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헤스페레틴(Hesperetin)과 사이클로덱스트린(Cyclodextrin) 포접 복합체의 항산화, 항염증, 항균 활성

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Antioxidant, anti-inflammatory, and antimicrobial activity of hesperetin and its cyclodextrin inclusion complexes

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요 약 : Hesperetin은 Hesperidin에서 유도되는 강한 항산화 기능의 플라보노이드 비당체이다. 본 연구에서는 Hesperetin과 이의 Cyclodextrin 포접 복합체에 대하여 항산화, 항염증 및 항균 활성을 비교하였다. Hesperetin은 Hesperidin에 효소처리하여 제조되었으며, Hesperetin/Cyclodextrin 포접체는 용매 증류법에 의해 β-Cyclodextrin 및 Hydroxypropyl-β-Cyclodextrin을 사용하여 제조되었다. Hesperetin에 비해 Hesperetin/Hydroxypropyl-β-Cyclodextrin 포접체의 용해도는 93.5배 증가하였고, Hesperetin/β-Cyclodextrin 포접체의 용해도는 22.5배 증가하였다. 항산화 분석에서 Hydroxypropyl-β-Cyclodextrin 포접체는 Hesperetin과 유사한 라디칼 소거 활성능을 보인 반면, β-Cyclodextrin 포접체는 Hesperetin 보다 약간 낮은 활성을 나타내었다. RAW 264.7 세포에 대한 세포독성은 Hydroxypropyl-β-Cyclodextrin 포접체, β-Cyclodextrin 포접체, Hesperetin의 순으로 세포독성이 낮았다. Hesperetin과 Cyclodextrin 포접체는 모두 세포내 산화질소(NO), 종양괴사인자-α(TNF-α) 및 인터루킨-6(IL-6)과 같은 염증 매개체를 감소시켰다. Hesperetin 및 Hydroxypropyl-β-Cyclodextrin 포접체는 상대적으로 β-Cyclodextrin 포접체 보다 더 효과적이었다. 피부 유해성 세균인 황색 포도상구균과 녹농균에 대해 억제 효과를 시험한 결과, 황색 포도상구균에 대해서는 Hesperetin = Hydroxypropyl-β-Cyclodextrin 포접체 > β-Cyclodextrin 포접체의 순서로 항균 효과를 나타내었으나, 녹농균에 대해서는 뚜렷한 억제효과를 나타내지 않았다. 결론적으로, Hesperidin의 비당체 형태인 Hesperetin과 이의 Cyclodextrin 포접체는 다양한 생물학적 활성을 보여주었으며, 용해도가 높은 Hydroxypropyl-β-Cyclodextrin 포접체가 β-Cyclodextrin 포접체에 비해 상대적으로 더 높은 활성을 나타내었다.

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주제어 : 헤스페레틴, 사이클로덱스트린 포접체, 용해도, 항산화, 항염증, 항균

Abstract : Hesperetin(HT) is a potent antioxidant flavonoid aglycone derived from hesperidin(HD). The antioxidant, anti-inflammatory, and antimicrobial activities of HT and its cyclodextrin(CD) inclusion complexes were compared in *vitro*. HT was prepared by enzymatic hydrolysis of HD, and HT/CD complexes were prepared using β-cyclodextrin(β-CD) and hydroxypropyl-β-cyclodextrin(HP-β-CD) by solvent co-evaporation method. The solubility of the HT/HP-β-CD inclusion complex increased 93.5-fold compared to HT, and the solubility of HT/β-CD increased 22.5-fold. The HT/HP-β-CD inclusion complex showed a similar effect as HT on radical scavenging activity in antioxidant assays, whereas the HT/β-CD inclusion complex showed slightly lower activity than HT. Cytotoxicity was low in the following order; HT/HP-β-CD, HT/β-CD, and HT in murine macrophage RAW264.7 cells. Treatment with HT and HT/CD inclusion complexes reduced the levels of inflammatory mediators such as nitric oxide(NO), tumor necrosis factor-α (TNF-α) and interleukin-6(IL-6) in the cells. HT and HT/HP-β-CD inclusion complex were more effective than HT/β-CD inclusion complex at relatively low concentrations. Inhibitory effects were tested on skin-pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and they showed an antimicrobial effect on *S. aureus* in the order of HT = HT/HP-β-CD 〉 HT/β-CD, but they did not show any significant inhibitory effect on *P. aeruginosa*. In conclusion, HT, the aglycone form of HD, and its CD inclusion complexes showed various biological activities. HT/HP-β-CD inclusion complex, which is the highly soluble form of HT, showed relatively higher activity compared to HT/β-CD inclusion complex.

