American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Scavenge ABTS and Inhibition of Elastase Enzyme Activity from Ethanol Extract of Pineapple (*Annas cosmusus* (L.) Merr) Core

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Abstract

The exact cause of aging is still unknown, there are some theories which has been directed. Excess ROS (Reactive oxygen species) or UV radiation can precipitate activation of elastase which degrades elastin. This study was aimed to investigate anti-elastase and antioxidant activity of ethanol extract from pineapple core. Determination of Antioxidant activity was using ABTS Methods while Inhibition of Elastase assay was using Elastase Enzyme from porcine pancreases. The result of this study was express by Mean ± SD and analysed by One-Way ANOVA test and followed by the Post Hoc Test with the Tukey HSD test, while IC_{50} was determined by simultaneous analysis of linear regression. At the highest concentration (50 ug/ml), The scavenge activity of ABTS' were 36.13 ± 2.82 % for ethanol extract of pineapple core and 93.91 ± 3.25 % for luteolin compound. Same as Scavenge ABTS Activity, at the highest concentration (66.67 ug/ml), the elastase inhibition activities were 78.73 ±3.08 % for ethanol extract of pineapple core and 86.30 ± 1.78 % for luteolin compound. The result of Post Hoc test for ABTS activity and inhbition of elastase was shown significant differences (P value < 0.05) of percentage activity at various concentration. Based on IC_{50} value of Ethanol extract from pineapple core that antioxidant activity (IC_{50}: 72.73±4.31) was strong while inhibition of elastase enzyme (IC50:16.79±1.62) was very strong. Antioxidant and antielastase activity of ethanol extract from pineapple core aren't potent as luteolin compound.

Keywords: Elastase Inhibition; ABTS; Pineapple core; Luteolin.

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1. Introduction

Aging is a process become older. Aging can be occur in human, some of animals, and fungi, however such as bacteria, plant, and some simple animal can be potentially immortal biologically. More obviously aging can be defined as single cell in organism which have stopped to divided (Cell Aging) or in population of a species (Population aging) [1].The exact cause of aging is still unknown, there are some theories which has been directed. One of these theory is accumulation of damage which can cause failure of biology system. Other theory includes programmed aging concept which is internal process (such as DNA methylation) contributed to cause aging process [1]. On the other hand, there are some factors which affect biology aging process. These factors are categorized in to 2 main categories (Programmed and depend on damage). Factors which are programmed have had biology time includes growth and development process and gene expression. While Factors which are depend on damage process includes damage that are come from internal or environment of organism and cause cumulative damages [2, 1]. In the normal skin production of elastase and collagenase enzyme are balanced. Excess ROS (Reactive oxygen species) or UV radiation can precipitate activation of elastase which degrades elastin. Elastin are major component of connective tissue and tendon. Elastin and collagen are major component which responsible to form dermis tissue. Due to elastase activity which are degrades elastin in the skin, it will cause skin wrinkles [3] The aging process is more obvious in the skin than other organs. The treatments which are aimed to prevent reverse aging process are spent a lot of daily expenses [4] Natural products can be used as solution for slow down aging process which consist of phytochemical [5]. Luteolin is an antioxidant from flavonoids class of photochemistry which has been studied and others effects of luteolin are anti-tumor, and anti-inflammatory [6]. There are many natural products which contain phytochemicals, one of them is a pineapple core which is useless leftover products. Several studies have reported the contents of flavonoids and phenols which function as antioxidants in their skin and flesh [7]. There is little information about biological activity of ethanol extract from pineapple core especially antioxidant and elastase inhibitory properties. So this study was aimed to investigate anti-elastase and antioxidant activity of ethanol extract from pineapple core.

2. Methods

2.1. Materials

Pineapple Core (*Ananas cosmosus (L.) Merr*), ethanol 70%, luteolin compound, 2,2-Azinobis(3-etilbenzatiazolin)-6-sulfonat (ABTS), dymethilsufoxide (DMSO), Elastase from porcine pancreases (Sigma 45124), N-Sucanyl-Ala-Ala-Ala-p-nitroanilide, elastase substrate (Sigma 54760), Trizma base, Phamacia Biotech, 17-1321-01, Hydrocholic acid solution (Merck 109057), sodium chloride.

