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"The new anti-aging agent : Blue color Houttuynia Cordata by fermentation"

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1. Introduction

Skin health and beauty are important essential factors representing overall "well being" as well as the recognition of "health" in humans[1]. For healthy skin, anti-aging is fundamental factor that is closely related prevention of free-radical[2]. Houttuynia cordata(HC) is a perennial plant spread widely across many countries in Asia and it has been used as traditional medicine and also as food[3].

However, the novel blue color HC by fermentation and its anti-aging efffects has not been investigated. In this study, we examined anti-aging efficacy evaluation of fermented blue color HC, versus normal green color HC.

2. Materials and Methods

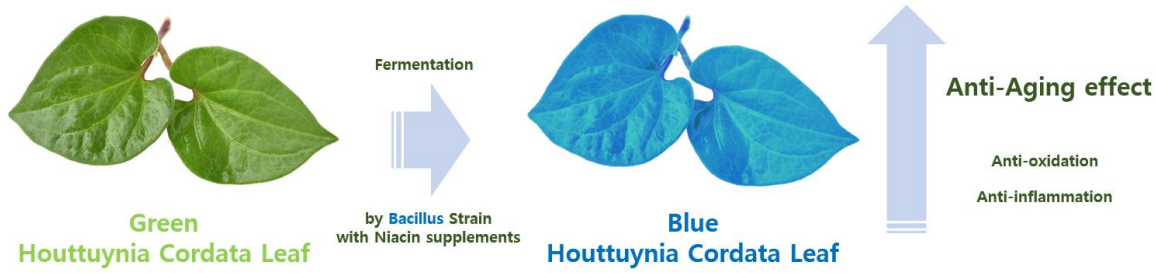
Chemicals and reagents

Nitroblue tetrazolium chloride (NBT), 2,2-diphenyl-1-picrylhydrazyl(DPPH) free radical, xanthine, xanthine oxidase, lipopolysaccharide (LPS), griess reagent (1% (w/v) sulfanilamide, 0.1% (w/v) naphylethylenediamine in 2.5% (v/v) phosphoric acid), 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl-2H-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and sodium nitrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other solvents and chemicals were of analytical grade and were purchased from J-T Baker Co. (Phillipsburg, NJ, USA) or Sigma.

Plant material and sample preparation

HC leaves were purchased from the Sanchung local plantation which located Jirisan mountain, Korea. And bacillus niacin (KCCM42963) was purchased from Korea Culture Center of Microorganisms, Korea. The leaves were dried in dry oven at 70 ℃ during 12 h. Dried leaves were ground and sieved through standard 16-mesh sieve. In case of fermented blue HC sample, dried leaves were fermented by *Bacillus niacini* strain at 30℃ during 72 h with chlorella vulgaris and spirulina platensis for niacin supplements. and then dried in dry oven at 85℃ during 12 h(Figure 1). These normal green HC and fermented blue HC samples were reflux extracted and lyophilized and then used for the experiments.

Figure 1. Graphic abstract



DPPH(free radical)-scavenging assay

The DPPH(free radical)-scavenging activities were analyzed by using the Blois [4] method with minor modifications. In brief, DPPH solution (0.2 mM in methanol) was added to each concentrated sample of the same volume. After 15 min at room temperature, the optical density at 540 nm was measured with a microplate reader (Thermomax; Molecular Devices, Sunnyvale, CA, USA). The control was a DPPH sample solvent mixture (1:1, v/v) and the blank was a methanol concentrated extract mixture (1:1, v/v). The free radical-scavenging activities (FSC%) were calculated as follows:

FSC% = 100 × (1 − [(experimental optical density − blank optical density) / control optical density].

Superoxide anion radical-scavenging assay with NBT

The superoxide anion radical-scavenging activities were analyzed by using the Furuno et al. [5] method with minor modifications. In brief, xanthine (3 mM), ethylenediaminetetraacetate (15 mM), NBT (0.72 mM) and each concentrated sample were added to 50 mM KH_2PO_4 buffer (pH 7.4) and incubated for 10 min at 25 ℃. After incubation, 1 U/ml xanthine oxidase was added to the reaction mixture, which was then incubated again at 25 ℃ for 30 min. The optical density at 540 nm was measured using a microplate reader (Thermomax; Molecular Devices, Sunnyvale, CA, USA). The superoxide anion radical scavenging activities were expressed as the percent inhibition (IC%).

