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Potential ABTS Reducing and Anti-collagenase Activity of Scutellarein and Apigenin

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Abstract

In developed and developing countries, the aging population growth rapidly and the consumption of natural product which rich antioxidant increasing. The aim of this study was to investigate the ABTS reducing activity and anti-collagenase activity of scutellarein and apigenin. This study used ABTS reducing activity for antioxidant assay and collagenase inhibitory activity for antiaging assay. Scutellarein (16.77 µg/ml) has higher antioxidant activity through ABTS reducing activity compared with apigenin (80.19 µg/ml). Scutellarein (99.20 µg/ml) has higher antiaging activity through collagenase inhibitory activity compared with apigenin (211.67 µg/ml). Scutellarein has very strong antioxidant activity through ABTS reduction activity and strong antiaging property particularly as collagenase inhibitor. Apigenin has strong antioxidant activity through ABTS reduction activity and weak antiaging property particularly as collagenase inhibitor.

Keywords: Scutellarein; apigenin; ABTS; anti-collagenase; Antioxidant; antiaging.

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1. Introduction

In the 20th century, the aging population is growing rapidly both in developed and developing countries [1]. The skin aging process is developed from a combination of intrinsic aging (age-related) and extrinsic aging (environmental factors) [2]. Benchmarks and wrinkles are the combination from ultraviolet (UV) light and poor lifestyle to accelerate the skin depression [2,3]. Ultraviolet radiation (UVR) like UVB can penetrate to the epidermis, destructive the skin function and keratinocyte development [4]. UVA can induce ROS that caused DNA damage [5]. Mitogen-activated protein kinase (MAPK) pathway activated by ROS and increasing MMP production that degrades collagen [6]. In this 21st century, antioxidant intake is increasing [7]. Fruits, vegetables, whole grains, and herbs are rich of antioxidants like vitamin C, vitamin E, carotenoids and phenolics [8]. Antioxidant donates hydrogen atom or proton to free radical so that the oxidation process can be slowed down [9]. Bioactive compounds in the natural sources have been used widely in cosmeceuticals substance to prevent skin aging [10]. The antiaging activity plays a role in stimulating collagen synthesis useful for improving skin appearance by restoring skin firmness and elasticity [11]. Scutellarein, apigenin, linalool, ursolic acid, estragole are rich in *Ocimum basilicum* L. leaf, they have applied for tropical cream because of their antiaging and antioxidant activity [12,13,14]. Arterbery and his colleagues [15] reported that apigenin use in cosmetical products contributed to improving skin firmness and skin health. Gu and his colleagues [16] reported that scutellarein has antioxidant activity particularly as DPPH scavenging activity. The aim of this study was to investigate the potential of scutellarein and apigenin as antioxidant sources particularly as ABTS reducing activity and antiaging sources particularly as the collagenase inhibitor.

2. Experimental Section

Materials

Materials used in this study are ABTS (Sigma A1888), Potassium persulfate, Distilled water, PBS 1x, Dimethylsulfoxide (DMSO), Scutellarein, Apigenin, N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGPA) (Sigma F5135), Collagenase from *Clostridium histolyticum* (Sigma C8051), Tricine, Calcium chloride, Sodium Chloride and Hydrochloric acid solution.

Instrumentation

Instruments used in this study are Multiscan Go Reader, Micropipette ((1-10 µl, 50- 200 µl, 100-1000 µl), Tips (1-10 µl, 50- 200 µl, 100-1000 µl), 96 well plate, Falcon tube (15 ml and 50 ml), Analytical Balance, Eppendorf tube 1.5 ml, Vortex, pH meter, Beaker glass, Spatula, and Incubator.

