Trad. Med. J., September-December 2020
Vol. 25(3), p 182-189
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 30-08-2020
Revised : 15-12-2020
Accepted : 23-12-2020

In Vitro Anti-Wrinkle and Tyrosinase Inhibitory Activities of Grapefruit Peel and Strawberry Extracts

Endang Lukitaningsih*, Syamsu Nur, Fadilah Qonithah, Andi Zulbayu, Rina Kuswahyuning, Rumiyati
Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

The research aims to analyse the antioxidant, anti-tyrosinase and anti-wrinkle activities from grapefruit (Citrus Maxima L) and strawberry extracts. Samples were extracted by maceration using 96% ethanol and ethyl acetate, subsequently. The Ferric Reducing Antioxidant Power (FRAP) and β-carotene bleaching (BCB) were used to measure the antioxidant activity. The effect of anti-wrinkle was determined by testing the inhibition of elastase and collagenase enzyme, while anti-tyrosinase activity was analysed using mushroom tyrosinase enzyme. The results showed that strawberry extracts in ethanolic (SE) and ethyl acetate (SEA) have antioxidant activity in FRAP (EC_{50} = 404.39 ± 3.27 µg / mL and 1978.65 ± 37.25 µg/mL) and BCB (IC_{50} = 292.30 ± 4.69 µg/mL and 671.11 ± 6.74 µg/mL). Whereas the grapefruit peel extracts both in ethanolic (GPE) and ethyl acetate (GPEA) have antioxidant activity in FRAP (EC_{50} 219.47 ± 71.96 µg / ml and 309.44 ± 95.76 µg/ml) and BCB (EC_{50} 245.19 ± 162.47 µg/ml and 567.54 ± 95.31 µg/ml). As positive standards for FRAP antioxidant analysis were quercetin and vitamin C which has IC_{50} respectively 18.97 ± 4.50 µg/mL and 24.47 ± 1.44 µg/mL. While in BCB analysis, Butylated Hydroxy Toluene (BHT) used as positive standard (IC_{50} 38.68 ± 5.70 µg/mL). The samples of SE, SEA, GPE and GPEA showed tyrosinase inhibitory activity which the IC_{50} values were respectively 492.68 ± 1.43; 2658 ± 48.08; 3312.5 ± 222.74; 2985.17 ± 122.80 µg/ml. Kojic acid (IC50 111.52 ± 0.42 µg/ml) is used as positive standard in this study. In addition, SE, SEA, GPE and GPEA were able to inhibit elastase and collagenase enzymes, although their activities were still lower than the positive standard used in this study. Elastastinal in concentration 50 µg/mL giving elastase inhibition about 71.71 ± 0.81 µg/mL, while vitamin C in the same concentration showed collagenase inhibition about 66.79 ± 1.23 µg/mL. It can be concluded that the extract of strawberry and grapefruit peel has antioxidant, anti-tyrosinase and anti-wrinkle activity through inhibition of elastase and collagenase enzymes; thus, they can be used as antiaging cosmetic ingredients.
Keywords: *Citrus maxima* L; strawberry; antioxidant; anti-wrinkle; anti-tyrosinase

INTRODUCTION

Skin aging, induced by intrinsic and extrinsic factors (photo aging), causes a loss of structural integrity and decreased skin function (Lephart, 2018). The main contributor to the two ageing processes is oxidative stress which damages the cells and the extracellular matrix of the skin (Giampieri *et al.*, 2014; Karim *et al.*, 2014). Oxidative stress occurs due to an imbalance between the production of reactive oxygen species (ROS) and antioxidants in the body (Abirami *et al.,* 2014). Skin care and treatment are very important in order to make the skin healthy and free from any diseases (Allen and Tracy, 1995).

In a normal condition, skin produces elastase and collagenase enzymes. Moreover, the increased levels of ROS will accelerate the production of both enzymes, and it will hasten the degradation of elastin and collagen which is in the extracellular matrix of the dermis. On the other hand, the UV exposure will also trigger skin pigmentation since the production of melanin by tyrosinase enzyme is increased (Apak *et al.*, 2007; Apraj and Pandita, 2016). The inhibitor of tyrosinase enzyme such as hydroquinone, kojic acid and azelaic acid are used against skin pigmentation disorders (Aumeeruddy-Elalfi *et al.*, 2016).

