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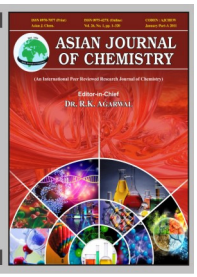

Evaluation of Free Radical Scavenging Activity of Iridoid Aucubigenin and its Glycoside

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The iridoid aucubigenin and its glycoside aucubin have been investigated for their *in vitro* free radical scavenging activity. Various methods, such as the scavenging activities towards DPPH radical, superoxide anion radical and hydroxyl radical, were efficiently established. The results showed that both compounds exhibited significant differences in free radical scavenging activity.

Keywords: Iridoid, Aucubigenin, Aucubin, Free radical scavenging.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, are generated under physiological and pathological stresses in human body. Reactive oxygen species can cause oxidative damage of DNA, proteins, lipids and small cellular molecules. Many human diseases, such as cancer, diabetes, cardiovascular diseases, inflammations and neurological disorders, are the results of the oxidative damage by ROS[1-3]. The antioxidants act as radical scavengers, can reduce oxidative stresses, inhibit lipid peroxidation and protect human body from oxidative damage[4]. Numerous active compounds in natural plants such as carotenoids, polyphenolics and flavonoids were found to possess antioxidants activities[5,6].

Iridoid represents a group of natural constituents with a monoterpene cyclic system and exists usually as glycosidic forms in nature. Among them, aucubin, a cyclopentano[c]pyran iridoid glycoside with a highly unstable aglycone, has been known to have a variety of biological activities[7]. As an antioxidant, aucubin is known to possess antioxidative capacity by protecting human dermal fibroblasts against photoaging which is attributable to MMP-1-mediated dermal damage when irradiated with UVB and by the protective effect on lipid peroxidation and activities of antioxidant defense systems and to conduct immunohistochemical evaluation of pancreas in streptozotocin-induced diabetic rats[8,9]. It is worth to notice that most biological activities of aucubin were assessed in the presence of a β-glucosidase which hydrolyzed aucubin to its unstable aglycone aucubigenin. However, there is no information available regarding the radical scavenging activity of aucubigenin.

In the previous paper, we have improved the procedure for the extraction of aucubigenin from the enzymatic hydrolysis of aucubin in order to obtain the stable pure crystal form and we also reported the structural study of aucubigenin by a single crystal X-ray analysis[10]. Therefore, in this study, we examined the possible radical scavenging activity of aucubigenin, the antioxidant activity was evaluated using different radical scavenging assays, such as DPPH radical scavenging, superoxide anion radical scavenging and hydroxyl radical scavenging assay, compared with its glucoside aucubin. The results described herein are the continuation of our biological activity study on the iridoid compounds.

EXPERIMENTAL

UV spectra were recorded on Model U-2001 UV/VIS Spectrophotometer. DPPH, Tris and EDTA-2Na were purchased from Sigma chemical company, USA. 30 % hydrogen peroxide, sodium salicylate, pyrogallol, 2-thiobarbituric acid and other chemicals in the studies were of highest quality commercially available from local suppliers (Xi' an, China). Water was obtained using the Millipore Simplicity water purification system (Millipore SAS 67120Molsheim, France). Aucubin, colourless and transparent crystals, isolated from the seeds of *Eucommia ulmoides Oliv.* in our laboratory (Biomedicine Key Laboratory of Shaanxi Province, Northwest University). Colourless and transparent crystals of aucubigenin were

obtained from the enzymatic hydrolysis of aucubin by β-glucosidase (EC 3.2.1.21). All other reagents were of analytical grade and used without further purification.

DPPH radical scavenging activity assay: The free radical scavenging activities of the samples were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH method with minor modification[11]. Briefly, 1 mL of sample solutions in MeOH of various concentrations was added to 4 mL of 25 μg/mL MeOH solution of DPPH. After vortexing, the mixture was incubated for 0.5 h at room temperature. Absorbance at 517 nm was measured at the end of incubation period. The decreasing of the DPPH solution absorbance indicated an increase of the DPPH radical scavenging activity. The difference in absorbance between test sample and control expressed as per cent inhibition was taken as the activity.