2.1 ABTS Reducing Activity Assay

Mix the ABTS 14 mM and potassium persulfate 4.9 mM with a volume ratio 1:1 for 12-16 hours in dark room at room temperature to produce ABTS solution. Add PBS 1X to the mixture until the solution's absorbance was 0,7 ± 0,02 at 745 nm wavelength. In brief, 2 µL various level of Scutellarein and Apigenin added to each well, then add 198 µL ABTS solution into the well. The absorbance was measured at 745 nm wavelength after the

plate incubated for 6 minutes at 37°C [17].

$$\%\,\boldsymbol{Scavenging} = \frac{\boldsymbol{c-s}}{\boldsymbol{c}}\,\boldsymbol{x}\,\mathbf{100}\%$$

c: control absorbance (without sample), s: sample absorbance

2.2 Collagenase Inhibitory Activity Assay

The mixture consists of 30 µL sample (0.78-50 µg/mL), 10 µL collagenase from Clostridium histolyticum (0.1 mg/mL) and 60 µL Tricine buffer (50 mM Tricine, 10 mM calcium chloride, 400 mM sodium chloride, pH 7.5) incubated for 20 minutes at 37°C. After incubated for 20 minutes, add 20 µL FALGPA substrate (1 mM) into the mixture. Absorbance was measured at 335 nm wavelength [17].

$$\%\,Inhibition = \frac{c-s}{c}\,x\,100\%$$

c: control absorbance (without sample)

s: sample absorbance

3. Result and Discussion

3.1 ABTS Reducing Activity Assay

From table 1 showed that at 50.00 µg/mL, the ABTS reducing activity of scutellarein was 99.02 ± 0.06, at 25.00 µg/mL was 66.78 ± 2.99, at 12.50 µg/mL was 39.53 ± 1.88, at 6.25 µg/mL was 34.57 ± 4.94, at 3.13 µg/mL was 31.36 ± 2.90, at 1.56 was 25.42 ± 2.56. at 50.00 µg/mL, the ABTS reducing activity of apigenin was 30.88 ± 0.42, at 25.00 µg/mL was 16.93 ± 0.14, at 12.50 µg/mL was 10.42 ± 0.72, at 6.25 µg/mL was 4.26 ± 0.51, at 3.13 µg/mL was 2.47 ± 0.25, at 1.56 was 0.55 ± 0.09. Table 1 indicated that an increase in the concentration level was directly proportional to the increase of the ABTS reducing activity.

Table 1: ABTS reducing activity of scutellarein and apigenin

Final concentration (µg/ml)	Mean of ABTS reducing activity (%)	
	Scutellarein	Apigenin
50.00	99.02 ± 0.06^{e}	30.88 ± 0.42^{e}
25.00	66.78 ± 2.99^{d}	16.93 ± 0.14^{d}
12.50	39.53 ± 1.88^{c}	10.42 ± 0.72^{c}
6.25	$34.57 \pm 4.94^{b,c}$	4.26 ± 0.51^{b}
3.13	$31.36 \pm 2.90^{a,b}$	$2.47 \pm 0.25^{a,b}$
1.56	25.42 ± 2.56^{a}	0.55 ± 0.09^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test).

From table 2 showed the IC_{50} value of ABTS reducing activity from scutellarein (16.77 ± 0.15 µg/mL) was lower than apigenin (80.19 ± 0.99 µg/mL). The IC_{50} is the concentration of scutellarein and apigenin needed to reduce half of ABTS. Table 2 indicated that scutellarein needed 16.77 ± 0.15 µg/mL and apigenin needed 80.19 ± 0.99 µg/mL to reduce ABTS activity. Based on the result indicated that scutellarein had ABTS reducing activity higher than apigenin.

Table 2: IC_{50} value of ABTS reducing activity of scutellarein and apigenin

Sample	Equation	R^2	IC50 (µg/mL)	IC50 (µg/mL)
Scutellarein (1st repetition)	Y = 1.5219x + 24.292	0.98	16.89	16.77 ± 0.15
Scutellarein (2nd repetition)	Y = 1.5369x + 24.474	0.97	16.61	
Scutellarein (3rd repetition)	Y = 1.4843x + 25.038	0.99	16.82	
Scutellarein (mean)	Y = 1.5144x + 24.601	0.98	16.77	
Apigenin (1st repetition)	Y = 0.6104x + 1.0496	0.98	80.19	80.19 ± 0.99
Apigenin (2nd repetition)	Y = 0.6108x + 0.7413	0.99	80.65	
Apigenin (3rd repetition)	Y = 0.6269x + 0.6366	0.99	78.74	
Apigenin (mean)	Y = 0.616x + 0.8091	0.99	80.19	

