with a 5% CO_2 atmosphere.

2.4. Preparation of medicinal herb vesicles

The vesicles containing medicinal herbs have been prepared by the hydration method (Collier et al., 2016). The content of phosphatidylcholine (PC) was fixed to 1 g for vesicle production. Ethanol 2 g was added to 1 g of PC, and the mixture was heated to 60℃ with stirring. After adding a surfactant, the mixture was further stirred for 2 minutes, and then 0.05 g of the herbal extract was dissolved in a solvent. After the addition of the extract, the mixture was stirred for about 5 minutes and 10 mL of distilled water was added to form a hydrated liquid crystal. Distilled water 20 mL was added to the vessel at a rate of 1 mL/min and vesicles containing herbal medicines were prepared by sonication for 15 minutes to induce dispersion of the finished vesicles.

2.5. Entrapment efficiency of vesicles contained medicinal herbs

2.5.1. Standard curve

The stock solution was prepared by adding 10 mg of each of 5 medicinal herbs used in the experiment to 10 ㎖ of distilled water. After dilution with distilled water, standard curve was prepared using a UV visible spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences).

2.5.2. Measurement of entrapment efficiency

2 mL of the completed vesicle was removed using a 0.45 μm filter, and the vesicle membrane was destroyed by adding an excess amount of ethanol. After that, ethanol was completely evaporated and 1 mL of ethanol (cold) and 0.5 mL of dimethyl sulfoxide (DMSO) were added. After shaking for 60 minutes, the substances added during the vesicle

production were extracted. The extraction concentration for each medicinal product was then calculated and the collection efficiency was calculated using the following formula (Oskuee et al., 2016).

$$\text{Entrapment efficiency (\%)} = \frac{\text{extract concentration in vesicle}}{\text{initial extract concentration}} \times 100$$

2.6. Characterization of prepared vesicles

Particle size and zeta potentials of prepared Vesicles were measured using dynamic light scattering (Zetasizer Nano, ZS90, Malvern, German) to confirm the prepared vesicles containing medicinal herbs and the stability of vesicles. The pH of the vesicles was measured using a pH meter (STARTER2100, OHAUS).

2.7. Free radical scavenging activity

2.7.1. DPPH assay

DPPH radical scavenging activity of vesicles was measured for antioxidant activity. DPPH solution of 0.1 mmol/L was prepared by dissolving DPPH in 1:1 solution of methanol and distiled water. 100 μL of DPPH solution prepared in a 24-well plate and 100 μL of each vesicles containing herbal medicines were mixed at concentrations of 2.5%, 5%, 10% and 20%, and then reacted for 24 hours. After stirring for 1 minute, the absorbance was measured at 540 nm using Multilabel Counter (VICTOR 3, Perkin Elmer). BHA (1 mg/mL) was used as an experimental control, and the electron donating ability was expressed by the absorbance reduction ratio of the sample solution added and the no added sample (Saquib et al., 2013).

2.7.2. ABTS + assay

For the measurement of $ABTS^+$ radical scavenging activity, $ABTS^+$, 7.4 mM and 2.6 mM potassium persulfate were reacted overnight to form $ABTS^+$. The $ABTS^+$ solution was diluted with distilled water to have an absorbance value of 1.4-1.5 at a wavelength of 745 nm. 1 mL of the diluted solution, 50 μL of the sample was added to each vesicle containing 2.5%, 5%, 10%, and 20% concentrations of the medicinal herbs, and the absorbance was measured 30 minutes later. The degree of erasure was expressed as a percentage compared with the negative control to which no sample was added (Shaheen et al., 2016).

2.8. NO assay

NO concentration in the cell culture was measured by microplate assay. RAW 264.7 cells were inoculated into a 24-well plate at a density of 1.1×10^5 cells/well, and 100 μL of vesicles containing medicinal herbs were added at concentrations of 2.5%, 5%, 10% and 20%. One hour after incubation, 1 μg/mL lipopolysaccharide (LPS) was treated to induce NO production. After incubation for 24 hours, 100 μL of the cell culture medium was mixed with 100 μL of Griess reagent [1% sulfanilamide/0.1% N-(1-naphthyl)] and ethylenediamine dihydrochloride /2.5% H_3PO_4], and incubated at room temperature for 10 minutes. V_{max} 96-well microplate spectrophotometer to measure the absorbance at 540 nm. NO concentrations were calculated using the standard curve of sodium nitrite (Bae et al., 2005).