DPPH radical scavenging activity (%) was calculated as follows: DPPH radical scavenging activity rate (%) = $[1 - (A_{sample} - A_{blank})/A_{control}] \times 100$. Where $A_{control}$ is the absorbance without samples, A_{sample} is the absorbance of the samples addition and A_{blank} was the absorbance of samples blank.

Superoxide anion radical scavenging activity assay: The superoxide radical scavenging activity was evaluated at 25 ºC using the spectrophotometric monitoring of the inhibition of pyrogallol autoxidation according to the published method with some modifications[12]. Briefly, sample solutions of different concentrations were added to 1.5 mL of Tris-HCl-EDTA buffer (0.1 mol/L, pH 8.2). The mixture was shaked and 0.3 mL of pyrogallol solution (6.0 mmol/L) was added into the test tube. The absorbance of the reaction mixture was measured at 420 nm every 30 s using a spectrophotometer until the reaction proceeded to 3 min. The superoxide anion radical scavenging activity was determined as the percentage of inhibiting pyrogallol autoxidation, which was expressed by the oxidation degree of a sample group in comparison to that of the pyrogallol control.

The scavenging activity on superoxide radical was expressed using the following equation: Superoxide anion radical scavenging rate (%) = $[1 - (A_{sample} - A_{blank})/A_{control}] \times 100$. Where $A_{control}$ is the absorbance of the Tris-HCl buffer with pyrogallol, A_{sample} is the absorbance of the samples addition and A_{blank} was the absorbance of samples blank to eliminate interference.

Hydroxyl radical scavenging activity assay: Hydroxyl radicals were generated by Fenton reaction and the scavenging activity was measured according to a literature procedure with a minor modification[13]. The reaction mixture was consisted of 1 mL of $FeSO_4 \cdot 7H_2O$ (10 mmol/L), 0.8 mL of H_2O_2 (40 mmol/L), 0.5 mL of distilled water, 1.0 mL of samples in various concentrations and 0.2 mL of sodium salicylate (5 mmol/L). The total mixture (3.5 mL) was incubated at 37 ºC for 1 h and then the absorbance of the mixture was recorded at 562 nm.

The scavenging activity was calculated using the equation: Hydroxyl radicals scavenging rate (%) = $[1 - (A_{sample} - A_{blank})/A_{control}] \times 100$. Where $A_{control}$ is the absorbance without samples, A_{sample} is the absorbance of the samples addition and A_{blank} is the absorbance without sodium salicylate.

Statistical analysis: All the experiments were done in triplicate. The data were recorded as mean ± standard deviations. Analysis of variance was performed by One-way ANOVA procedure of PASW Statistics 18 (SPSS version 18.0 for Windows, SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

DPPH radical scavenging activity: DPPH method measures the radical scavenging activity in organic systems and has been used extensively as a prescreening method for new antioxidants from natural resources, due to its stability, simplicity, rapidity and reproducibility[14]. Aucubin showed little DPPH radical scavenging activity. However, a dose response relationship is found in DPPH scavenging activity of aucubigenin and an increase in concentration is synonymous of an increase in scavenging capacity. In comparison with 7.17 % of radical scavenging activity of aucubin, aucubigenin exhibited moderate radical scavenging activity, being 52.01 % at a concentration of 2.5 mg/mL (Fig. 1). The IC_{50} value (the concentration with 50 % scavenging activity) of scavenging activity on DPPH radical was found to be 2.35 mg/mL for aucubigenin. The result revealed that aucubigenin, which is the aglycone of aucubin, could be a potential inhibitor that may react with free radicals.

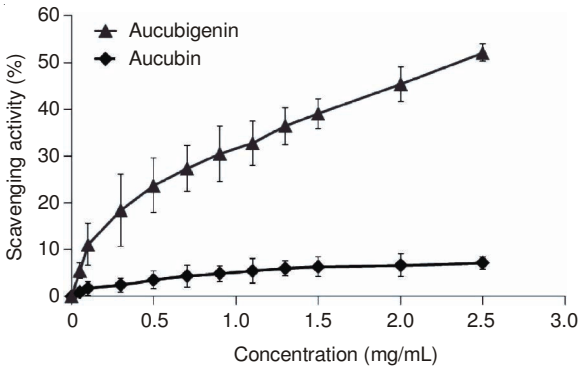

Fig. 1. DPPH radical scavenging activities of aucubigenin and aucubin. Values are means ± SD of three determinations