Today, there has been many researchers reported that natural resources such as fruits and vegetables containing polyphenol can be as a potential antioxidant. Other important substances derived from our diet, included vitamin A, C, E can also act as antioxidant (Khrisnamurthy and Wadhwani, 2012; Pullar *et al.,* 2017; Lephart, 2018). All of these compounds are beneficial to reduce the risk of skin ageing, because of antioxidant and anti-inflammatory activity on the skin (Bae *et al.*, 2009; Azzini *et al.*, 2010; Ben Ahmed *et al.*, 2016). Strawberry already known containing many bioactive, such as vitamin C, β-carotene, quercetin, anthocyanin, vitamin E and polyphenols (phenols, flavonoids, phenolic acids,

***Corresponding author : Endang Lukitaningsih**
Email : lukitaningsih_end@ugm.ac.id

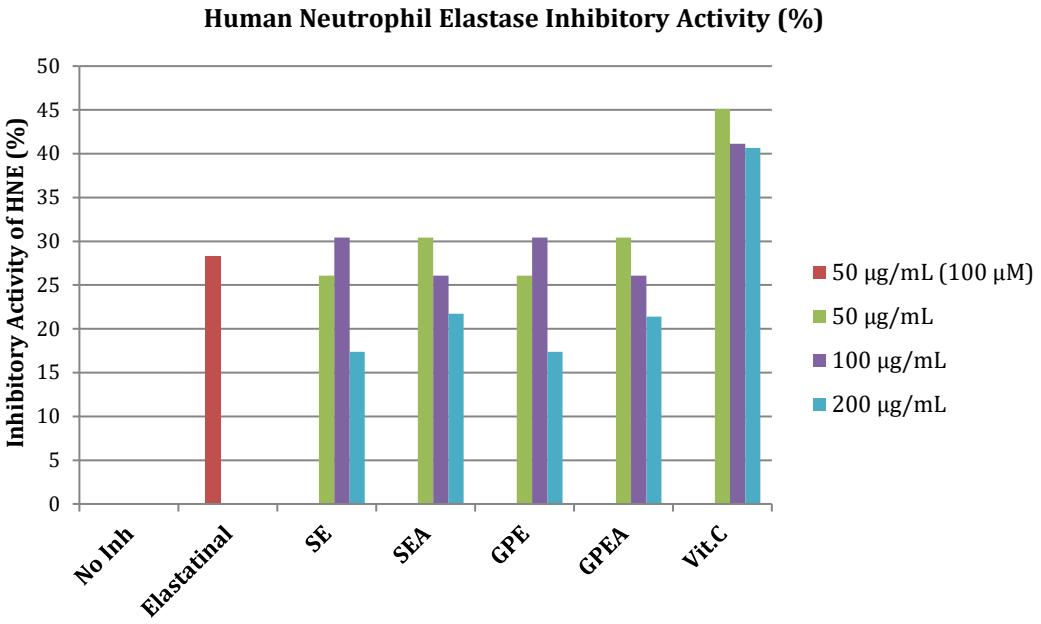

Figure 1. HNE inhibitory activity of extracts, vitamin C (as positive standard) and elastatinal at three different concentrations (n=3)

ligands and tannins) (Chang *et al.*, 2013; Ben Ahmed *et al.*, 2016). *Citrus Maxima* L or grapefruit which is known as Papanus, and is spread widely throughout India. The wooden peel and root of *C. maxima* L. contains β-sitosterol and acridine alkaloids. Essential oils from leaves and raw fruits contain limonene, neroli, neroli acetate and geraniol. These plants have been commonly used for medical treatment such as sedatives for anxiety, convulsive cough, treatment of haemorrhagic diseases and epilepsy. *C. maxima* L. also contains a high compound of polyphenol; hesperidin and naringin which show various pharmacological activities that have been previously studied (Cuvelier *et al.,* 1992), which is as an inhibitor of tyrosinase, acetylcholinesterase and β-glucuronidase enzymes (Karim *et al.*, 2014). Another research claimed that grapefruit (*C. maxima* L.) can be used as skin moisturizer and is made in cream form. Additionally, vitamin A and C in grapefruit are also able to fight free radical well (Xu *et al.*, 2008).