2.9. Tyrosinase inhibition assay

Tyrosinase activity was measured to examine whitening activity. In the 96-well plate, vesicles containing 0.06 M sodium phosphate buffer (pH 6.8), 2 mg/mL L-DOPA and 200 unit/mL tyrosinase and 2.5%, 5%, 10%, and 20% concentrations of the each vesicle containing herbal medicine. Considering that the color of the sample affects the experimental results, 0.06 M PBS solution was added instead of 200 unit/mL tyrosinase to make a color control. The absorbance was measured at 490 nm with an ELISA reader (Wallac 1420, USA) at 30℃ for 1 hour every 10 minutes, and the amount of melanin production

Table 1. Characterization of vesicles contained with medicinal herbs

Medicinal herbs	Size (nm)	Zeta potential (mV)	pH
Lonicera japonica	592.87±6.56	-39.60±0.90	6.46
Houttuynia cordata	531.60±6.88	-45.63±0.96	6.74
Uncaria sinensis (Oliv.) Havil	463.60±10.61	-34.93±1.04	6.94
Gardenia jasminoides for. Grandiflora	599.53±23.94	-46.23±1.08	6.25
Scutellaria baicalensis	164.07±2.14	-31.37±0.53	7.15

was calculated (de Freitas et al., 2016).

$$\text{Tyrosinase Inhibition (\%)} = 1 - \left[\frac{\text{presence of inhibitor}}{\text{absence of inhibitor}} \right] \times 100$$

2.10. Statistical analysis

The experimental results in this study are expressed as mean(Standard Deviation , SD). Each experiment was repeated at least three times. The data values for each experiment were tested for significance at P-value <0.05.

3. Results and Discussion

3.1. Characteristics of vesicles containing medicinal herbs

The characteristics of vesicles containing medicinal herbs were measured by Dynamic Light Scattering (DLS). The size of vesicles containing golden-and -silver honeysuckle, fish mint, lianas, gardenia, and Baikal skullcap were measured as 592.87 ± 6.56 nm, 531.60 ± 6.88 nm, 463.60 ± 10.61 nm, 599.53 ± 23.94 nm and 164.07 ± 2.14 nm, respectively. Zeta potential values indicating the stability of the vesicles to the flocculation were -39.60 ± 0.90 mV (golden-and-silver honeysuckle), -45.63 ± 0.96 mV (fish mint), -34.93 ± 1.04 mV (lianas), -46.23 ± 1.08 mV (gardenia), 31.37 ± 0.53 mV (Baikal skullcap).

The pH of the vesicles are known to be the most stable at pH 5.5 to 7.0, and pH value of the prepared vesicles qre in the range of 6.3-7.2, confirming that the vesicle is in a stable range (Table 1).

3.2. Measurement of entrapment efficiency

A standard curve was drawn to measure the degree of entrapment of medicinal herbs. The absorption wavelength of each vesicles containing golden-and -silver honeysuckle, fish mint, lianas, gardenia, and Baikal skullcap were 332 nm, 309 nm, 312 nm, 325 nm and 348 nm, respectively. As shown in Table 2, the average entrapment efficiency was 80.2% at the minimum and 84.2% at the maximum, which are higher than 80.0% in each vesicles.

3.3. Analysis of DPPH and $ABTS^+$ radical electron donating activity

Many natural substances, including medicinal herbs, have a high antioxidant activity. It plays an important role in maintaining metabolism and homeostasis in our body. Ultimately, it plays a very

Table 2. Entrapment efficiency on vesicles contained with medicinal herbs

	Lonicera japonica	*Houttuynia cordata*	*Uncaria sinensis (Oliv.) Havil*	*Gardenia jasminoides for. Grandiflora*	*Scutellaria baicalensis*
EE(%)	84.2	80.5	81.7	80.2	81.0

