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Antioxidant and Inhibition of Elastase Effect of Scutellarein and Apigenin

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Abstract

Aging process in the skin was begun from the time when one is born, especially skin. Cutaneous aging can be caused by intrinsic and/or extrinsic factors. Wrinkles, thinning, and roughening of skin are part of skin aging. It becomes important looking for natural sources to prevent aging process. Apigenin and Scutellarein are potential phytochemistry which have antioxidant and anti-elastase effects. The purpose of this study is investigate antioxidant and anti-elastase effects of Apigenin and Flavonoid. Determination of Antioxidant Activity of Apigenin and Scutellarien was using FRAP Methods while anti-elastase activity was determined by inhibition of Elastase from Porcine Pancreas. The result of this study was expressed by Mean ± SD and analyzed by One Way analysis of variance (ANOVA) and Turkey HSD Post Hoc Test as a Further Analysis. Both of Scutellarein or Apigenin had the most potent antioxidant activity at the highest concentration (50 µg/ml). The FRAP Activity of Scutellarein or Apigenin at Highest concentration are 250.22 ±2.3 and 29.05 ±5.88 µg Fe (II), respectively. Antioxidant activity of Scutellarein are more potent than apigenin at various concentration and was shown differences at P value < 0.05. Same like antioxidant activity, anti-elastase activity of Scutellarein and apigenin has most potent inhibition effect at the highest concentration (66.67 µg/ml) which are 87.96 ± 1.63 and 28.33 ± 4.45 %, respectively while at various concentration of apigenin and scutellarein had differences percent inhibition of elastase at P value < 0.05. Scutellarein and Apigenin had antioxidant and anti-elastase activity. Each concentration of scutellarein had more potent antioxidant and anti-elastase activity than apigenin.

Keywords: Apigenin; Scutellarein; Antielastase; Inhibition of Elastase; Anti-elastase; Antioxidant; FRAP Methods; Elastase from Porcine Pancreas.

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1. Introduction

Cutaneous aging can be caused by intrinsic and/or extrinsic factors. The intrinsic factors is come from inevitable physiological process which cause thin and dry skin, fine wrinkles and gradual dermal atrophy. In the other hands, extrinsic factors also play important role in aging process especially long-term exposure to solar ultraviolet (UV) which is referred to as photoaging [1].

Aging process in the skin was begun from the time when one is born, especially skin. Skin aging is obviously seen more than other organ and affected most of woman. These are spent amount of daily expense for cosmetics and pharmaceuticals which are attempting to prevent and reverse skin aging [1]. Wrinkles, thinning, and roughening of skin are part of skin aging. Elastase is a protease enzyme. These enzyme will degrades elastin and to be modulated by oxidative stress which are inducted by Reactive Oxygen Species [2].

Based on these facts, it becomes important looking for natural sources to prevent aging process. Apigenin and Scutellarein are potential phytochemistry which have antioxidant and antielastase effects. Apigenin is a flavonoid compound as part of flavon family. On the other hand, scutellarein is phytochemical which can be found in some *Scutellaria*. *Scutellaria* is a part of *Lamiaceae* Family with 350-400 species [3]**.** The purpose of this study is investigate antioxidant and anti-elastase effects of Apigenin and Flavonoid.

2. Material and Methods

2.1. Materials

Scutellarein (Chengdu Biopurify, BP1277), Apigenin (Chengdu Biopurify, BP0177), 2,4,6-Tripyridyl-s-Triazine (TPTZ) (SigmaT-1253), FeCl3, buffer acetat (pH 3,6), Elastase enxyme from porcine pancrease (Sigma 45124), N-Sucanyl-Ala-Ala-Ala-p-nitroanilide, elastase substrat, (Sigma 54760), Trizma base, Phamacia Biotech, 17-1321-01, and Hydrocholic acid solution (Merck 109057).

