Elastase, Hyaluronidase and Tyrosinase inhibitor activities antiaging of Curcuma mangga Val. extract and its fractions

D Pujimulyani1*, L Suryani1, A Setyawati1, A Amalia2, R L Qodaniah2, H S W Kusuma2 and W Widowati3

1Faculty of Agroindustry, University of Mercu Buana Yogyakarta, Jl Wates Km. 10.5, Argomulyo, Sedayu, Bantul, Daerah Istimewa Yogyakarta 55753, Indonesia
2Aretta Medika Utama, Biomolecular and Biomedical Research Center, Jl Babakan Jeruk 2, No 9, Bandung 40163, West Java, Indonesia
3Medical Research Centre, Faculty of Medicine, Maranatha Christian University, Jl. Prof drg. Suria Sumantri No. 65, Bandung 40164, West Java, Indonesia

* Email: dwiyati2002@yahoo.com

Abstract. Curcuma mangga Val. has bioactive compounds such as curcumin. Curcumin is a natural antioxidant and known as antiaging agent. Curcumin can stimulate the synthesis of collagen type I, inhibit melanogenesis and help to maintain correct skin hydration. This show C.mangga Val. has potential as antioxidant and antiaging agents. This research aims to find out the antiaging activities of C. mangga ethanolic extracts (CMEE) and its fractions. In this study, the antiaging activity was performed by measuring inhibition of elastase, hyaluronidase, and tyrosinase inhibitory activity assay. Ascorbic acid using as a control. In elastase inhibitory activity, ascorbic acid has the highest activity (15.53 µg/mL) but ethyl acetate fraction of C. Mangga (EACM) also has high activity (26.34 µg/mL) compared to other fractions and CMEE. In hyaluronidase inhibitory activity, ascorbic acid has the highest activity (27.67 µg/mL) but CMEE also has high activity (80.04 µg/mL) compared to other fractions, while in tyrosinase inhibitory activity EACM has the highest value (40.34 µg/mL) compared to ascorbic acid (65.99 µg/mL). In summary, C. mangga extracts have anti-hyaluronidase, while EACM also has good antiaging properties through anti-elastase and antityrosinase activity.

1. Introduction
Aging of the skin is induced by both intrinsic, and extrinsic factors [1]. Reactive oxygen species (ROS) play an important role in skin aging. In the skin, about 1.5-5% of the consumed oxygen is converted into ROS by intrinsic processes [2]. Two other main events associated with intrinsic skin aging are a decrease in the replicative ability of cells and increased degradation of the extracellular matrix [3]. Aging has a quite different appearance depending if either dermis or epidermis is considered. In the dermis the disruption of the extracellular matrix plays the most obvious role which is true for intrinsic as well as extrinsic aging. The results are fine wrinkles due to the reduction of collagen, elastic fibers, and hyaluronic acid [3]. Elastase, collagenase, hyaluronidase, and tyrosinase, are very interesting enzymes due to their direct implications in skin aging and as therapeutic hits [4].

Curcuma mangga is commonly called “temu mangga” or “kunir putih” in Indonesia belongs to the Zingiberaceae family [5]. The genus Curcuma is widely distributed in tropical Asia and Australia [6].
**C. mangga** rhizomes are the one kind of herbal medicinal plants contain some bioactive compounds such as curcuminoid (curcumin, bisdemethoxycurcumin, bismethoxycurcumin) and phenolic [5][7]. The rhizome of **C. mangga** has been reported to possess some pharmacological activities such as antioxidant [5][7] and anticancer [8]. Thus, in this study we used **C. mangga** ethanol extracts and its fractions to evaluate the antiaging activities through inhibitory of hyaluronidase, elastase and tyrosinase activity.

2. **Material**
The main material in this research was white saffron (**Curcuma mangga** Val.), which had yellow color on its roots, flavor like mango, and it was obtained from Sedayu, Bantul, Yogyakarta. Chemicals used were methanol, ethyl acetate (E Merck), aquabidestilata (Ika Pharmindo) and distilled water.

The instruments used in this study were vacuum rotary evaporator or rotavapor (Heidolph VV 2000), sartorius scale, autoclave, UV-VIS spectrophotometer (Genesys-20), incubator, vacuum filter, centrifuge, 0.45-μm-milex filter, micro factor, homogenizer, blender and High Performance Liquid Chromatography (HPLC) Knauer with C18 column, Photodiode array detector (DAD)UV 6000LP, Smartline pump and ChromGate 3.1.6 software.