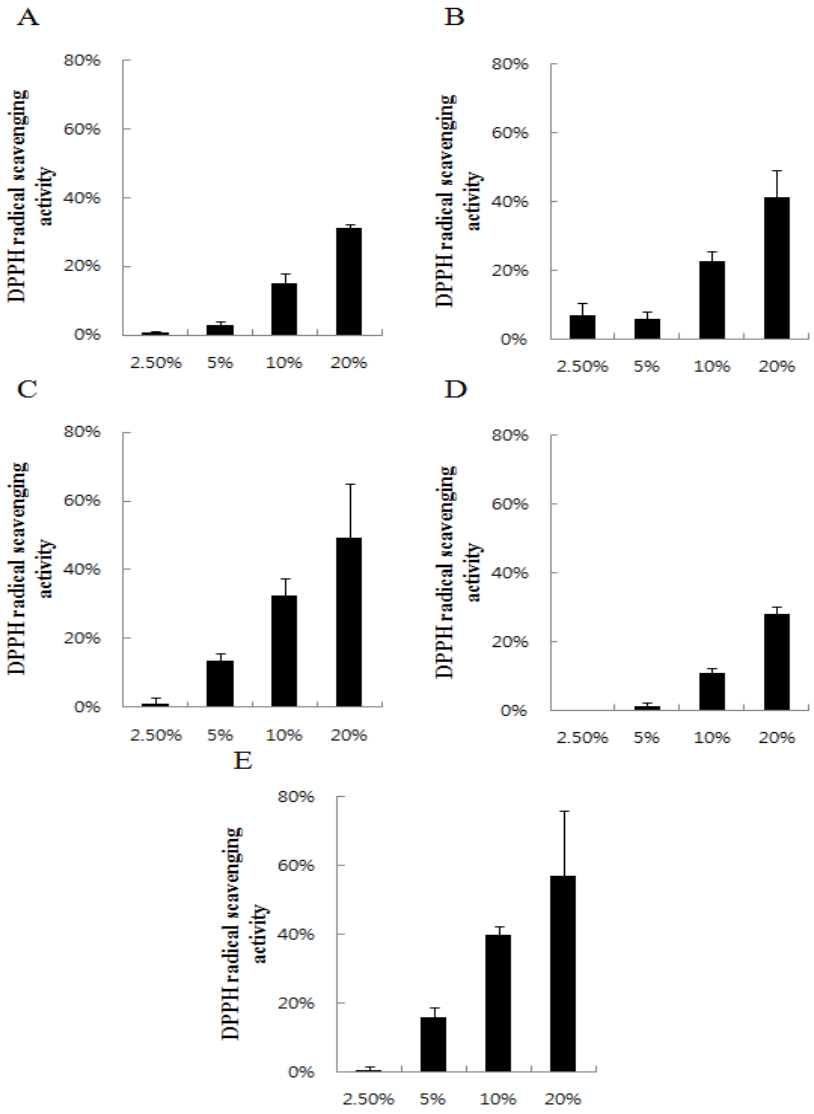

Fig. 1. DPPH radical scavenging activity on vesicles. Vesicles contained with (A) *Lonicera japonica*, (B) *Houttuynia cordata*, (C) *Uncaria sinensis* (Oliv.) Havil, (D) *Gardenia jasminoides for. Grandiflora and* (E) *Scutellaria baicalensis*.

important role against ROS that is the basis of our body's physiological activity and is generated in the human body.

As shown in Fig. 1, the levels of the vesicles containing the herbal medicines against the antioxidant activity are as follows. The vesicles containing golden-and-silver honeysuckle were 1%, 2%, 16% and 33%, and the vesicles with fish mint were 4%, 10%, 25%, 50% and the vesicle with lianas were 0%, 15%, 37%, and 68%. The vesicle including the rest of gardenia were 0%, 0%, 12%, and 37%, the vesicle with Baikal skullcap shown 0%, 14%, 41%, 76%, respectively.