Nitric oxide (NO) inhibition assay

For evaluation of anti-inflammation effects, the nitric oxide(NO) assay on lipopolysaccharide(LPS) induced RAW 264.7 cells was performed by Yoon et al.,[6] method with minor modifications. Raw 264.7 cells (1.8 x 10^5 cells/mL) were placed in a 48-well plate and cultured for 18 h. After treating with lipopolysaccharide (LPS), an inflammatory response inducer, at a concentration of 1 μg/mL, the samples were treated at different concentrations and cultured for 24 h. The amount of NO produced was measured in the form of NO_2^- present in the cell culture medium using Griess reagent (1 % (w/v) sulfanilamide, 0.1 % (w/v) naphylethylenediamine in 2.5 % (v/v) phosphoric acid). 100 μL of cell culture supernatant and 100 μL of Griess reagent were mixed and reacted for 10 min, and then the absorbance was measured at 540 nm. The amount of NO produced was compared with sodium nitrite ($NaNO_2$) as a standard.

Statistical analysis

All experiments were performed in triplicate. The data are presented as the mean ± standard deviation (S.D.). The means were ranked by Student's multiple-range t-tests. Statistically significant differences were indicated at a level of $p < 0.05$.

3. Results

in vitro anti-oxidative efficacy evaluation

The DPPH (free radical) scavenging activities were analyzed by using the Blois[4] method with minor modifications. And also, the superoxide anion radical-scavenging activities were analyzed by using the Furuno et al.,[5] method with minor modifications. Both of evaluations, the highest free radical-scavenging activity (50% of the FSC%) and superoxide anion radical-scavenging activity were observed in the fermented blue HC extract than normal green HC extract(Figure 2).

in vitro anti-inflammation evaluation

For evaluation of anti-inflammation effects, the nitric oxide(NO) assay on lipopolysaccharide(LPS) induced RAW 264.7 cells was performed by Yoon et al.,[6] method with minor modifications. The cytotoxicity was not observed on all concentration samples. The fermented blue HC extract showed excellent concentrate-dependent increases NO inhibition effects than normal green HC extract(Figure 3).