The IC_{50} was used to classified antioxidant activity of scutellarein and apigenin. If the IC_{50} less than 50 µg/mL is very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a moderate antioxidant, over than 150 µg/mL is a weak antioxidant [3]. Li and his colleagues [18] reported that the IC_{50} value of scutellarein was 18.3 ± 1.2 µM, which indicated that scutellarein has very strong antioxidant activity. Martinez and his colleagues [19] reported that the IC_{50} value of apigenin was 379.7 µg/mL, which indicated that apigenin isolated from *Alchornea coelophylla* pax & k. Hoffm. leaf extract has weak antioxidant activity. From the study, scutellarein has antioxidant activity through ABTS reducing activity higher than apigenin. Apigenin plays a major role in protecting cells from the breakdown of DNA double-stranded caused by oxidative stress [20]. Scutellarein plays a role in transferred electron to quench the free radical and reduced the activity [18].

3.2 Collagenase inhibitory activity assay

From table 3 showed that at 250 µg/mL, the collagenase inhibitory activity of scutellarein was 92.83 ± 0.37, at 125 µg/mL was 60.63 ± 1.47, at 62.5 µg/mL was 50.57 ± 1.92, 31.25 µg/mL was 26.78 ± 0.54, at 15.625 was 20.29 ± 1.00, at 7.8125 was 17.18 ± 0.18 At 250 µg/mL, the collagenase inhibitory activity of apigenin was 54.05 ± 7.98, at 125 µg/mL was 42.71± 0.31, at 62.5 µg/mL was 33.41 ± 0.50, 31.25 µg/mL was 29.48 ± 1.83, at 15.625 was 25.12 ± 1.17, at 7.8125 was 21.10 ± 0.06. Table 3 indicated that an increase in the concentration level was directly proportional to the increase of the collagenase inhibitory activity.

Table 3: Anti-collagenase activity of scutellarein and apigenin

Final concentration (ug/ml)	Mean of collagenase inhibitory activity (%)	
	Scutellarein	Apigenin
250	92.83±0.37[f]	54.05±7.98[d]
125	60.63±1.47[e]	42.71±0.31[c]
62.5	50.57±1.92[d]	33.41±0.50[b]
31.25	26.78±0.54[c]	29.48±1.83[ab]
15.625	20.29±1.00[b]	25.12±1.17[ab]
7.8125	17.18±0.18[a]	21.10±0.06[a]

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test). From table 4 showed the IC_{50} value of collagenase inhibitory activity from scutellarein (99.20 ± 0.94 µg/mL) was lower apigenin (211.67 ± 46.81 µg/mL). the IC_{50} is the concentration of scutellarein and apigenin needed to inhibit half of collagenase. Table 4 indicated that scutellarein needed 99.20 ± 0.94 µg/mL and apigenin needed 211.67 ± 46.81 µg/mL to inhibit collagenase activity. Based on the result indicated that scutellarein had collagenase inhibitory activity higher than apigenin.

Table 4: IC_{50} value of anti-collagenase from scutellarein and apigenin

Sample	Equation	R^2	IC50 (µg/mL)	IC50 (µg/mL)
Scutellarein (1st repetition)	Y = 0.3061x + 19.46	0.96	99.77	99.20 ± 0.94
Scutellarein (2nd repetition)	Y = 0.309x + 19.685	0.94	98.11	
Scutellarein (3rd repetition)	Y = 0.3091x + 19.179	0.94	99.71	
Scutellarein (mean)	Y = 0.3081x + 19.441	0.95	99.19	
Apigenin (1st repetition)	Y = 0.1536x + 22.724	0.98	177.58	211.67 ± 46.81
Apigenin (2nd repetition)	Y = 0.0921x + 25.59	0.80	265.04	
Apigenin (3rd repetition)	Y = 0.1407x + 22.93	0.98	192.40	
Apigenin (mean)	Y = 0.1288x + 23.748	0.95	203.82	