As the concentration of vesicles increased, the

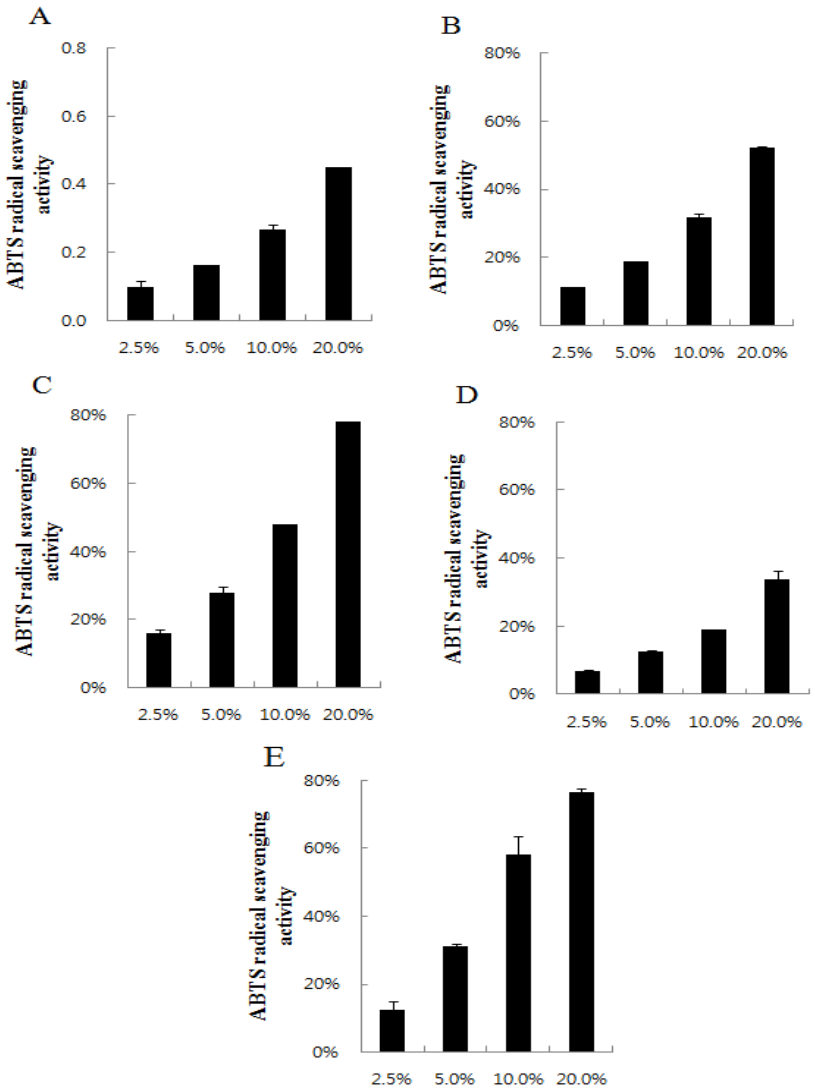

Fig. 2. $ABTS^{+}$ radical scavenging activity on vesicles. Vesicles contained with (A)*Lonicera japonica*, (B) *Houttuynia cordata*, (C) *Uncaria sinensis* (Oliv.) Havil, (D) *Gardenia jasminoides for. Grandiflora and* (E) *Scutellaria baicalensis.*

antioxidant activity were increased in a concentration -dependent manner. The maximum values were 33% (golden-and-silver honeysuckle), 50% (fish mint), 68% (lianas), 37% (gardenia), and 76% (Baikal skullcap). The DPPH radical electron donating activity were found to be 33~76% depending on the type of herbal medicines contained, and the activity of the Baikal skullcap-containing vesicle was the highest

The $ABTS^{+}$ radical scavenging activity are shown in Fig. 2. The vesicles containing golden-and-silver honeysuckle were 10%, 16%, 27% and 45% and the