2.2. Instruments

pH meter (OHAUS, Starter 300 pH Portable), Beaker glass, Multiskan Go Reader (Thermo Fisher Scientific), Micropipet (1-10 µl, 50- 200 µl, 100-1000 µl) (Eppendorf), Tips (1-10 µl, 50- 200 µl, 100-1000 µl) (NEPTUNE), 96 well-plate (TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Analytical Balance (AXIS), Tube Effendorf 1,5 ml (SPL 60015-1), Vortex (WiseMix VM-10), and Rotator (Thermo Fisher Scientific).

2.3. Preparation of Sample Concentration

For the purpose of these study, there were two serial concentration of sample in this study as shown by the following table 1.

Tabel 1: The Concentration of Apigenin and Scutellarein

Assay	Concentration (µg/mL)
Antioxidant assay	50, 25, 12.50, 6,25, 3,13, and 1,56
Antielastase assay	66.67, 33.33, 16.67, 8.83, 4.17, and 2.08

2.4. Antioxidant assay using FRAP (Ferric Reducing Antioxidant Power) Activity

Mixed the 7.5 µl of samples (Apigenin, Schutellarein, and $FeSO_4$) and 142.5 µl FRAP in the well plates (well sample and well blank). Concentration of $FeSO_4$ which were used in this assay includes: 7.80 µg/mL, 3.90 µg/mL, 1.95 µg/mL, 0.98 µg/mL, 0.49 µg/mL, 0.24 µg/mL. Amount of 142.5 µl DMSO were added as a solvent for the sample into well sample. Each of these well plates were incubated for 6 minutes in 37^{o}C. After that, the absorbance of these well plates were measured by microplate reader (λ = 745 nm). The antioxidant activity were determined from the absorbance of these well plates by $FeSo_4$ standard regression curve [4, 5, 6, 7].

2.5. Antielastase assay using Elastase Enzyme from porcine pancrease

Mixed 5 µl of Elastase enzyme from porcine pancreases (0.01 mg/mL, Sigma 45124) with 135 µl tris buffer for well control, 124 µl tris buffer for well sample, and 130 µl tris buffer for well blank. Amount of 10 µl samples were added in to well sample and well blank. Each of these well plates were incubated for 15 minutes in 25^{o}C. After that, each of these well plates were added 10 µl of $SucAla_3$-pNa. Furthermore, these well plates were incubated as before. For the last, the absorbance of these well plates are measured by microplate reader (λ = 410 nm). Percent of Inhibition of Elastase are determined by following formulation [8, 5, 6, 7, 9]:

$$\frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \times 100\ \%$$

2.6. Statistical Analysis

All data of antioxidant and anti-elastase activity were expressed as Mean ± SD for each concentration of sample. The result of this study was analyzed by One Way analysis of variance (ANOVA) and Tukey HSD Post Hoc Test as a Further Analysis. P value less than 0.05 were considered statistically significant.

3. Results

3.1. Antioxidant Activity

Antioxidant activity of Apigenin and Scutellarein was analyzed by FRAP methods. The result of antioxidant assay of apigenin and scutellarein were shown in following table 2.

Table 2: Antioxidant activity of Scutellarein and Apigenin (Mean, Post Hoc Tet Tukey HSD)

Concentration (µg/ml)	FRAP Activity (µg Fe(II)) [Mean ± SD]	
	Scutellarein	**Apigenin**
50	250.22 ±2.31^{f}	29.05 ±5.88^{d}
25	199.58 ±1.60^{e}	22.17 ±2.66^{cd}
12.5	149.60 ±0.51^{d}	16.68 ±0.43^{bc}
6.25	118.70 ±0.92^{c}	13.25 ±1.70^{ab}
3.125	88.65 ±2.13^{b}	11.00 ±0.35^{ab}
1.5625	75.85 ±0.48^{a}	6.38 ±0.19^{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).

Based on the table 2 above, the most potent antioxidant activity was shown at scutellarein than apigenin for each concentrations. Antioxidant activity in Scutellarein samples had the highest antioxidant activity at 50 µg/ml concentration with FRAP activity was 250.22 ±2.31 µg Fe(II) and the lowest antioxidant activity at 1.5625 µg/ml concentration with FRAP activity was 75.85 ±0.48 µg Fe (II). Furthermore, the result of Post hoc test was shown differences at P Value < 0.05 which meant at various concentration of apigenin and scutellarein shown differents FRAP activity and the FRAP Activity was gradually increased as well as increase of concentration of samples.