The IC_{50} was used to classified anti-collagenase activity of scutellarein and apigenin. If the IC_{50} less than 50 µg/mL is very strong anti-collagenase, 50-100 µg/mL is a strong anti-collagenase, 101-150 µg/mL is a moderate anti-collagenase, over than 150 µg/mL is a weak anti-collagenase [3] Kristina and his colleagues [21] reported that the IC_{50} value of lycopene was 85.09 ± 1.81 µg/mL, which indicated that lycopene has strong anti-collagenase activity. Widowati and his colleagues [22] reported that the IC_{50} value of myricetin was 11.65 ± 2.08 µg/mL, which indicated that myricetin has very strong antioxidant. From our finding, that bioactive compounds did not always have strong anti-collagenase activity. According to Arterbery and his colleagues [23] in tropical products apigenin improve firmness, elasticity, maintain hydration, decrease MMP-1 and MMP-2 production. The bioactive compound can boost the collagen synthesis and reduce the collagenase activity

through the collagen breakdown [21]. Clinical application of the present study is to investigate the antiaging effect of scutellarein in topical product compared with apigenin.

4. Conclusions

Scutellarein has very strong antioxidant activity through ABTS reduction activity and strong antiaging activity particularly as collagenase inhibitor, apigenin has strong antioxidant activity through ABTS reduction activity and weak antiaging property particularly as collagenase inhibitor. Scutellarein has antioxidant activity through ABTS reduction activity and antiaging activity through collagenase inhibitory activity better than apigenin.

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References

[1] AW. Pathath. “Theories of aging”. The International Journal of Indian Psychology, vol. 4(3), pp. 15-22. 2017.

[2] YH. Huang, PY. Wu, KC. Wen, CY. Lin and HM. Chiang. “Protective effects and mechanisms of Terminalia catappa L. methanolic extract on hydrogen-peroxide-induced oxidative stress in human skin fibroblast”. BMC Complementary and Alternative Medicine, vol. 18(266), pp. 1-9. 2018.

[3] Djohan, Kristina, AH. Henderson, INE. Lister, E. Girsang and E. Fachrial. “Comparison of antioxidant and anti-hyaluronidase activity of tomato (Solanum lycopersicum L.) extract and lycopene”. ASRJETS, vol. 52(1), pp. 49-56. 2019.

[4] ACM. Arango, SVF. Munoz and G. Sanclemente. “Mechanisms of Skin Aging”. IATREIA, vol. 30(2), pp. 160-70. 2017.

[5] U. Panich, G. Sittithumcharee, N. Rathviboon and S. Jirawatnotai. “Ultraviolet Radiation-Induced Skin Aging: The Role of DNA Damage and Oxidative Stress in Epidermal Stem Cell Damage Mediated Skin Aging”. Stem Cells International, vol. 2016, pp. 1-14. 2016.

[6] S. Zhang and E. Duan. “Fighting against skin aging: The way from bench to beside”. Cell Transplantation, vol. 27(5), pp. 729-38. 2018.

[7] C. Simioni, G. Zauli, AM. Martelli, M. Vitale, G. Sacchetti, A. Gonelli and LM. Neri. “Oxidative stress: Role of physical exercise and antioxidant nutraceuticals in adulthood and aging”. Oncotarget, vol. 9(24), pp. 17181-98. 2018.

[8] M. Sardarodiyan and AM. Sani. “Natural antioxidants: Sources, extraction and application in food systems”. Nutrition & Food Science, vol. 46, pp. 363-73. 2016.

[9] WD Fitriana, S. Fatmawati and T. Ersam. “Uji aktivitas antioksidan terhadap DPPH dan ABTS dari fraksi-fraksi daun kelor (Moringa oleifera)”. Prosiding Simposium Nasional Inovasi dan Pembelajaran

Sains, Bandung, Indonesia, 2015.