METHODOLOGY

Materials

The sample of grapefruit (*C. Maxima* L.) was obtained from Bantul Residence, Yogyakarta, Indonesia. The ripped strawberry sample was obtained from strawberry farm in Bandung, West Java, Indonesia.

Chemicals used in this research include: Ethanol (JT-Baker, USA), ethyl acetate (JT-Baker, USA), dimethyl sulfoxide (DMSO), 10% SDS, HCl, 28 N-succinyl- (ala) 3-p-nitroanilide (Sigma, USA) and buffers Tris-Cl (Sigma, USA), tyrosinase (mushroom) enzyme (sigma, USA), enzyme Neutrophil Human Elastase (Enzo Life Science, USA), elastatinal inhibitor (Enzo Life Science, USA), MeOSuc-AAPV-pNa substrate, enzyme Matrix Metalloproteinase-1 (MMP-1, Enzo Life Science, USA)), MMP-substrate (BML-P125-9090) (Enzo Life Science, USA), NNGH inhibitors (Enzo Life Science, USA), kojic acid, ascorbic acid (Merck, Germany), L-Dopa, iron (II) sulphate (FeSO4. 7H2O) (sigma, USA), 2,4,6-tripiridyl-s-triazine (TPTZ) (sigma, USA), FeCl3 (sigma, USA), linoleic acid, Tween 20 (sigma, USA), β-carotene (sigma, USA), butyl hydroxy toluene (BHT) (sigma, USA), sodium acetate.

Instrumentation

Glassware, laminar air flow cabinet (Labconco, Kansas), centrifuge (Sorvall, USA), UV-Vis spectrophotometer (Shimadzu, Japan), ELISA Reader (Bio-rad Benchmark, Japan and Corona electric type SH-1000), rotary evaporator (IKA, Germany), micro pipettes (Socorex, Switzerland), analytical balance (Sartorius, Germany), vortex (Shimadzu, Japan), Ovens (Memmert, Germany), incubator (Heraeus, Germany), 96 plate well (pyrex).

Table I. Persen yield of SE, SEA, GPE and GPEA extracts

No	Extract	Yield (%)
1	Ethanolic Extract of Strawberry (SE)	1.58
2	Ethyl Acetate Extract of Strawberry (SEA)	0.31
3	Ethanolic Extract of Grapefruit Peel (GPE)	4.60
4	Ethyl Acetate Extract of Grapefruit Peel (GPEA)	3.51

Table II. Phenolic and Flavonoid content in Extract

Sample	Phenolic Content (% equivalent to Gallic acid)	Flavonoid Content (% equivalent to Quercetin)
Ethanolic Extract of Strawberry (SE)	3.5±0.04	2.58±0.01
Ethyl Acetate Extract of Strawberry (SEA)	2.12±0.04	1.38±0.04
Ethanolic Extract of Grapefruit Peel (GPE)	4.22+0.06	11.25+0.06
Ethyl Acetate Extract of Grapefruit Peel (GPEA)	2.38+0.07	5.95+0.02

Sample Preparation and extraction

The first step was to clean 5 kilograms of fresh strawberries by washing it in flowing water, and then blended them using blender. The blended sample was then extracted using 96% of ethanol and ethyl acetate by maceration method. Every 100 grams of the sample was extracted by 300 mL of the ethanol and then stand it be for 1x24 hours. Next, the sample was then filtered and the residue was macerated again using 96% of ethanol. The following step was to macerate the residue again by ethyl acetate using the same method. Each extracted result from the maceration was then evaporated using rotary evaporator until it got the strawberry extract of ethanolic and ethyl acetate (SE and SEA) which was ready to test the activity.

For the grapefruit, the first step was to wash the 5 kg of grapefruit by washing it in flowing water. After that, the sample was peeled and peels were dried using an oven at 50^{o} C until it became dried. The dried sample was then weighed and macerated for 1x24 hours using 96% of ethanol solvent as much as 5L for each 1kg of sample. The maceration and residue were separated using filter, then the residue was macerated again by 96% ethanol with the same method in three times repetition. Then the residue was macerated again by ethyl acetate solvent for 1x24 hours and then was filtered. Residue was macerated again by ethyl acetate solvent with the same method in three times repetition. The ethanol and ethyl acetate maceration were evaporated using a rotary evaporator until it got concentrated extract of grapefruit peel in ethanol and ethyl acetate (GPE and GPEA, respectively) which was ready to be tested. The extract yield was then measured using the below formula:

$$\text{Yield \%} = \frac{\textit{the weight of extract obtained}}{\textit{the weight of sample}} \text{ X } 100\%$$