Keywords : Hesperetin, Cyclodextrin inclusion complex, Solubility, Antioxidant, Ant-inflammatory, Antimicrobial

1. Introduction

Flavonoids are a large class of polyphenolic secondary metabolites widely distributed in plant [1]. Hesperidin(HD, hesperetin 7- rutinoside) is a flavanone glycoside composed of an aglycone hesperetin(HT) and 6-O-α-L-rhamnosyl-D-glucose, which is found abundantly in citrus fruits, such as lemons, limes, and oranges. Many studies have been conducted on the absorption, bioavailability and pharmacokinetics of HD and its aglycone HT. These studies have shown that orally ingested HD in humans is absorbed in the form of the aglycone HT after the rutinose is removed by intestinal microbial enzymes, and the HT is further converted to glucuronidated or sulfated metabolites that is detectable in the blood[2,3]. HD and HT have been reported to have a wide range of pharmacological properties, including anti-inflammatory, antimicrobial, antithrombotic and anticarcinogenic activity [4-9]. Recently, HD and HT have studied for their anti-viral activity, and beneficial effects on the skin[10-12]. However, HD and HT are limited in their expansion into industrial applications in pharmaceutical, food, and cosmetic fields due to their low solubility. Various experimental studies have been conducted to overcome the poor solubility and enhance biological effects. An improvement in water solubility and antioxidant activity has been reported in HD mixed with chitogoligosaccharide[13]. Effect of modifying the sugar moieties of HD has been studied extensively, and glycosylation is considered one of the efficient methods to increase water solubility and bioavailability[14,15]. Various

nanoparticles loaded with HD have been applied to enhances solubility and bioavailability [16].

Cyclodextrins(CDs) are cyclic oligosaccharides composed of 6(α-CD), 7(β-CD) and 8(γ-CD) glucose monomers which are linked by α-(1, 4) bonds. CDs have a hydrophobic inner cavity and a hydrophilic outer surface, and are well-known as host molecules that form inclusion complexes with various guest molecules[17]. CD/drug complexes are receiving special attention in numerous pharmaceutical applications because they can increase the water solubility and bioavailability of the guest compounds[18]. Properties of HD or HT/CD inclusion complexes has been reported in many studies, however, the property and bioavailability was not fully understood[19]. The purpose of this study is to investigate whether HT and HT/CD complexes with different solubilities can have different effects on biological activity such as antioxidant, anti-inflammatory, and antimicrobial activity.

2. Experiment

2.1. Materials and Chemicals

Purified HD (>95%) extracted from *Citrus aurantium* L. was obtained from IBT Co., Ltd.(Gunpo, Korea). A hydrolytic enzyme, Plantase AK^{TM}, was obtained from Bision Biochem Corp.(Seoul, Korea) for preparation of HT. Standard HD and HT were purchased from Sigma-Aldrich Co.(St. Louis, MO, USA). β-CD and HP-β-CD were obtained from Wacker Chem. Korea(Seoul, Korea).

Reagents for antioxidant assays, 1,1-Diphenyl-2-picrylhydrazyl(DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) were purchased from Sigma-Aldrich Co.(St. Louis, MO, USA). For in *vitro* anti-inflammatory assays, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) and lipopolysaccharide(LPS) were also purchased from Sigma Chemical Co.(St. Louis, MO, USA). Dulbecco's modified eagle's medium (DMEM) and Fetal bovine serum(FBS) were purchased from Hyclone(Logan, UT, USA). A detection kit for nitric oxide(NO) was a product of iNtRON Biotechnology(Seongnam, Korea), and ELISA kits for tumor necrosis factor-α(TNF-α) and interleukin-6(IL-6) were purchased from Thermo Fisher Scientific. For antimicrobial test, resazurin was purchased from Sigma-Aldrich Co.(St. Louis, MO, USA), andmicrobial culture media were purchased from BD Biosciences(Heidelberg, Germany). All other reagents used were first-grade reagents.