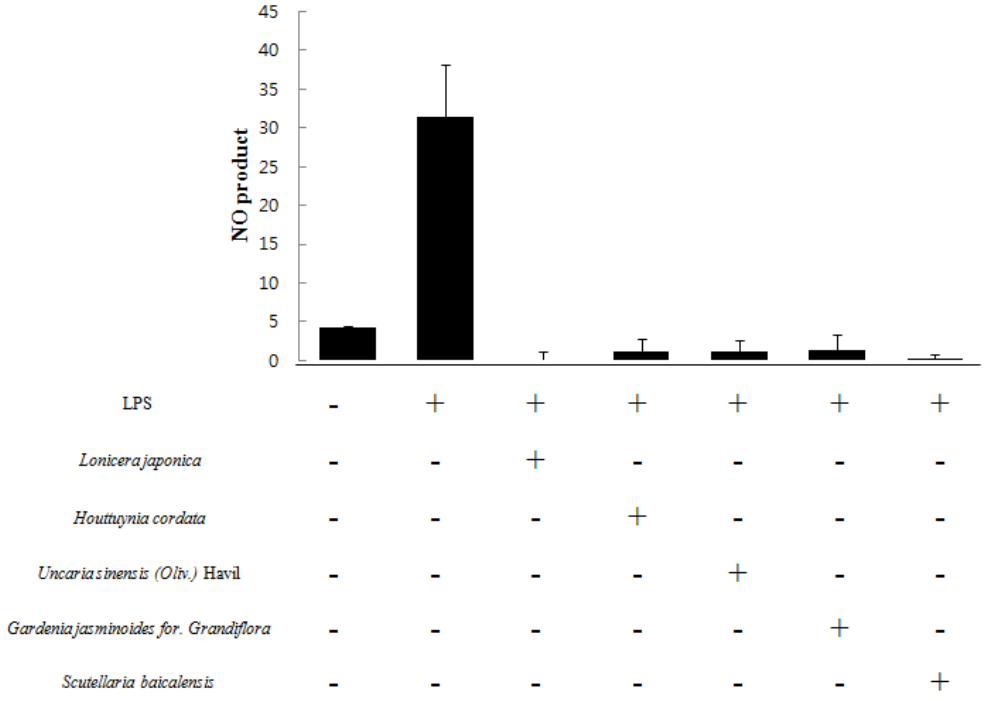

Fig. 3. Effects of the vesicles contained with medicinal herbs on nitric oxide production in lipopolysaccharide stimulated RAW264.7 macrophages.

vesicles containing fish mint were11%, 19%, 32% and 52%, And 78% respectively. The vesicles containing lianas were 7%, 12%, 19% and 34%, and the vesicles containing gardenia were 11%, 19%, 2% and 52%. Finally, the vesicles containing Baikal skullcap shown to have the radical scavenging activity of 12%, 31%, 58% and 76%, respectively.

As based on 20% concentration of vesicles containing herbal medicine, $ABTS^{+}$ radical scavenging activity was 34-78%. The radical scavenging activity of vesicles are increased in a concentration dependent manner. The results of DPPH radical electron donating activity also show similar antioxidant activity.

3.4. Measurement of NO production inhibition

Nitric Oxide (NO) is a free-radical produced by L-arginine as a substrate due to intracellular intoxication or cytokine stimulation. It is an important marker involved in inflammation induction mechanism.

In this study, RAW 264.7 macrophage cells were treated with LPS to induce stimulation, which resulted in the production of large amounts of NO. After LPS treatment and NO production, each vesicles containing herbal medicines were treated with 20% concentration and their changes were confirmed. As shown in Fig. 3, the production of NO of 31 μM was induced after treatment with LPS, and the amounts of NO produced were 0 μM (golden-and-silver honeysuckle), 1.1 μM (fish mint), 1.2 μM (lianas), 1.3 μM (gardenia) and 0 μM (Baikal skullcap), respectively, after treatment with vesicles containing each herbal medicine. Vesicles containing herbal medicines had high NO production inhibitory