Determination of phenolic and flavonoid content

Determination of flavonoid content was conducted by the aluminium chloride colorimetric method (Chang *et al.*, 2002; Ahmad *et al.*, 2015; Baba and Malik, 2015; Klungsupya *et al.*, 2015). Briefly, 50 µL of extract was made up to 1 mL with methanol, then mixed with 4 mL of distilled water and incubated for 5 min. After that followed by adding 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with distilled water. The mixture was allowed again to stand for 15 min, and measured absorbance at 510 nm. The total flavonoid content was calculated from a calibration curve, and the data was expressed as mg quercetin equivalent per 100 g dry weight.

Determination phenolic content was performed according to Folin – Ciocalteu's method. Briefly, put 0.05 mL of extract and 0.45 mL distilled water into flask, and then mixed with 2.5 mL of 1:10 2 N Folin – Ciocalteu's phenolic reagent (Sigma Aldrich). Thereafter 2 mL of 7.5% (w/v) sodium carbonate was added and heated at 50 °C for 5 minutes. After that, absorbance was read at 760 nm with a UV/vis spectrophotometer (Varion Cary IE) in room temperature. Total phenolic content was estimated according to a Gallic acid

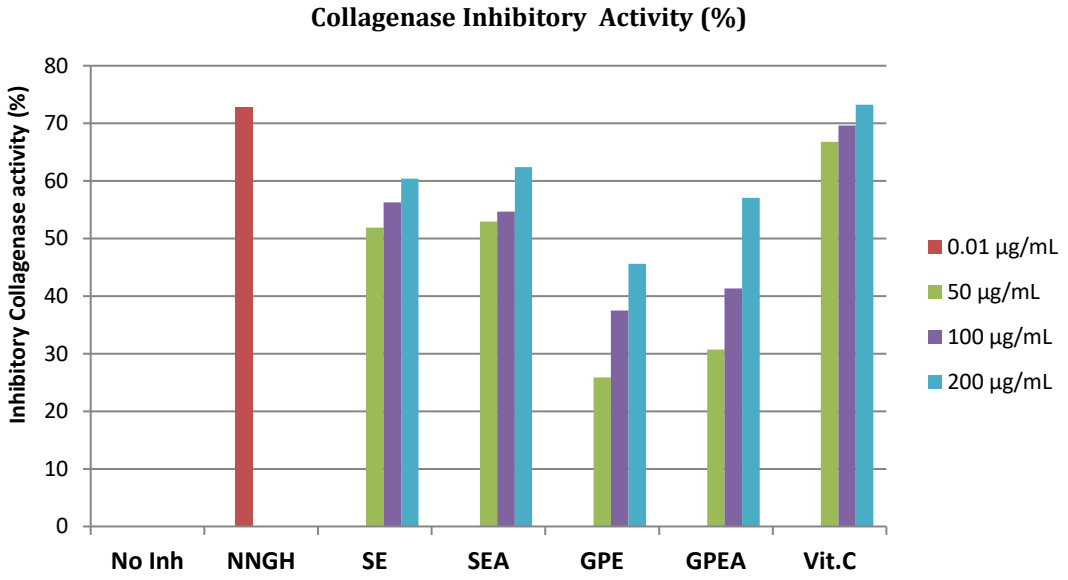

Figure 2. Collagenase inhibitory activity of extracts, vitamin C (as positive standard) and NNGH at three different concentrations (n=3)

Table III. Antioxidant of samples determined by using FRAP and BCB method

Sample	IC_{50} (µg/mL) (FRAP)	IC_{50} (µg/mL) (BCB)
Ethanolic Extract of Strawberry (SE)	404.39 ± 3.27	292.30 ± 4.69
Ethyl Acetate Extract of Strawberry (SEA)	1978.65 ± 3.27	671.11 ± 6.74
Ethanolic Extract of Grapefruit Peel (GPE)	219.47 ± 71.96	245.19 ± 162.47
Ethyl Acetate Extract of Grapefruit Peel (GPEA)	309.44 ± 95.76	567.54 ± 95.31
Quercetin	18.97 ± 4.50	135.56 ± 6.80
Vitamin C	24.47 ± 1.44	
Butylated hydroxy Toluene (BHT)	-	38.68 ± 5.70