2.2. Preparation of HT and HT/CD inclusion complexes

2.2.1. Preparation of HT

HT was prepared by enzymatic hydrolysis of HD. In brief, HD was dissolved in 0.1N NaOH solution to a final concentration of 1%(w/v). After dissolving HD, the pH was immediately adjusted to 6.5-7.0 and a hydrolytic enzyme was added to a final concentration of 1%(w/v). After reacting at 50℃ and pH 6.5 for 16 hours, the precipitate was collected and dissolved in absolute ethanol. The ethanol solution was filtered through a filter(0.45 μm) to remove insoluble debris and the filtrate was evaporated and resuspended in water. Insoluble debris was removed from the ethanol solution by filtration(0.45 μm), and the filtrate was evaporated and resuspended in water. The final precipitate was collected and dried. The conversion rate of HD to HT was calculated as the ratio of the amount of HD reacted from the amount of HD initially used.

2.2.2. CD inclusion complex formation

HT/CD inclusion complexes were prepared by co-evaporation method[20] with slight modification. CDs and HT were reacted in a 1:1 molar ratio. In brief, β-CD or HP-β-CD was dissolved in distilled water to obtain a saturated solution. Then, the ethanol solution

of HT was slowly added into the CD solution while stirring. The mixture was stirred at 40° C until the ethanol evaporated, and the resulting mass was freeze-dried. The dried powder was washed with ethanol and dried in vaccum. The inclusion rate of HT and CDs were calculated as the ratio of the amount of HT in the HT/CD inclusion complexes to the amount of HT initially used.

2.3. Analysis of HT and HT/CD inclusion complexes

2.3.1. Determination of HT

The content of HT and HD were analyzed using a HPLC system(Chromaster 5110, Hitachi, Japan). An analytical column(CAPCELL PAK C18 UG120 S5, 5 μm, 2.0×150 mm, OSAKA SODA, Osaka, Japan) was used and the mobile phase was binary eluents of water(0.1% formic acid) and acetonitrile(0.1% formic acid) under gradient conditions: 0 min(77% water: 23% acetonitrile), 3.5 min(40%:60%), 9 min (40%:60%), 23 min(40%:60%) and 24 min (77%:23%), 35 min(77%: 3%). The flow rate was maintained at 0.3 mL/min, the column temperature was 25℃, and the detection wavelength was 280 nm. For the further efficacy tests, HT molar concentrations were adjusted equally.

2.3.2. Identification of HT by LC-MS

HT obtained through enzymatic reaction were identified by analyzing fragmentation in LC-MS spectra. Chromatographic separation was conducted on an HPLC system(LC-20AD, Shimadzu, Kyoto, Japan) based on the HPLC analysis method described. LC/MS detection was conducted on Q-TOF Premierometer (Waters, Milford, MA, USA) system with an electrospray ion source in the positive mode. The ion transitions for confirmation and quantification were m/z 303(precursor ion) → 153(product ion) for HT.

2.3.3. Identification by FT-IR

Chemical properties of HT and HT/CD inclusion complexes were analyzed using a FT-IR Spectrometer(Nicolet iS5, Thermo Fisher Scientific, Waltham, MA, USA). Spectral scanning was taken in the wavelength region between 400 and 4000 cm^{-1} with a resolution of 2 cm^{-1}.

2.3.4. Determination of solubility

The solubility test was performed at 25℃ (298 K). Excess amounts of HT or HT/CD inclusion complexes were added to 5 mL of distilled water and rotated for 24 hours at a constant speed of 60 rpm using a laboratory rotator(MX-RD-E, LKLab Korea, Namyangju, Korea). Then, the solutions were filtered through 0.2 μm filters and analyzed by the HPLC. Solubilization coefficients(SCs) were calculated as the amount of HT in solubilized CD inclusion complex to the amount of total HT added in water.

2.4. Determination of Antioxidant Activity

2.4.1. DPPH Radical Scavenging Assay

Antioxidant analysis using DPPH radical scavenging ability was performed with some modifications to the methods of Ratha *et al.*[21]. DPPH was dissolved in ethanol at a concentration of 0.4 mM, and 0.2 mL of the diluted sample was mixed with 3.8 mL of DPPH solution. After reacting for 30 min, when the color of the reaction was stabilized, the absorbance was measured at 525 nm using a spectrophotometer(S22, Biochrom, UK). The scavenging activity of DPPH radical was expressed as a percentage of the difference between the control and the sample group.

2.4.2. ABTS Radical Scavenging Assay

Antioxidant analysis using ABTS radical scavenging ability was performed with some modifications to the methods of Re *et al.*[22].