[10] A. Nurrochmad, Wirasti, A. Dirman, E. Lukitaningsih, A. Rahmawati and N. Fakhrudin. "Effects of antioxidant, anti-collagenase, anti-elastase, anti-tyrosinase of the Extract and Fraction from Turbinaria decurrens Bory". Indonesian J. Phar, vol. 29(4), pp. 188-97. 2018.

[11] L. Yulianti, E. Mardliyati, K. Bramono, HJ. Freisleben. "Asiaticoside induces cell proliferation and collagen synthesis in human dermal fibroblasts". Univ Med, vol. 34(2), pp. 96-103. 2015.

[12] MA. Ch, SB Naz, A. Sharif, M Akram and MA. Saeed. "Biological and Pharmalogical Properties of the Sweet Basil (Ocimum basilicum)". BJPR, vol. 7(5), pp. 330-9. 2015.

[13] S. Zhara and Y. Iskandar. "Review artikel: Kandungan senyawa kimia dan bioaktivitas Ocimum Basilicum L". Farmaka, vol. 15(3), pp. 143-52. 2017.

[14] EPG. Grijalva, MAP. Salas, NL Lopez, MSC. Mendoza, GV. Olivo and JB. Heredia. "Flavonoids and phenolic acids from oregano: Occurrence, biological activity and health benefits". Plants, vol. 7(2), pp. 1-23. 2018.

[15] VE. Arterbery, S. Gupta. "Apigenin as an anti-aging skin treatment". J. Clin. Cosmet. Dermtol, vol. 2(2), pp.1-8. 2018.

[16] T. Gu, Y. Zhong, YT. Lu, Y. Sun, ZX. Dong, WY Wu, ZH. Shi, NG. Li, X. Xue, F. Fang, HM Li and YP. Tang. "Synthesis and bioactivity characterization of scutellarein sulfonated derivative". Molecules, vol. 22(1028), pp. 1-8. 2017.

[17] W. Widowati, N. Fauziah, H. Herdiman, M. Afni, E. Afifag, HSW. Kusuma, H. Nufus, S. Arumwardana and DD. Rihibiha. "Antioxidant and anti aging assays of Oryza Sativa extract, vanillin and coumaric acid". Journal of Natural Remedies, vol. 16(3), pp. 88-99. 2016.

[18] Q. Liu, X. Li, X. Ouyang and D. Chen. "Dual effect of glucuronidation of a Pyrogallol-type phytophenol antioxidant: A comparison between scutellarein and scutellarin". Molecules, vol. 23(3225), pp. 1-13. 2018.

[19] CA. Martinez, OM. Mosquera and J. Nino. "Apigenin glycoside: an antioxidant isolated from Alchornea coelophylla pax & k. Hoffm. (euphorbiaceae) leaf extract". Univ. Sci, vol. 21(3), pp. 245-57. 2016.

[20] M. Gulluce, F. Orhan, D. Yanmis, T. Arasoglu, Z. Guvenalp and LO. Demirezer. "Isolation of a flavonoid, apigenin 7-O-glucoside, from Mentha longifolia (L.) Hudson subspecies longifolia and its genetoxic potency". Toxicol. Ind. Health, vol. 31(9), pp. 1-10. 2013.

[21] Kristina, AH. Henderson, Djohan, INE. Lister, E. Girsang and E. Fachrial. "Antioxidant and anticollagenase activity of tomato (Solanum lycopersicum L.) and lycopene". ASRJETS, vol. 52(1), pp. 57-66. 2019.

[22] W. Widowati, AP. Rani, RA. Hamzah, S. Arumwardana, E. Afifah, HSW. Kusuma, DD. Rihibiha, H. Nufus and A. Amalia. "Antioxidant and antiaging assays of Hibiscus sabdariffa extract and its compounds". Natural Product Sciences, vol. 23(3), pp. 192-200. 2017.

[23] A. Arterbery, S. Gupta. "Apigenin as an anti-aging skin treatment". J. Clin. Cosmet. Dermatol, vol. 2(2), pp. 1-8. 2018.