Table IV. The determined result of anti-tyrosinase activity from the sample

Sample	IC_{50} Anti tyrosinase (µg/mL)
Ethanolic Extract of Strawberry (SE)	492.68 ± 1.43
Ethyl Acetate Extract of Strawberry (SEA)	2658.00 ± 48.08
Ethanolic Extract of Grapefruit Peel (GPE)	3312.5 ± 222.74
Ethyl Acetate Extract of Grapefruit Peel (GPEA)	2985 ± 122.80
Kojic acid	111.52 ± 0.42

standard curve (20-100 mg/mL), results were expressed as milligrams of Gallic acid equivalent (GAE) per 100 g of dry weight (Chang *et al.*, 2002; Ahmad *et al.*, 2015; Klungsupya *et al.*, 2015).

Antioxidant activity test using FRAP

The Ferric Reducing Antioxidant Power (FRAP) test was conducted according to method (Apak *et al.*, 2007) with a slight modification. Firstly, prepare ferric chloride solution (3 mM in 5 mM citric acid) and TPTZ solution (2,4,6-tripyridyl-s-triazine; 1 mM in 0.05 M HCl). The comparison of quercetin and vitamin C were made with several series of concentrations which are 7.81 µg/ml; 15.63 µg/ml; 31.25 µg/ml; 62.50 µg/ml and 125 µg/ml. Then, the samples (SE, SEA, GPE and GPEA) were prepared into each concentration of 5 µg/ml, 25 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml. $FeSO_4$ was used to obtain the standard curve and it was made by the concentration of 2.5 µg/ml; 5 µg/ml; 10 µg/ml; 20 µg/ml and 40 µg/ml. Using pipette,

it was obtained 30 µl of each sample and standard, after that it was put into 96 well plate and then added with 140 µl TPTZ solution. Each of the samples was added with 30 µl $FeCl_3$ and measured the absorbance at a wavelength of 620 nm. The sample absorbance data was calculated against the $FeSO_4$ solvent series and its antioxidant capacity was determined as equivalent to µM Fe^{3+} which was reduced to µM Fe^{2+}.

Antioxidants Activity Test Using BCB

The β-carotene bleaching (BCB) test was conducted in accordance to method (Apak *et al.*, 2007) with a slight modification. Beta-carotene solution was made by dissolving 2.0 mg of beta-carotene powder in 0.2 ml of chloroform, 0.2 ml of linoleic acid, 2 ml of tween 20 and distilled water to a volume of 100.0 ml, and then it was shaken until it became a clear solution. Butyl hydroxyl toluene (BHT) and quercetin were used as a standard and then made as concentration series. For quercetin, the concentration series were 7.81 µg/ml; 15.63 µg/ml; 31.25 µg/ml; 62.50 µg/ml; 125 µg/ml and 250 µg/ml. For the standard of BHT, the used concentration series were 3.9 µg/ml; 7.8 µg/ml; 15.6 µg/ml; 31.3 µg/ml and 62.5 µg/ml. Furthermore, the samples were made in the concentration series of 50 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml and 1000 µg/ml. From each sample and standard 20 µl were piped into 96 plate wells and then it was added with 180 µl BCB solution. It was then incubated for 30 minutes at 50°C and the absorbance was observed at a wavelength of 450 nm in the 0th minute until the 120th minute with a 30-minute interval. The antioxidant activity was calculated based on the activity of different degradation of the test sample with degradation to the control (only beta-carotene). The rate of degradation of the sample was calculated with the formula:

The rate of the sample degradation = ln (a/b) x 1/t

Which: ln = natural log; a = Absorbance at 0 minute; b = Absorbance at 120-minute; t = time (minute).