The ABTS+ reagent was used as a potassium persulfate solution(140 mM) diluted with absolute ethanol until the absorbance at 734 nm reached 0.700 ± 0.02. An aliquot of 0.2 mL sample was added to 5 mL of ABTS+ reagent and after 6 min, the absorbance was measured at 734 nm using a spectrophotometer (S22, Biochrom, UK). The scavenging activity of ABTS radical was expressed as a percentage of the difference between the control and the sample group.

2.5. Effects on inflammatory mediators and Pro-inflammatory Cytokines

2.5.1. Cytotoxicity

A macrophage cell line, RAW 264.7 cells were provided by Korea Cell Line Bank (Seoul, Korea). Cells were cultured in DMEM containing 10% FBS and 100 μg/mL of penicillin/streptomycin. The cytotoxicity of HT and HT/CD inclusion complexes were determined by MTT assay[23]. In brief, cells were cultured in 96-wells at a density of approximately 5×10^4 cells/well and then incubated for 24 hours in a CO_2 incubator. Different concentrations of HT and HT/CD inclusion complexes were added to the wells and maintained for 1 hr, then LPS was added to final concentration of 1 μg/mL and incubated for 24 hours at 37℃. After the removal of the medium in the wells, 100 μL of MTT solution was added to each well and maintained for 2 hours at 37℃. Then DMSO was added to the wells and the absorbance was measured at 540 nm in a microplate reader(Epoch2, Bio Tek, Winooski, VT, USA). Cell viability was expressed as a percentage of the difference in absorbance at 540 nm between the control and the sample group.

2.5.2. Measurement of effect on NO

NO levels were measured in RAW 264.7 cells treated/untreated with HT and HT/CD inclusion complexes. Cells were pre-cultured at a density of 5×10^4 cells/well in 96-wells and treated with various concentrations of HT and HT/CD inclusion complexes for 1 hr. To induce inflammation, LPS(final concentration 1 μg/mL) was added and incubated at 37℃ for 24 hours. NO levels were measured using a NO detection kit according to the manufacturer's protocol. Absorbance was measured at 540 nm using a microplate reader.

2.5.3. Measurement of effect on TNF-α and IL-6

The amounts of Pro-Inflammatory Cytokines, TNF-α and IL-6 were measured in RAW 264.7 cells treated/untreated with HT and HT/CD inclusion complexes. Cell culture conditions for TNF-α and IL-6 assay were almost identical to those used for NO assay. The concentrations of TNF-α and IL-6 in the medium were measured respectively using ELISA kits according to the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader..

2.6. Determination of Antibacterial activity

In vitro antibacterial activity was tested by agar diffusion method[24,25] against Gram-positive *Staphylococcus aureus*(KCTC 3881), and Gram-negative *Pseudomonas aeruginosa* (KCTC 2513). Tryptic soy agar was used for cultivation of *S. aureus* and *P. aeruginosa.*

2.7. Statistical Analysis

Most of the experimental procedures were carried out in triplicate, and values were expressed as mean ± standard deviation. One-way analysis of variance(ANOVA) was performed using SPSS software(version 22, SPSS Inc., Chicago, IL, USA). A significant difference between treatments was tested at $p < 0.05$ using Duncan's multi-range test.

3. Results and Discussion

3.1. Preparation of HT and HT/CD inclusion complexes

3.1.1. Enzymatic Conversion of Hesperidin to HT

Hesperetin, the aglycone of hesperidin, was obtained by removing of rutinose, which is the rhamnosyl glucose moiety from hesperidin. The reaction was carried out using an hydrolase containing various glucosidase activities. More than 95% of hesperidin was converted to HT in this experiment. Through repeated separation in water and ethanol, HT of more than 95% purity was obtained.

3.1.2. Mass Spectral Fragmentation of HT

The HT obtained by the enzymatic hydrolysis was identified through LC-MS analysis. In the spectra, the protonated molecular ion of HT at m/z 303 produced the fragment ion at m/z 153 by breaking down(Fig. 1).

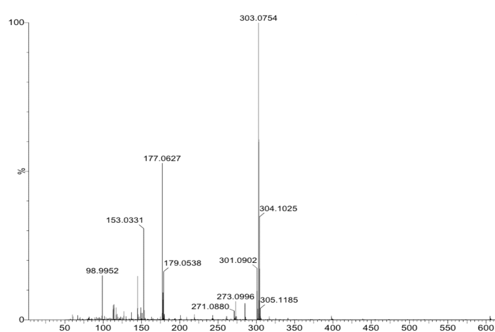

Fig. 1. Mass spectrum of prepared HT by LC-MS analysis.