3.2. Anti-elastase Activity

Anti-elastase activity of Apigenin and Scutellarein was analyzed by Elastase Enzyme from Porcine Pancreas. The result of anti-elastase assay of apigenin and scutellarein were shown in following table 3.

Table 3: Anti-elastase activity of Scutellarein and Apigenin (Mean, Post Hoc Tet Tukey HSD)

Concentration (µg/ml)	Inhibition of Elastase (%) [Mean ± SD]	
	Scutellarein	**Apigenin**
66.67	87.96 ± 1.63^{e}	28.33 ± 4.45^{d}
33.33	60.90 ± 1.40^{d}	21.33 ± 0.48^{c}
16.67	45.72 ± 1.98^{c}	18.47 ± 0.47^{b,c}
8.33	29.17 ± 1.33^{b}	13.08 ± 0.53^{a,b}
4.17	25.07 ± 0.26^{a,b}	11.43 ± 1.35^{a}
2.08	22.51 ± 2.01^{a}	10.95 ± 0.24^{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).

Based on the tabel 3 above, the most potent anti-elastase activity was shown at scutellarein than apigenin for each concentrations. Anti-elastase activity in Scutellarein samples had the highest percent inhibition of elastase at 66.67 µg/ml concentration with percent inhibition was 87.96 ± 1.63 % and the lowest antielastase activity at 2.08 µg/ml concentration with percent inhibition was 22.51 ± 2.01 %. Furthermore, the result of Post hoc test was shown differences at P Value < 0.05 which meant at various concentration of apigenin and scutellarein shown differents percent inhibition of Elastase and the Inhibition of Elastase was gradually increased as well as increase of concentration of samples.

4. Discussion

Ocimum basilicum L. is one type of vegetables which also called basil and originates Africa, India, and Asia, however, it is also cultivated all over the world. The phytochemical constituents are essential oil, apigenin, luteolin, oglucotiside-apigenin, 7-O-Glukoronida, luteolin 7-O-Glucoronida, flavon C-glucoside orientin, molludistin, and ursolat acid which can act as anti-bacteria [10]. Refer to Figure 1 and 2 below, antioxidant and anti-elastase activity from scutellarein and apigenin increase linearly as well as elevation of each samples concentrations.