2.2. Preparation of Samples

Pineapple core as sample which were used in this study were obtained from Tambaksari Village, Jalan Cagak District, Subang Regency, and West Java Province. The sample was sorted and cleaned, then dried using food dehydrator to obtain simplicia. Then simplicia was mashed and weighed 200 grams, extracted by maceration method with 70% ethanol as solvent. Every 24 hours the filtrates were collect until the ethanol filtrate became

colourless for 3 days. Then the filtrate was evaporated with a rotary evaporator at 50^{o}C until the filtrate become concentrated [8, 9, 10, 11]**.**

2.3. Antioxidant Assay Using Scavenge ABTS Activity

Added 2 µl sample into each of well on the 96 well micro-plates, then added 198 µl $ABTS^{++}$ solution. The Absorbance was measured using micro-plates reader (λ = 745 nm) which had been incubated at 30^{o}C for 6 minutes. Percent of scavenge ABTS was measured using following formulation. Others than percent of scavenge, Median Inhibitory Concentration (IC_{50}) was also measured [8, 9]:

$$\%Scavenge = [(Abs_{control}-Abs_{sample})/Abs_{control} \times 100\%] \quad (1)$$

2.4. Inhibition of Elastase Assay using Elastase Enzyme from Porcine Pancreas

Added 10 µl sample into orbital shaker, then added 125 µl buffer (pH = 8) and 5 µl Elastase Enzyme (0.2-0.5 unit). After that the mixture was incubated for 15 minutes at room temperature. For the last, added 10 µl substrate (4 mM) and incubated again for 15 minutes at room temperature. The absorbance was measured at 410 nm wavelength. Percent of elastase inhibition was measured using following formulation. Others than percent of inhibition, Median Inhibitory Concentration (IC_{50}) was also measured [9].

$$\%Inhibition = [(Abs_{control}-Abs_{sample})/Abs_{control} \times 100\%] \quad (2)$$

2.5. Statistical Analysis

Data was analysed by One-Way ANOVA test and followed by the Post Hoc Test with the Tukey HSD test with a confidence level of 95% (α = 0.05). Percent of scavenge ABTS and inhibition of elastase were followed to analyse by simultaneous analysis of linear regression then determined the value of Inhibition Concentration 50 (IC_{50}).

3. Result and Discussion

Table 1: Percentage of Scavenge ABTS Activity from Ethanol Extract of Pineapple and Luteolin (Mean, Post Hoc Test Tukey HSD)

Concentration (ug/ml)	Average Scavenge Activity of ABTS (%)	
	Ethanol Extract of Pineapple Core	Luteolin
50.00	36.13 ±2.82^{e}	93.91 ± 3.25^{e}
25.00	22.01 ± 0.57^{d}	54.57 ± 0.85^{d}
12.50	19.28 ± 0.61^{c}	32.76 ± 3.77^{c}
6.25	13.06 ± 0.87^{b}	15.38 ± 1.17^{b}
3.13	7.45 ± 3.21b	10.47 ± 0.88^{a,b}
1.56	6.88 ± 3.51^{a}	5.84 ± 1.34^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05

The average of scavenge ABTS activity was analysed using Post Hoc Test Turkey HSD as show in table 1 below. Based on tabel 1 above, the scavenge activity of ethanol extract of pineapple core was lower than luteolin compound at various concentration except at lowest concentration (1.56 ug/ml). At the highest concentration (50 ug/ml), The scavenge activity of ABTS' were 36.13 ± 2.82 % for ethanol extract of pineapple core and 93.91 ± 3.25 % for luteolin compound. While at the lowest concentration (1.56 ug/ml), scavenge activity of ABTS' were 6.88 ± 3.51% for ethanol extract of pineapple core and 5.84 ± 1.34 % for luteolin compound. Furthermore, the result of Post Hoc test was shown differences at P value < 0.05 which meant at various concentration of ethanol extract from pineapple core and luteolin had different average scavenge activity of ABTS, except at 6.25ug/ml and 3.13 ug/ml concentration (as look at same small letter in the table) and the Scavenge activity of ABTS was gradually increased as well as increase of concentration of sample. The median inhibition concentration of ABTS was plotted using Linear regression. Result of IC50 Value from linear regression were shown in table 2 below.