3.1.3. FT-IR Spectra of HT and HT/CD inclusion complexes

Characteristic absorption bands of hesperetin and hesperidin were present in the FT-IR spectra, and they were respectively identified using the OMNIC software library(Thermo Fisher Scientific, Waltham, MA, USA). In the FT-IR spectra, the characteristic bands of HT at 1200～1400 cm^{-1} disappeared in the IR spectrum of both HT/CD inclusion complexes (Fig. 2).

3.1.4. Solubility of HT and HT/CD inclusion complexes

It has been reported that changes in the solubility of flavonoids can affect their biological efficacy[26]. Many studies have demonstrated that increasing flavonoid solubility can increase in vitro and in *vivo* efficacy[27]. CD inclusion complexes have been developed and studied for improved solubility and efficacy. However, solubility and efficacy vary depending on the CD used, inclusion methods, and conditions[28]. In this experiment, the solubility of HT was about 5.15 ㎍/mL. The solubility of HT in the CD inclusion complexes increased drastically, reaching approximately 116 μg/mL in HT/β-CD and approximately 480 μg/mL in HT/HP-β-CD. The solubilization coefficient(SC) values of HT in CD inclusion complexes are shown in Table 1.

3.2. Antioxidant Activity of HT and HT/CD inclusion complexes

To evaluate the antioxidant activity, the radical scavenging activities of DPPH and ABTS were measured. In the DPPH assay, treatments with HT and HT/CD inclusion complexes showed a concentration-dependent radical scavenging effect(Fig 3, a), with HT and HT/HP-β-CD showing higher effects than HT/β-CD inclusion complexe. The median scavenging concentration(SC_{50}) was 1645.18 ± 1.24 μM for HT, 3627.04 μM ± 0.17 for HT/β-CD, and 1871.00 ± 0.28 μM for the HT/HP-β-CD inclusion complex, respectively, while the SC_{50} of the positive control, ascorbic acid, was 161.72 ± 0.05 μM.

The ABTS assay results showed similar pattern to the DPPH assay results, showing the antioxidant effect of HT and HT/HP-β-CD

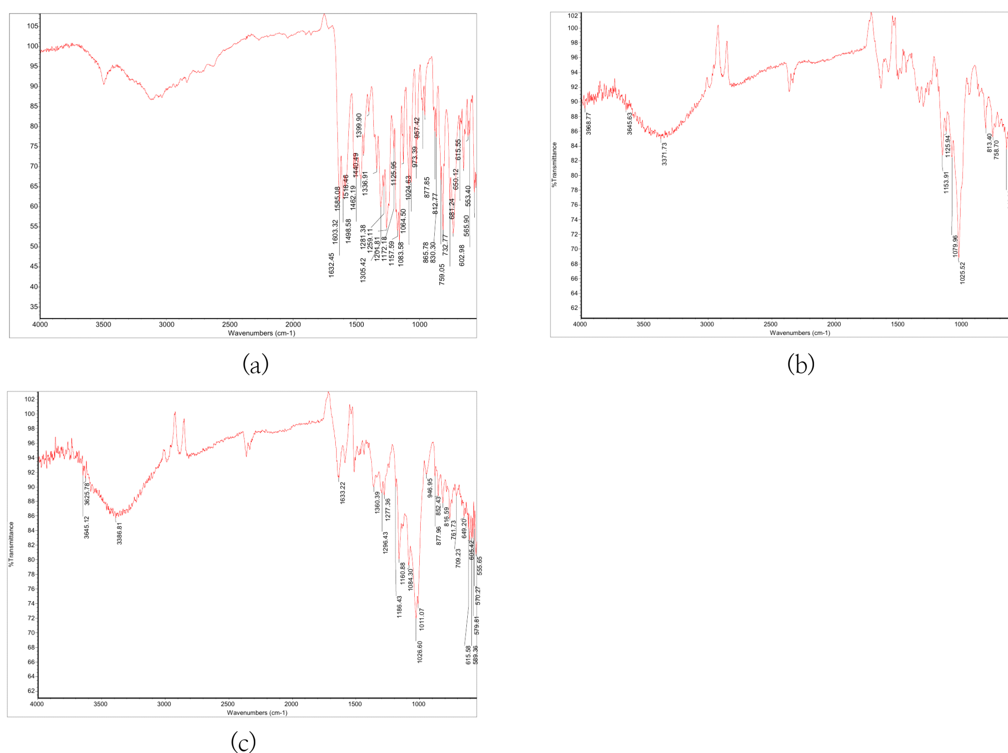

Fig. 2. FT-IR spectra of HT and HT/CD inclusion complexes. (a) HT, (b) HT/β-CD inclusion complex, (c) HT/HP-β-CD inclusion complex.