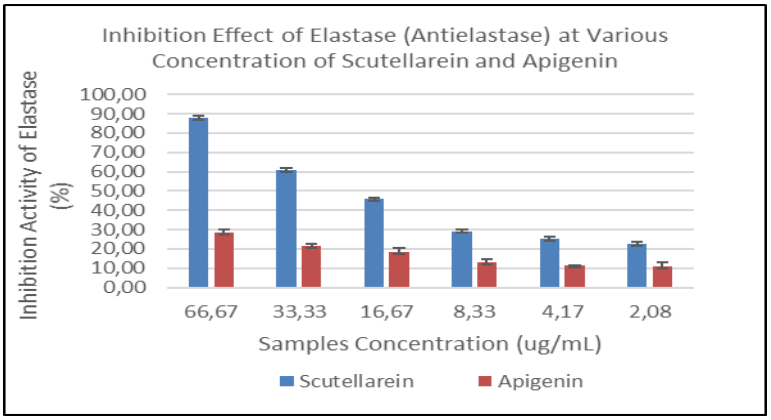

Figure 1: Histogram of Elastase Inhibition from Scutellarein and Apigenin

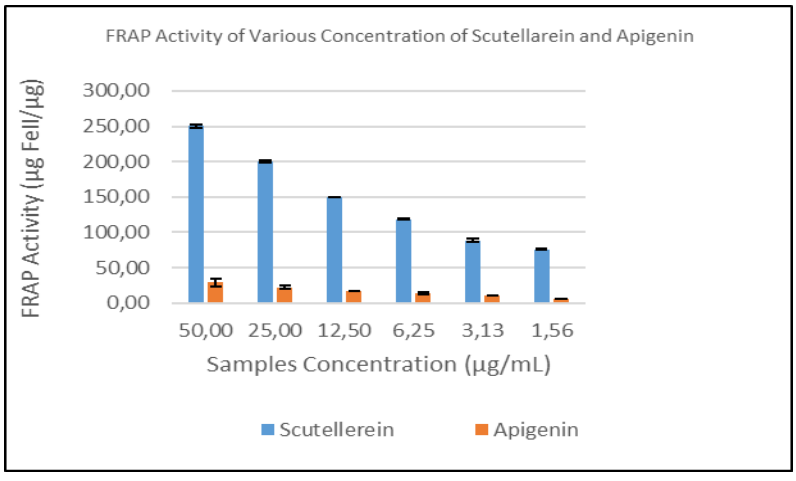

Figure 2: Histogram of Antioxidant from Scutellarein and Apigenin

Based on figures above, apigenin has less antioxidant and anti-elastase activity than scutellarein. But, anti-elastase effect of apigenin has lower enhancement effect when the concentration elevates. On the other hand, antioxidant activity of apigenin has similar pattern as scutellerein.

Apigen and scutellarein are part of flavonoid family which are found mainly in basil leaves (*Ocimum basilicum L.*) [10, 3]. Apigenin is a trihydroxyflavone which is flavone substituted by hydroxyl groups at 4', 5, and 7 while scutellarein is also a flavone which derives from an apigenin substituted with hydroxyl groups at C-4', 5, -6, and -7 [11, 12]. Flavone is one type of flavonoid compound which has various pharmacology properties. One of its pharmacology properties is radical scavenging which can prevent injury cause by free radical in various way and one is the direct scavenging of free radical. Due to hydroxyl group in flavonoid compound, the free radical are made in active [13]. Because of more number hydroxyl group in the scutellerein than apigenin, antioxidant activity of scutellarein become more potent than apigenin. The structures of apigenin and scutellerein are shown in the following figure 3.

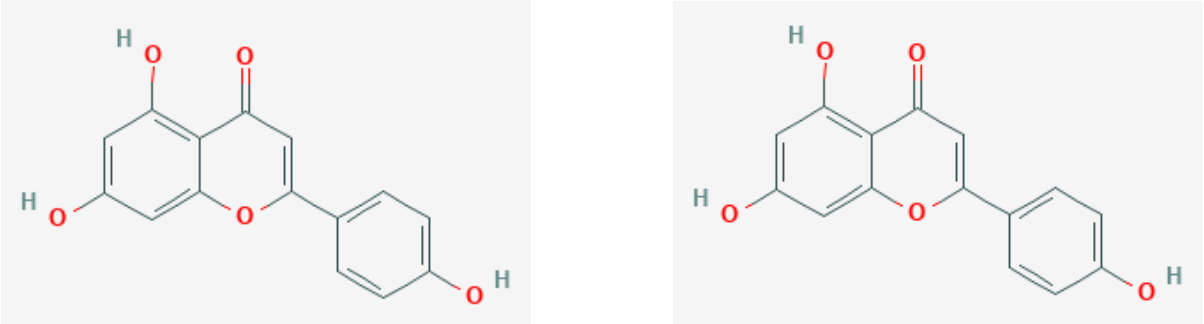

Figure 3: Structure of Apigenin (Left) and Scutellarein (Right)

Elastase is a serine protease which is primarily responsible for degradation of Elastin in Extracellular matrix. Since collagen and elastin mainly maintain skin structural integrity and elasticity, depletion of collagen and elastin contribute in wrinkles and skin aging. These enzyme is modulated by oxidative stress which are inducted by reactive oxygen species [14, 2]. Antioxidant activity of scutellarein is more potent than apigenin, so the scutellarein has more effect on reducing oxidative stress which will modulate inhibition of elastase activity.