Table 2: IC50 Value of Scavenge ABTS Activity from Ethanol Extract of Pineapple Core and Luteolin Compound

Sample	**Equation**	**R^2**	**IC_{50} (µg/mL) from Equation**	**IC_{50} (µg/mL)** **Mean ± SD**
Ethanol Extract of Core Pineapple				
1st Repetion	y = 0.5505x + 7.2323	0.92	77.69	72.73±4.31
2nd Repetion	y = 0.577x + 9.7061	0.98	69.83	
3rd Repetion	y = 0.6086x + 6.9876	0.91	70.67	
Mean	y = 0.5787x + 7.9753	0.95	72.62	
Luteolin Compound				
1st Repetion	y = 1.7534x + 6.4709	0.97	24.83	24.42±0.37
2nd Repetion	y = 1.8457x + 5.1368	1.00	24.31	
3rd Repetion	y = 1.84x + 5.6221	0.99	24.12	
Mean	y = 1.813x + 5.7433	0.99	24.41	

Based on table 2 above, IC_{50} value of ethanol extract from pineapple core (72.73±4.31%) was higher than luteolin compound (24.42±0.37%). It meant that a higher concentration of ethanol extract from pineapple core was needed to inhibit half of ABTS than Luteolin compund. The average of Elastase Inhibition Activity activity was analysed using Post Hoc Test Turkey HSD as show in table 3 below.

Table 3: Percentage of Elastase Inhibition from Ethanol Extract of Pineapple and Luteolin (Mean, Post Hoc Test Tukey HSD)

Concentration (ug/ml)	Average Elastase Inhibition Activity (%)	
	Ethanol Extract of Pineapple Core	Luteolin
66.67	78.73 ±3.08^{e}	86.30 ± 1.78^{d}
33.33	63.72 ±1.06^{d}	73.04 ± 0.40^{c}
16.67	54.44 ± 3.45^{c}	60.36± 1.16^{b}
8.33	45.60 ± 0.89^{b}	59.23 ± 5.04^{b}
4.17	39.78 ± 1.00^{a}	49.51 ±2.45^{a}
2.08	37.26 ± 0.51^{a}	46.74 ± 0.95^{a}

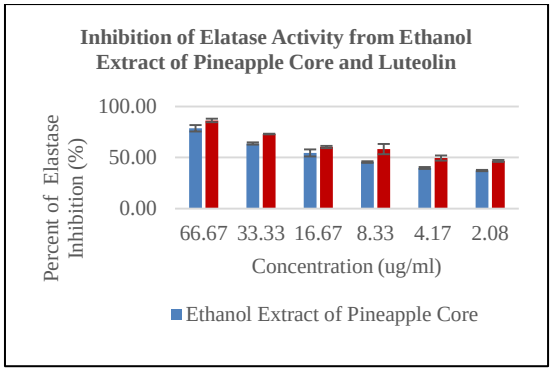
Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$

Based on tabel 3 above, elastase inhibition activity of ethanol extract of pineapple core was lower than luteolin compound at various concentration. At the highest concentration (66.67 ug/ml), the elastase inhibition activities were 78.73 ± 3.08 % for ethanol extract of pineapple core and 86.30 ± 1.78 % for luteolin compound. While at the lowest concentration (2.08 ug/ml), elastase inhibition activities were 37.26 ± 0.51% for ethanol extract of pineapple core and 46.74 ± 0.95 % for luteolin compound. Furthermore, the result of Post Hoc test was shown differences at P value < 0.05 which meant at various concentration of ethanol extract from pineapple core and luteolin had different average elastase inhibition activity. However, in some concentration of luteolin escpecially at lower concentration, elastase inhibition activity were not significantly different. While ethanol extract of pineapple core had no significant different at only two lower concentration samples. The median inhibition concentration of elastase enzyme was plotted using Linear regression. Result of IC_{50} Value from linear regression were shown in table 4 below.