Table 1. Solutility of HT and its CD inclusion complexes in water

Cyclodextrin complex	Solubilization coefficient(SC) of hesperetin
None (Hesperetin alone)	1.0 ± 0.02
β-Cyclodextrin complex	22.5 ± 0.13
Hydroxypropyl-β-cyclodextrin complex	93.5 ± 0.12

higher effects than HT/β-CD inclusion complexes(Fig. 3, b). However, the SC_{50} values were much lower compared to the DPPH assay. The SC_{50} of the ABTS assay was 672.04 ± 0.11 μM for HT, 1828.17 ± 0.23 μM for HT/β-CD, and 898.42 ± 0.13 μM for the HT/HP-β-CD inclusion complex, respectively, while the SC_{50} of of the positive control ascorbic acid was 104± 0.07 μM.

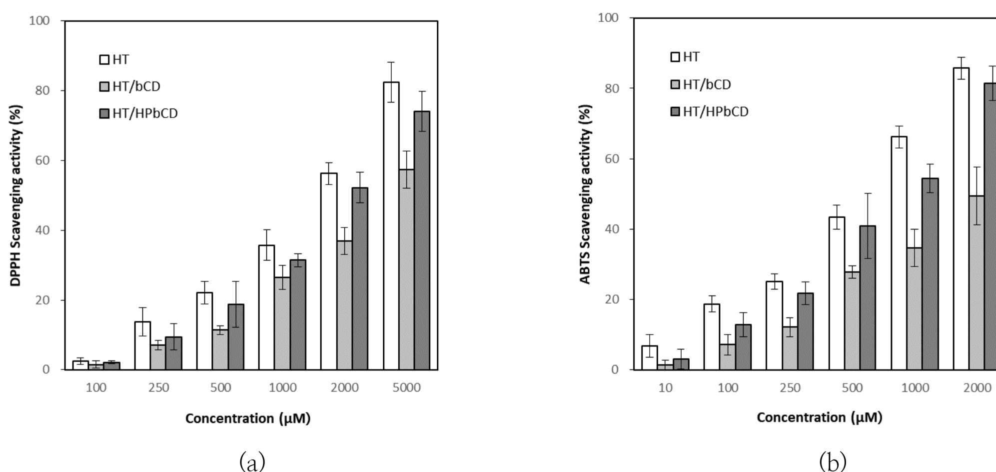

Fig. 3. Antioxidant activity of HT and HT/CD inclusion complexes. Results were expressed as a mean ± SD (n=3). (a) DPPH radical scavenging activity, (b) ABTS radical scavenging activity.

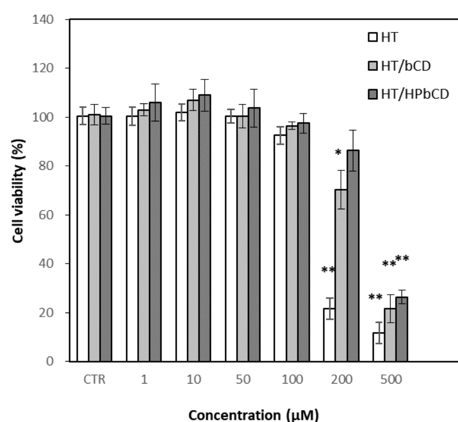

Fig. 4. Effect of HT and HT/CD inclusion complexes on RAW 264.7 cell viability. Cells were treated with HT and HT/CD inclusion complexes, respectively, for 1 hr and incubated for 24 hours with LPS(final concentration of 1 μg/mL_. Results were represented as mean ± SD (n=3). Significant differences from the control(*p 〈 0.05; **p 〈 0.01). CTR; Control(LPS)