Figure 2. Anti-oxidative effects of normal green HC and fermented blue HC

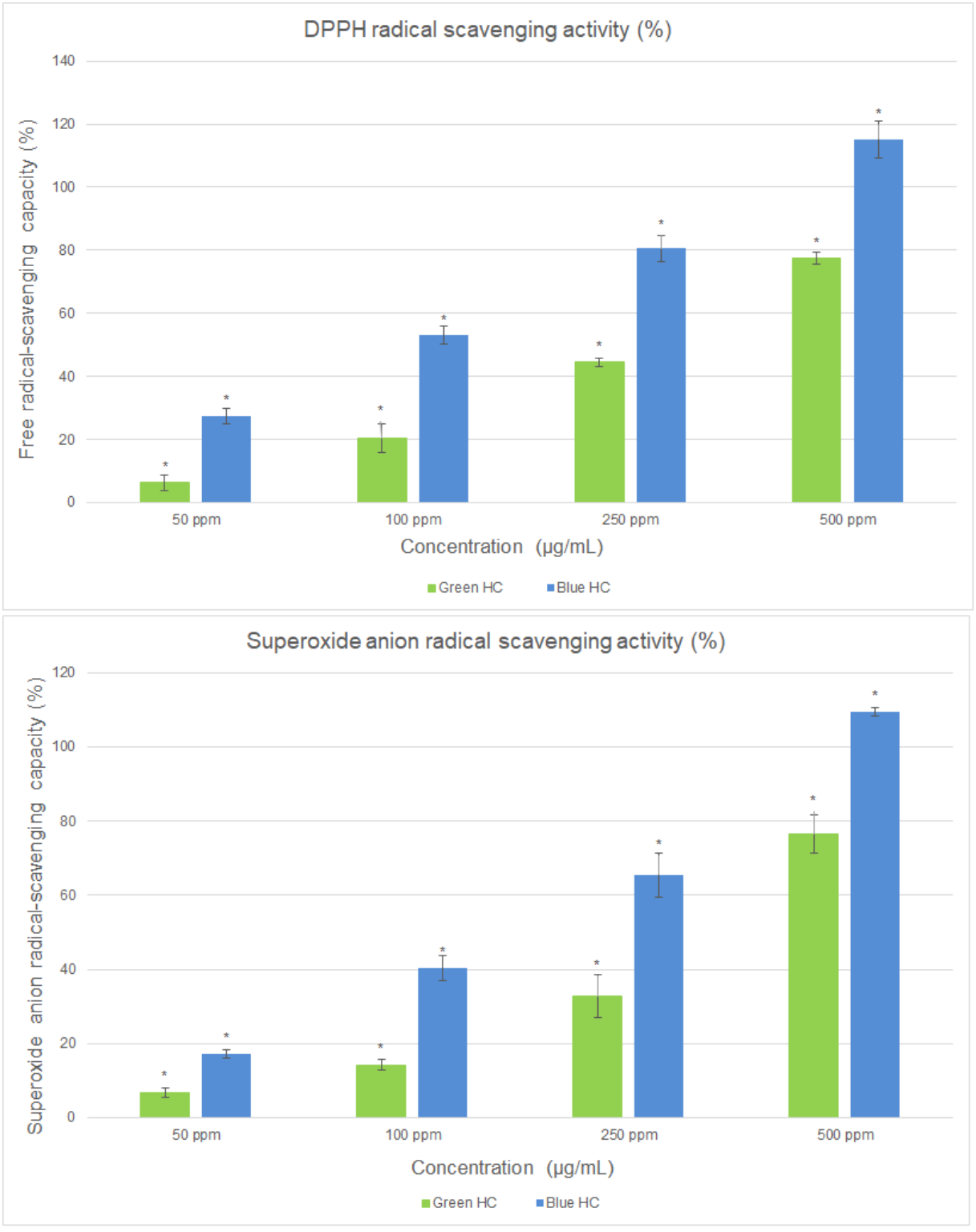
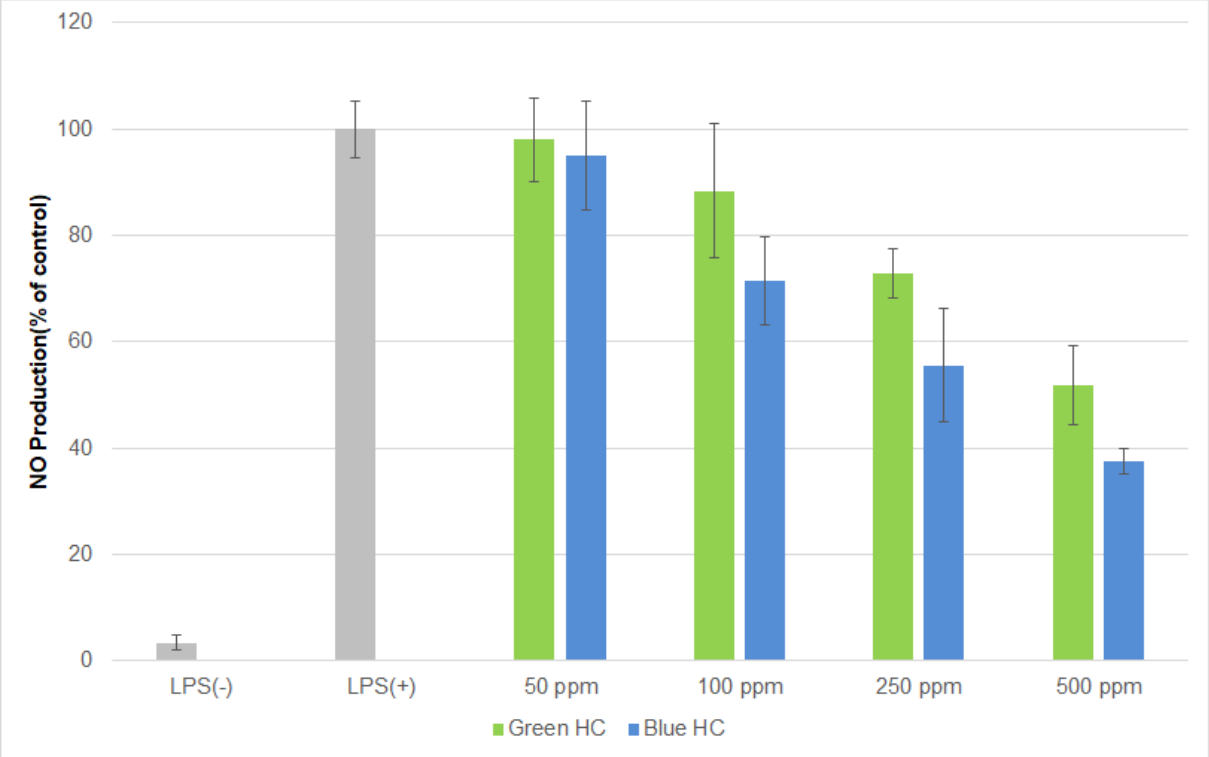


Figure 3. Anti-inflammation effects of normal green HC and fermented blue HC



4. Discussion and Conclusion

In this study, we have attempted to examined cosmetic efficacy evaluation of fermented blue color HC by *Bacillus niacin* strain, versus normal green color HC. Compared with extract obtained by non-ferment(Green HC), the fermented blue HC extract had enormous enhancement of their free radical (2,2,-diphenyl-1-picrylhydrazyl) and superoxide anion radical scavenging activities and anti-inflammation activities. Further study is necessary to investigation in HC bioactive components variation by *Bacillus niacin* strain fermentation and *in vivo* skin efficacies.

5. References

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