Antioxidant activity (AA), described as inhibition relative to the control, is formulated as follow:

$$AA\% = \frac{degradation\ of\ control - degradation\ of\ sample}{degradation\ of\ control} \times 100\%$$

Anti-tyrosinase Activity Test

The test of inhibition activity of tyrosinase enzyme was conducted by the spectrophotometry method (Chang *et al.*, 2013; Lukitaningsih and Holzgrabe, 2014) using L-DOPA as substrate and kojic acid for the positive control. From each sample, the series of concentration was made from 50-1000 µg/mL, and 30 µL of each of the sample was obtained and added with 125 µL of phosphate buffer (0.1 M, pH 6.8) and 5 µL of tyrosinase enzyme (2500 units/mL). It was incubated for 30 minutes at 37ºC and added with 40 µL of L-DOPA (2.5 mM). The absorbance was observed at the wavelength of 515 nm. The measurement was also conducted for the blank solvent and positive control of kojic acid by using the same equation. The percentage of the inhibition was formulated using the formula:

$$\%\ \text{inhibition} = \frac{Absorbance\ of\ negative\ control - Absorbance\ of\ sample}{Absorbance\ of\ negative\ control}\ x\ 100\%$$

By the linier regression equation of y = a + bx, by which the x is the sample concentration and the y is the % of inhibition, the value of IC_{50} can be measured by as follow:

$$IC_{50} = \frac{50 - a}{b}$$

Anti-elastase Activity Test

The anti-elastase test was carried out based on the manual products of Drug Discovery kit Neutrophil Elastase Coulometric (Ya Luo *et al.,* 2011). The first was to prepare buffer solution containing 10 mM HEPES, 50 mM NaCl and 0,05% Tween 20 in DMSO. The sample solution (50-200 µg/mL) was piped 20 µL using pipette and added with 65 µL buffer solvent into 96 well plate. The elastatinal (100µM) was used as an inhibitor control, the 95 µL buffer solution as the blank and 85 µL buffer solvent as a negative control. The 10 µL of Neutrophil elastase enzyme (2,2 µU/µL) was added into the sample, negative control and inhibitor control (the enzyme was not added into the blank) solution. The mixture was incubated for 30 minutes at 37° C, it was then added with 5 µL of substrate (MeOSuc-Ala-Ala-Pro-Val-pNA, 100 µM) into each well and the absorbance was measured at the wavelength of 405nm which was observed for 10 minutes at every 1-minute interval. The obtained absorbance data (OD) was then plotted into graphic of observation time (minutes) against the absorbance (OD/minute) to acquire the linier regression equation. The percentage of the enzyme activity inhibition was obtained from inhibitor (sample) slope (v) against the control slope (v) by the formula:

$$\%\ \text{inhibition of enzyme activity} = 100\% - \left(\frac{V\ inhibitor}{V\ control}\ x\ 100\%\right)$$

Anti-collagenase Test

The anti-collagenase test was conducted according to manual product method from MMP-1 Colorimetric Drug Discovery Kit. The first ingredient was buffer solution containing 50 mM HEPES, 10 mM CaCl2, 0,05% Brij-35 and 1 mM DTNB in DMSO. The 20 µL of sample solution (5-1000 µg/mL) was piped and added with 50 µL of buffer solution into 96 well plate. NNGH inhibitor control (N-Isobutyl-N-(4-methoxyphenylsulfonyl) glycylhydroxamic acid; 1,3 µM) was used as comparator, 90 µL of buffer solvent as the blank and 70µL of buffer solution as negative control. Then 20 µL MMP-1 enzyme (153 mU/µL) was added into the well containing the sample, inhibitor and negative control (The enzyme was not added into the blank). The mixture was incubated for 30 minutes at 37^{o} C, then each well was added with 10 µL of substrate (thiopeptide, Ac- PLG- [2- mercapto- 4-methylpentanoyl]- LG-OC2H5; 100 µM) and the absorbance was measured at the wavelength of 410 nm. The observation was conducted for 10 minutes at every 1-minute interval. The obtained absorbance data (OD) was then plotted into graphic of observation time (minutes) against the absorbance (OD/minute) to acquire the linier regression equation. The percentage of the enzyme activity inhibition was obtained from inhibitor (sample) slope (v) against the control slope (v) by the formula:

% inhibition of enzyme activity = 100% - ($\frac{V\ inhibitor}{V\ control}$ x 100%)

CONCLUSION

The extract of strawberry flesh and grapefruit peel contains antioxidant activity and inhibition of tyrosinase, elastase and collagenase enzymes. The expression of enzyme inhibition could not be compared precisely against the positive control, considering that the expression could not be delivered in the units of mol/mL. The strawberry flesh extract has more antioxidant, anti-tyrosinase and anti-collagenase compared to the grapefruit peel extract. On the other hand, the extract of GPEA is potentially able to get developed as anti-aging cosmetics ingredients by elastase enzyme inhibition mechanism.