3.3. Effects of HT and HT/CD inclusion complexes on Inflammation

3.3.1. Cytotoxicity of HT and HT/CD inclusion complexes

Effect of HT and HT/CD inclusion complexes to RAW 264.7 cell viability was assessed based on MTT assay. The HT/HP-β-CD inclusion complex showed significantly less cytotoxicity than HT in RAW 264.7 cells, whereas the HT/β-CD inclusion complex showed slighly less toxicity than HT (Fig. 4). The HP-β-CD inclusion complex did not induce significant cell death at a concentration

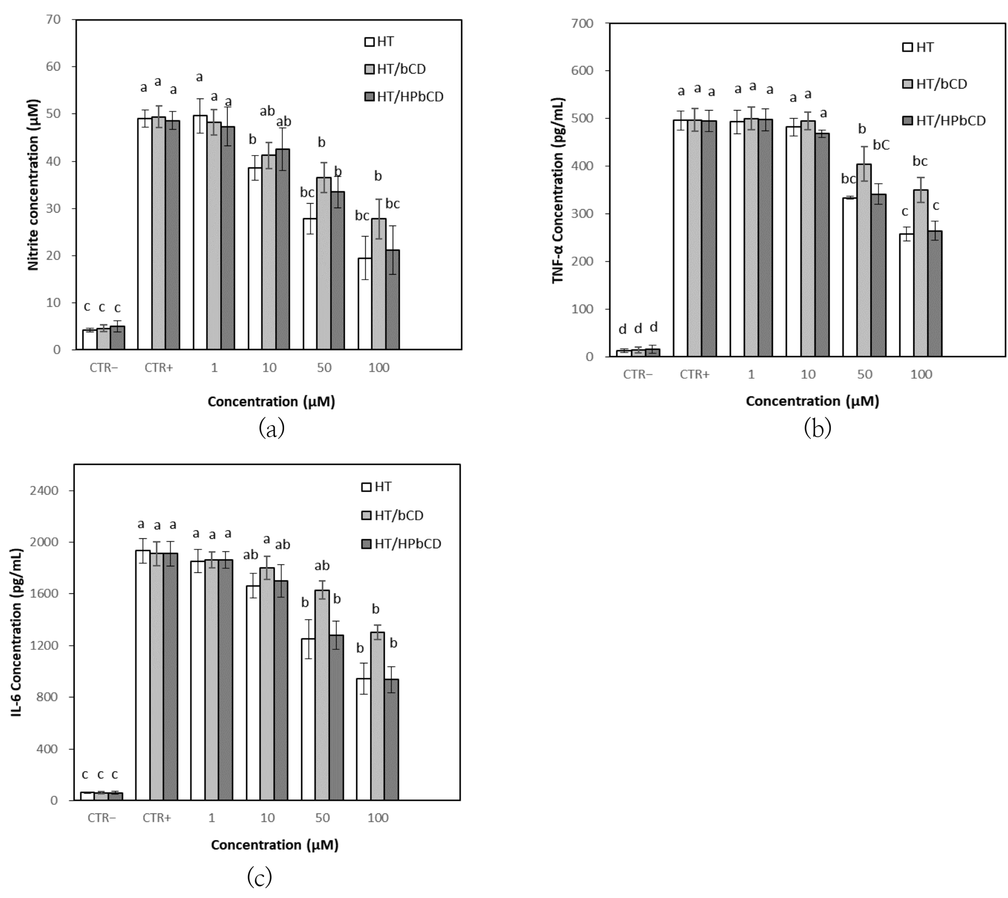

Fig. 5. Effect of HT and HT/CD inclusion complexes on the production of nitric oxide(NO), tumor necrosis factor-α(TNF-α) and interleukin 6(IL-6). RAW 264.7 cells were cultured with different concentrations of HT and HT/CD inclusion complexes for 24 hours with LPS(1 μg/mL). Results were represented as mean ± SD (n=3). (a) Effect on NO levels, (b) Effect on TNF-α levels, (c) Effect on IL-6 levels. Different letters (a, b, c, d) above the bars indicate significant differences($p < 0.05$), where ab or bc represents the intermediate significance value between a and b or b and c, respectively. CTR−; Negative control, CTR+; Positive control(LPS).

of 200 μM, but HT and β-CD inclusion complex showed significant cytotoxicity at this concentration. Anti-inflammatory assays were performed up to concentrations of 100 μM.

3.3.2. Effects on NO Levels

NO is a well-known inflammatory mediator, and its overproduction may play an important role in inflammatory processes[29]. The stimulation of cells with LPS induced significant NO production compared to untreated controls. Treatment with HT and its CD inclusion complexes began to suppress NO production from 10 μM, but the NO production was markedly decreased by treatment with HT and HT/HP-β-CD inclusion complex at concentrations up to 50 μM(Fig. 5, a).