5. Conclusion

Scutellarein and Apigenin had antioxidant and antielastase activity. Each concentration of scutellarein had more potent antioxidant and anti-elastase activity than apigenin. Each concentration of scutellarein and apigenin had a differences antioxidant and anti-elastase activity at P value < 0.05.

References

[1] S. Zhang dan E. Duan, "Fighting against Skin Aging: The Way from Bench to Bedside," Cell Transplantation, vol. 27, no. 5, pp. 729-738, 2018.

[2] I. K. Sundaram, D. D. Sarangi, V. Sundararajan, S. George dan S. S. Mohideen, "Poly Herbal Formulation

with Anti-Elastase and Anti-Oxidant Properties for Skin Anti-Aging," BMC Complementary and Alternative Medicine, vol. 18, no. 33, pp. 1-12, 2018.

[3] E. Minareci dan S. Pekonur, "An Important Euroasian Genus: Scutellaria L. Int," J. Sec. Metabolite, vol. 4, no. 1, pp. 35-46, 2017.

[4] W. Widowati, L. Darsono, J. Suherman, Y. Yelliantty dan Maesaroh, "High Performance Liquid Chromatography (HPLC)," Int. J Biosci, Biochem, Bioinform, vol. 4, no. 6, pp. 458-466, 2014.

[5] W. Widowati, N. Fauziah, H. Herdiman, M. Afni, E. Afifah, H. S. W. Kusuma, H. Nufus, S. Arumwardana dan D. D. Rihibiha, "Antioxidant and anti aging assays of Oryza sativa extracts, vanillin and coumaric acid," J. Nat. Remed, vol. 3, no. 88-99, p. 16, 2016.

[6] W. Widowati, A. P. Rani, R. A. Hamzah, S. Arumwardana, E. Afifah, H. S. W. Kusuma, D. D. Rihibiha, H. Nufus and A. Amalia, "Antioxidant and antiaging assays of Hibiscus sabdariffa extract and its compounds," Nat. Prod. Sci, vol. 23, no. 3, pp. 192-200, 2017.

[7] W. Widowatia, W. J. B, S. Nadya, A. Amalia, S. Arumwardana, H. S. W. Kusuma dan Y. Arinta, "Antioxidant and Antiaging Activities of Jasminum Sambac Extract, and its Compounds," J. Reports Pharmaceutic. Sci, vol. 7, no. 3, pp. 270-285, 2018.

[8] T. S. Thring, P. Hilli dan D. P. Naughton, "Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants`," BMC Complement Altern Med, vol. 9, no. 27, pp. 1-11, 2009.

[9] S. Utami, Q. R. Sachrowardi, N. A. Damayanti, A. Wardhana, I. Syarif, S. Nafik, B. C. Arrahman, H. S. W. Kusuma dan W. Widowati, "Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (Garcinia picrorrhiza Miq.) fruit as antiaging," J Herbmed Pharmacol, vol. 2, no. 88-93, p. 7, 2018.

[10] D. A. Larasati, "Efek Potensial Daun Kemang (Ocimum basilicum L.) sebagai Pemanfaatan Hand Sanitizer," Jurnal Majority, vol. 5, no. 5, 2016.

[11] National Center for Biotechnology Information, "Scutellarein, CID- 5281697," PubChem Database, [Online]. Available: https///pubchem.ncbi.nih.gov/compound/5281697. [Diakses 24 March 2019].

[12] National Center for Biotechnology Information , "Apigenin, CID=5280443," PubChem Database, [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/5280443. [Diakses 24 March 2019].

[13] A. N. Panche, A. D. Diwan dan S. R. Chandra, "Flavonoid: an Overview," Journal of Nutritional Science, vol. 5, no. e47, pp. 1-15, 2016.

[14] S. Pientaweeratch, V. Panapisal dan A. Tansirikongkol, "Antioxidant, Anti-Collagenase and Anti-Elastase Activities of Phyllanthus emblica, Manilkara zapota and Silymarin: an In Vitro Comparative Study for Anti-Aging Applications," Pharmaceutical Biology, vol. 54, no. 9, pp. 1865-1872, 2016.