Superoxide anion radical scavenging activity: The superoxide anion radical is the most common free radical generated *in vivo*. Pyrogallic acid can auto-oxidize in alkaline conditions to produce superoxide anion radical directly and the rate constant of this auto-oxidation reaction is dependent on the superoxide anion radical concentration. With the scavenging capability on superoxide anion radical, the test compound can significantly slow down this auto-oxidation reaction of pyrogallic acid[15]. The results were expressed as inhibitory rate of the superoxide productivity. The superoxide anion scavenging activity of aucubigenin is slightly stronger than that of aucubin, recorded around 51.20 % of inhibition rate at 2.67 mg/mL when compared with that of aucubin (41.71 %) (Fig. 2). The IC_{50} value was 2.60 mg/mL for aucubigenin. No IC_{50} value was found for aucubin. The result demonstrated that both aucubigenin and aucubin showed a moderate scavenging activity against superoxide anion radical.

Hydroxyl radical scavenging activity: Hydroxyl radicals are considered to cause the ageing of human body and some diseases, interact with the purine and pyrimidine bases of DNA as well as abstract hydrogen atoms from biological molecules.

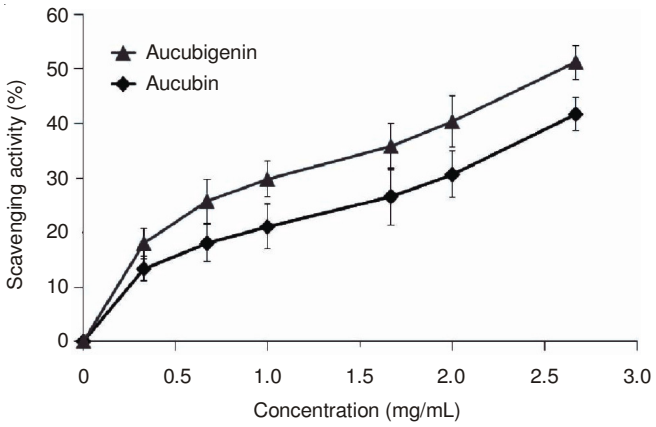

Fig. 2. Superoxide anion scavenging activities of aucubigenin and aucubin. Values are means ± SD of three determinations

The scavenging capacities of samples on hydroxyl radicals were evaluated according to the reaction of sodium salicylate and residual hydroxyl radical and these results were expressed as an inhibition rate. Hydroxyl radicals were generated by Fenton reaction in the system of $FeSO_4$ and H_2O_2 ($Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + {\cdot}OH$)[16]. However, aucubin showed 41.38 % of radical scavenging activity at a concentration of 2.5 mg/mL, while it was 6.38 % for aucubigenin (Fig. 3). The result indicated that aucubigenin appeared to have little activity on hydroxyl radical scavenging effects but aucubin exhibited moderate scavenging activity in a dose dependent manner.

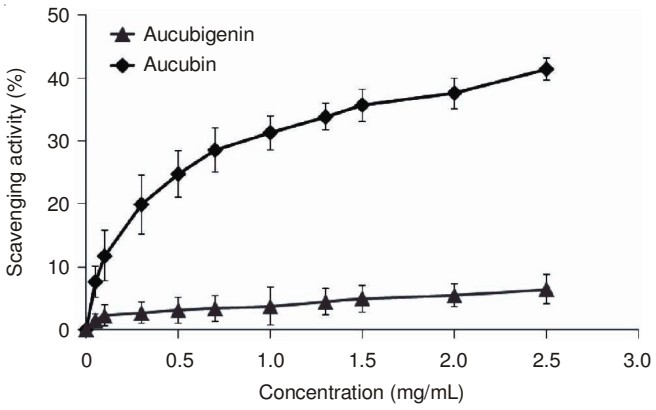

Fig. 3. Hydroxyl radical scavenging activities of aucubigenin and aucubin. Values are means ± SD of three determinations

Conclusion

In this study, we have focused on the free radical scavenging of iridoid aucubigenin and its glucoside. The results demonstrated for the first time that aucubigenin and aucubin show free radical scavenging activities at different magnitudes of potency. However, the mechanisms responsible for the radical scavenging activities of aucubigenin and aucubin are still unclear. To elucidate their antioxidant mechanisms, further study is in progress.

ACKNOWLEDGEMENTS

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