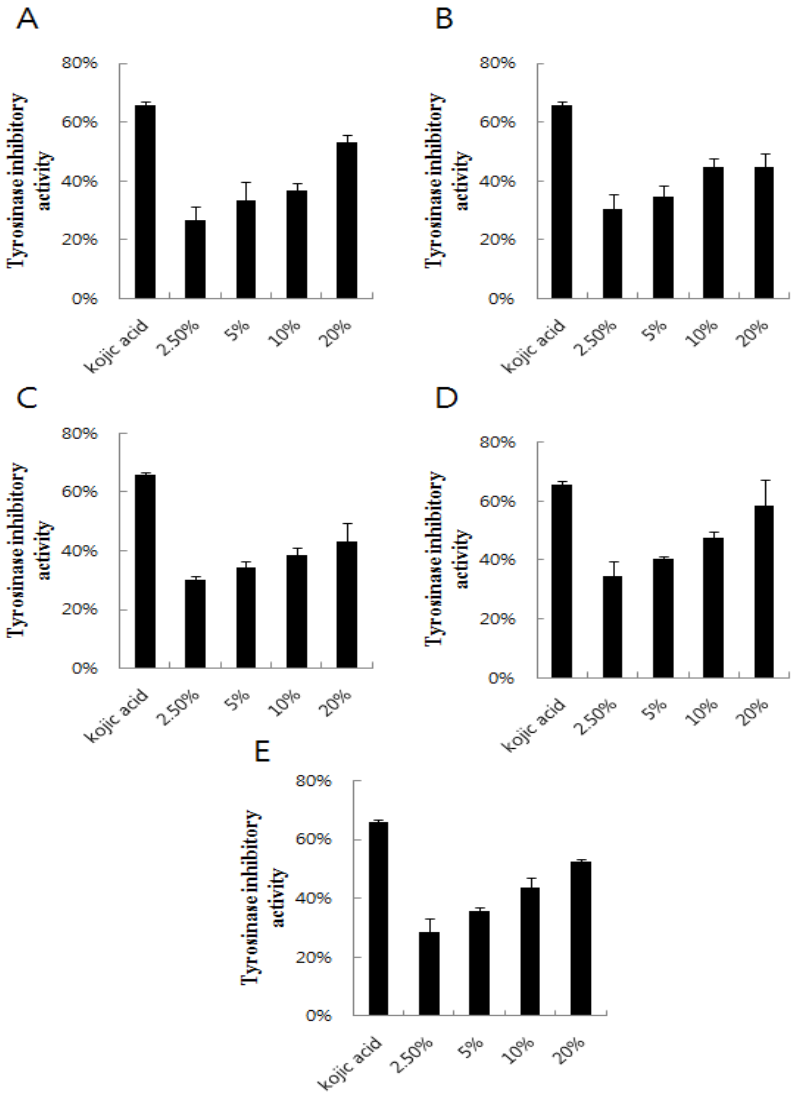

Fig. 4. Tyrosinase inhibitory activity of the vesicles. Vesicles contained (A) *Lonicera japonica*, (B) *Houttuynia cordata*, (C) *Uncaria sinensis* (Oliv.) Havil, (D) *Gardenia jasminoides for. Grandiflora and* (E) *Scutellaria baicalensis.*

ability. There was a significant difference between the control and the comparison results. Therefore, it was confirmed that the activity of each herbal medicine contained in the vesicles are well preserved.

3.5. Analysis of whitening effect of vesicles

Tyrosinase activity was measured to examine the whitening activity of vesicles used in this experiment. As shown in Fig. 4, the tyrosinase inhibitory activity of vesicles containing golden-and-silver honeysuckle were 27%, 33% mint), 37% and 53%, respectively. Other vesicles also showed concentration-dependent inhibition activity values.

When the concentration of vesicles are 20%, each vesicles showed inhibition activity of 43 ~ 58% and 58% of vesicle containing gardenia extract showed the highest. The inhibitory activity of tyrosinase inhibitory activity of kojic acid, a positive control, was close to 66%. It showed lower levels than the low activity of the kojic acid, but medicinal herbs are natural substances and when considered as compound extracts, it have a very high activity level compared to the single kojic acid. A

4. Conclusion

This study increases the need for antioxidants to reduce the free radicals caused by oxidative stress in the human body, and many studies are underway to more efficiently delivery of antioxidants. Therefore, there is growing interest in highly antioxidant-active natural products, particularly those from natural substances that are used as medicinal herbs. However, in order for these antioxidants to be delivered into the intended biomedical cells, a bio-delivery system supplementing the present formulation of the extract is required.

In this study, vesicles for intracellular delivery of highly efficient carriers were prepared, focusing on the mechanism of antioxidant, anti-inflammation, and whitening activity of medicinal herbs. The vesicles containing the herbal extracts were prepared by adding hot water extracts of medicinal herbs to the lipid-based vesicles, and their basic characteristics were examined.

In order to analyze the efficacy of vesicles containing herbal extracts, antioxidant activities such as DPPH, $ABTS^{+}$ assay and anti-inflammatory and whitening activities by NO assay were analyzed by tyrosinase inhibition assay.

In addition, Omija oil surfactant was added to this study for the production of environmentally friendly, biocompatible and highly efficient vesicle which is not shown in this paper. This suggests that the vesicle membrane has a more flexible shape and thus has a higher delivery efficiency.

In this study, the vesicles prepared containing medicinal herbs have excellent antioxidant, anti-inflammatory, and whitening effects. In addition, the vesicles prepared in this study were highly stability and effective in entrapping the active ingredients. Therefore, if commercial development is carried out through further research on this vesicle, it will help develop biocompatible cosmetics and skin care medicines that are very easy to absorb.

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