REFERENCES

Abirami, A., Nagarani, G. and Siddhuraju, P. (2014) 'potential of fresh juice from Citrus hystrix and C . maxima fruits', *Food Science and Human Wellness*. Beijing Academy of Food Sciences., 3(1), pp. 16–25.

Ahmad, A. *et al.,* (2015) 'Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of Piper nigrum Linn fruits', *Asian Pac. J. Trop. Biomed*. Hainan Medical University, 5(2), pp. 101–107.

Ben Ahmed, Z. *et al.,* (2016) 'Determination of optimal extraction conditions for phenolic compounds from: Pistacia atlantica leaves using the response surface methodology', *Analytical Methods*, 8(31), pp. 6107–6114.

Allen, D. and Tracy, P. (1995) 'No Title', *Journal oh Biological Chemistry*, 270(3), pp. 1408–1415.

Apak, R. *et al.,* (2007) 'Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay', *Molecules*, pp. 1496–1547.

Apraj, V. D. and Pandita, N. S. (2016) 'Evaluation of skin anti-aging potential of Citrus reticulata blanco peel', *Pharmacognosy Research*, 8(3), pp. 160–168.

Aumeeruddy-Elalfi, Z., Gurib-Fakim, A. and Mahomoodally, M. F. (2016) 'Kinetic studies of tyrosinase inhibitory activity of 19 essential oils extracted from endemic and exotic medicinal plants', *South African Journal of Botany*, 103, pp. 89–94.

Azzini, E. *et al.,* (2010) 'Bioavailability of strawberry antioxidants in human subjects', *British Journal of Nutrition*, 104(8), pp. 1165–1173.

Baba, S. A. and Malik, S. A. (2015) 'Determination of total phenolic and flavonoid content , antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume', *Integrative Medicine Research*. Taibah University, 9(4), pp. 449–454.

Badriyah and Hastuti, U. S. (2017) 'The effect of pomelo citrus (Citrus maxima var. Nambangan), Vitamin C and lycopene towards the number reduction of mice (Mus musculus) apoptotic hepatocyte caused of ochratoxin A', *AIP Conference Proceedings*, 1854(2017).

Bae, J. Y. *et al.,* (2009) 'Bog blueberry anthocyanins alleviate photoaging in ultraviolet-B irradiation-induced human dermal fibroblasts', *Molecular Nutrition and Food Research*, 53(6), pp. 726–738.

Chang, C. *et al.,* (2002) 'Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods', 10(3), pp. 178–182.

Chang, C. T. *et al.,* (2013) 'Chemical composition and tyrosinase inhibitory activity of

Cinnamomum cassia essential oil', *Botanical Studies*, 54(1), pp. 2–8.

Cuvelier, M. E., Richard, H. and Berset, C. (1992) 'Comparison of the Antioxidative Activity of Some Acid-phenols: Structure-Activity Relationship', *Bioscience, Biotechnology and Biochemistry*, 56(2), pp. 324–325.

Giampieri, F. *et al.,* (2014) 'Polyphenol-rich strawberry extract protects human dermal fibroblasts against hydrogen peroxide oxidative damage and improves mitochondrial functionality', *Molecules*, 19(6), pp. 7798–7816.

Inomata, S. *et al.,* (2003) 'Possible involvement of gelatinases in basement membrane damage and wrinkle formation in chronically ultraviolet B-exposed hairless mouse', *Journal of Investigative Dermatology*. Elsevier Masson SAS, 120(1), pp. 128–134.

Karim, A. A. *et al.,* (2014) 'Phenolic composition, antioxidant, anti-wrinkles and Tyrosinase Inhibitory Activities of Cocoa Pod Extract', *BMC Complementary and Alternative Medicine*, 14(381), pp. 1–13.

Khrisnamurthy, P. and Wadhwani, A. (2012) 'Antioxidant Enzymes and Human Health', in *Antioxidant Enzymes and Human Health*, pp. 1–18.