3.3.3. Effects on TNF-α and IL-6 Levels

To evaluate the anti-inflammatory properties of HT and HT/CD inclusion complexes, proinflammatory cytokines such as TNF-α and IL-6 were quantified in RAW 264.7 cells. Stimulation of cells with LPS induced significant TNF-α and IL-6 production compared to untreated controls. As in the NO production, HT and its CD inclusion complexes tested showed reduction in TNF-α and IL-6 production(Fig. 5, b, c). HT and HT/HP-β-CD inclusion complex treatment began to inhibit TNF-α and IL-6 production from 50 μM. TNF-α and IL-6 production was also decreased by HT/β-CD inclusion complex at concentrations up to 100 μM.

Inflammation and oxidative stress are closely related to various life-threatening diseases, including cancer, neurodegenerative diseases, and cardiovascular diseases[30,31]. Oxidative stress can activate certain transcription factors that induce the expression of many genes, including proinflammatory cytokines[32]. HT and HT/CD inclusion complexes showed concentration-dependent reductions in NO, TNF-α, and IL-6 levels. HT was more cytotoxic than HT/CD inclusion complexes but also had a higher anti-inflammatory effect. However, the HT/HP-β-CD inclusion complex showed a comparable activity to HT with relatively low cytotoxicity. These results will help in the application of HT/CD inclusion complexes.

3.4. Antimicrobial Activity of HT and HT/CD inclusion complexes

In this study, we tested two bacteria, *S. aureus* and *P. aeroginosa,* which can be act as skin pathogens under certain conditions. HT and HT/CD inclusion complexes showed higher antibacterial effects against the gram-positive *S. aureus* than gram-negative *P. aeroginusa*(Fig. 6).

Recent studies have shown that HD and HT have antibacterial activity[33]. The protective effects of HD and HT against toxicity caused by microorganisms and certain chemotherapy drugs have also been investigated[34]. In this study, hydrophobic HT and highly soluble HP-β-CD inclusion complexes showed similar activity. The lipophilicity of flavonoids is an important factor in their antibacterial activity, but high lipophilicity often does not always lead to high antibacterial activity[35,36]. CD inclusion complexes can be dissolved more easily in the medium. The high antibacterial activity of CD inclusion complexes can be assumed to be due to their high solubility.

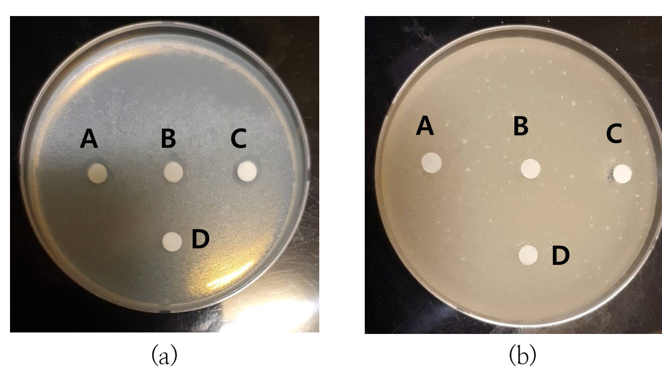

Fig. 6. Effect of HT and HT/CD inclusion complexes on the growth of *S. aureus* and *P. aeruginosa*. Equal amounts of HT equivalents were loaded onto the discs on agar plates. (a) *S. aureus*, (b) *P. aeruginosa.* A: HT, B: HT/β-CD, C: HT/HP-β-CD, D: Negative control.

4. Conclusion

HT and HT/CD inclusion complexes were prepared and their solubility and biological efficacy were compared. HT/HP-β-CD inclusion complex showed much higher water solubility compared to HT and HT/β-CD. HT, a poorly soluble flavonoid, and its CD inclusion complexes both showed high activity in DPPH and ABTS radical scavenging assays. Both HT and CD inclusion complexes also exhibited high anti-inflammatory effects in RAW 264.7 cells. Additionally, HT and CD inclusion complexes showed potential for antimicrobial applications. HT, the aglycone form of HD, is assumed to have various high biological activity, and increased solubility of CD inclusion complexes may affect these activities. Even though they exhibit similar activity, HT/CD inclusion complexes with improved solubility may have greater application than HT with lower solubility.

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