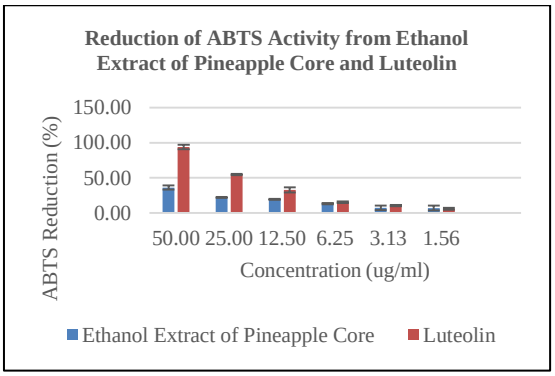
Table 4: IC_{50} Value of Elastase Enzyme from Ethanol Extract of Pineapple Core and Luteolin Compound

Sample	Equation	R^2	IC_{50} (µg/mL) from Equation	IC_{50} (µg/mL) Mean ± SD
Ethanol Extract of Core Pineapple				
1st Repetion	y = 0.6348x + 38.631	0.97	18.36	16.79±1.62
2nd Repetion	y = 0.6735x + 38.647	0.98	16.88	
3rd Repetion	y = 0.572x + 41.359	0.89	15.12	
Average	y = 0.6268x + 39.545	0.95	16.68	
Luteolin Compound				
1st Repetion	y = 0.6077x + 48.926	0.96	1.78	0.04±2.28
2nd Repetion	y = 0.5748x + 51.461	0.88	-2.54	
3rd Repetion	y = 0.5838x + 48.067	0.95	0.88	
Average	y = 0.5888x + 49.485	0.95	0.87	

Based on table 4 above, IC_{50} value of ethanol extract from pineapple core (16.79±1.62%) was higher than luteolin compound (0.04±2.28%). It meant that a higher concentration of ethanol extract from pineapple core was needed to inhibit half of Elastase Enzyme than Luteolin compund.



(A)



(B)

Figure 1: Histogram of Antixodant (A) and Inhibition of Elastase (B) from Ethanol Extract of Pineapple Core and Luteolin Compound

Figures 1 shows whether at various concentration of ethanol extract from pineapple core has antioxidant activity and inhibition of elastase which are less potent than luteolin as a comparison. Based on Taskeen and his colleagues (2010) study whether bioactive content of pineapple includes kaempferol 2.5 mg / kg, rhamnetin 7.0 mg / kg, luteolin 3.5 mg / kg and quercetin 2.5 mg / kg. The other active substances contained in pineapple such as anthocyanidin, flavan-3-ols (catechin, gallocatechin), flavononones (hesperetin, naringenin), flavones (apigenin, luteolin), flavonols (kaempferol, myricetin, quercetin). These compounds have antioxidant, anti-cancer and anti-mutagenic activity [12, 13]. In addition, other studies also report similar result which pineapple extract can be found carbohydrates, quinones, cardiac glycoside, terpenoids, phenols, and steroids, as well as small amounts of tannins, flavonoids, and coumarins [14]. The IC_{50} value is classified into very strong activities (IC_{50} less than 50µg / mL), strong activity (IC_{50} 50 - 100µg / mL), moderate activity (IC_{50} 101 - 150µg / mL), and weak activity (IC_{50} greater than 150µg / mL) [15, 10]. According to the results of the research as shown in tables 2 and 4 above that the antioxidant activity of ethanol extract from pineapple core as samples were strong, while antielastase of ethanol extract from pineapple core were very strong. These effect are because pineapple extract has phenols and flavonoids. In several studies it was shown that phenol and flavonoids had anti-elastase activity [16]. The presence of triterpenoid compounds in the extract is responsible for protecting the skin due to the presence of hydroxyl subtituents capable of inhibiting ROS, reducing metal ions, modulating protein

phosphorylation related to inhibition of enzyme activity and inhibition of lipid peroxidation [16, 17]. Luteolin is a pure compound which has the ability of antielastase activity. According to the research of Thring (2009) and Makarenko and Levitsky (2016), that the IC50 value of bioflavonoids such as luteolin has been reported to have activity against inhibition of elastase [18, 19].

4. Conclusion

Pineapple core are comorbid which has potential to become antioxidant and antielastase, but these aren't potent as luteolin compound. Further study is needed to improve antioxidant and antielastase effect of pineapple core for antiaging product.

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