Kim, J. H. *et al.,* (2009) 'Compounds with elastase inhibition and free radical scavenging activities from Callistemon lanceolatus', *Journal of Medicinal Plants Research*, 3(11), pp. 914–920.

Klungsupya, P. *et al.,* (2015) 'Determination of free radical scavenging, antioxidative DNA damage activities and phytochemical components of active fractions from lansium domesticum corr. Fruit', *Nutrients*, 7(8), pp. 6852–6873.

Lephart, E. D. (2018) 'Equol's anti-aging effects protect against environmental assaults by increasing skin antioxidant defense and ECM proteins while decreasing oxidative stress and inflammation', *Cosmetics*, 5(1), pp. 1–17.

Lukitaningsih, E. (2014) 'Bioactive Compounds in Bengkoang (Pachyrhizus Erosus) as Antioxidant and Tyrosinase Inhibiting Agents', *Indonesian Journal of Pharmacy*, 25(2), p. 68.

Menaa, F., Menaa, A. and Tréton, J. (2013) 'Polyphenols against Skin Aging', in *Polyphenols in Human Health and Disease*, pp. 819–830. doi: 10.1016/B978-0-12-398456-2.00063-3.

Nur, S., Rumiyati, R. and Lukitaningsih, E. (2017) 'Screening of Antioxidants, Anti-Aging and Tyrosinase Inhibitory Activities of Ethanolic and Ethyl Acetate Extracts of Fruit Flesh and Fruit Peel Langsat (Lansium domesticum Corr) In Vitro', *Majalah Obat Tradisional*, 22(1), p. 63.

Nurrochmad, A. *et al.,* (2018) 'Effects of antioxidant, anti-collagenase, anti-elastase, anti-tyrosinase of the extract and fraction from Turbinaria decurrens Bory', *Indonesian Journal of Pharmacy*, 29(4), pp. 188–199.

Ochiai, A. *et al.,* (2016). 'Rice bran protein as a potent source of antimelanogenic peptides with tyrosinase inhibitory activity', *Journal of Natural Products*, 79(10), pp. 2545–2551.

Poljšak, B., Dahmane, R. G. and Godić, A. (2012) 'Intrinsic skin aging: The role of oxidative stress', *Acta Dermatovenerologica Alpina, Pannonica et Adriatica*, pp. 33–36.

Pullar, J. M., Carr, A. C. and Vissers, M. C. M. (2017) 'The Roles of Vitamin C in Skin Health', (Figure 1).

Qonita, F. (2017). *Pemanfaatan Ekstrak Buah Strawberry (Fragaria X Ananassa (Duchesne Ex Weston)) Sebagai Bahan Kosmetik: Uji Aktivitas Antiaging dan Antibakteri Secara In Vitro*. Yogyakarta: Faculty of Pharmacy, Gadjah Mada University.

Vijaylakshmi, P. and Radha, R. (2015) 'An overview: Citrus maxima', *The Journal of Phytopharmacology*, 4(5), pp. 263–267. Available at: www.phytopharmajournal .com.

Wang, F. *et al.,* (2008) 'Effect of increased pigmentation on the antifibrotic response of human skin to UV-A1 phototherapy', *Archives of Dermatology*, 144(7), pp. 851–858.

Wong, S. K., Lim, Y. Y. and Chan, E. W. C. (2010) 'Evaluation of antioxidant, anti-tyrosinase and antibacterrial activities of selected Hibiscus species', *Ethobotanical Leaflets*, 14(June), pp. 781–796.

Xu, G. *et al.,* (2008) 'Juice components and antioxidant capacity of citrus varieties cultivated in China', *Food Chemistry*, 106(2), pp. 545–551.

Ya Luo, Hao-ru Tang *, Xiao-rong Wang, Yong Zhang, Z. L. (2011) 'Antioxidant properties and involved antioxidant compounds of strawberry fruit at different maturity stages', *Journal of Food, Agriculture and Environment*, 9(1), pp. 166–170.

Zulbayu, L. ode (no date) *Uji Aktivitas Antiaging dan Antibakteri Ekstrak Etanol dan Etil Asetat Kulit Buah Jeruk Bali (Citrus maxima L.) secara In Vitro*. Yogyakarta: Faculty of Pharmacy, Gadjah Mada University.