3. **Methods**
3.1. **Preparation of C. mangga Extract**
**C. mangga** plants were collected from the plantation located in Yogyakarta, Special Region of Yogyakarta, Indonesia. The extraction was performed by maceration method. C.mangga was dried, blanched, and milled then soaked in 70% distillated ethanol. The substance was filtered every 24 hours until colorless filtrate was gained. After that, the filtrate was evaporated using rotary evaporators (Stuart, RE300) to obtain ethanol extract, after it was done, the **C. mangga** extract (CME) was stored at -20 °C [9][10][11].

3.2. **Fractionation of C. mangga Ethanol Extract**
Fractionation of **C. mangga** ethanol extract was done using modified partition. **C. mangga** ethanol extract (25 g) was partitioned with n-hexane and water (1:1), obtained a hexane fraction of 3.77 g (18.85%), then the residue was partitioned with ethyl acetate and water (1:1), obtained an ethyl acetate fraction of 4.62 g (9.24%); the residue was then partitioned with butanol and water (1:1), obtained a butanol fraction of 2.40 g (4.8%); and the remaining residue was the water fraction of 11.93 g (23.86%) [12][13].

3.3. **Elastase inhibitory activity assay**
Elastase inhibitory activity was measured by a modified method of Sigma Aldrich and Thring et al. (2009) with some modifications. A mixture of 10 μL of various levels of samples (7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00 μg/mL and 0), 5 μL elastase enzyme from porcine pancreas (Sigma 45124, USA) (0.5 mU/mL in the cold distilled water) and 125 μL Tris buffer was pre-incubated for 15 min at room temperature. Mix solution was added N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide (SucAla3-pNA) substrate [Sigma 54760, USA] and then incubated for 15 min at room temperature. Absorbance was measured immediately after incubation time with 410 nm wavelength [9][14][10].

% Elastase inhibition = (1-B/A) x 100%
A = Sample absorbance
B = Control absorbance

3.4. **Hyaluronidase Inhibitory Activity**
Hyaluronidase inhibitory of activity was measured by the modified method of Sigma Aldrich and Tu and Tawata (2015). A mix of 25 μL of various level of samples (7.81, 15.63, 31.25, 62.50, 125.00,
250.00, 500.00 μg/mL and 0) and 3 µL hyaluronidase from bovine testes type I-S [Sigma H3506, USA] was pre-incubated for 10 min at 37 °C and then added 12 µL phosphate buffer (300mM, pH 5.35) for 10 min at 37 °C. Afterward 3 µL hyaluronic acid substrate [Sigma H5542, USA] was added and incubated for 45 min at 37°C. The decomposition reaction of hyaluronic acid was stopped by adding 100 µL acidic albumin solution in each well. Mixed solution incubated at room temperature for 10 min, then absorbance was measured at 600 nm wavelength [15] [10] [9].

Quantification of inhibition activity by the formula:

\[
\% \text{Hyaluronidase inhibition} = \left(1 - \frac{B}{A}\right) \times 100\% 
\]

where A is sample absorbance, and B is controlled absorbance.

3.5. Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity assay was measured by the modification method by [16] and [15] with some modifications. Sample was prepared in 96 well-plate and added potassium phosphate buffer (140 µL, 20 mM, pH 6.8), 20 µL sample with various concentrations (4.69, 9.38, 18.75, 37.50, 75.00, 150.00, 300.00 µg/mL), and 20 µL tyrosinase enzyme from fungi (125 U/ml in potassium phosphate buffer). The mixture was incubated at room temperature for 15 min. After incubation the mixture was added 20 µL L-DOPA (1.5 mM), and incubated at room temperature for 10 min. Tyrosinase inhibitory activity was measured by quantifying level of DOPA that was formed at 470 nm wavelength.

Tyrosinase Inhibitory Activity (%): \((A - B)/A \times 100\)

For A is control absorbance and B is sample absorbance.

3.6. Statistical analysis

In this study, the statistical analysis was performed using SPPS software (version 17.0). The data were analyzed by analysis of variance (ANOVA) continued with Tukey HSD post hoc test, \(p<0.05\) was considered as statistically significant. Values are expressed as means ± standard deviation.

4. Result

In this study, the antioxidant and antiaging properties of CMEE and those fractions (from 4 solvents) were assessed as antiaging activities due to elastase, hyaluronidase, and tyrosinase inhibitory activity.

4.1. Elastase Inhibitory Activity

Elastase inhibitory activity is one of the measurement methods in antiaging activity. The result of CMEE and fractions in elastase inhibitory activity was performed in Figure 1 and Table 1.

![Figure 1. Elastase inhibitory activity of C. mangga extracts, its fractions and ascorbic acid.](attachment:image.png)
Figure 1 show that EACM has the highest in elastase inhibitory activity (117.80%) compared to other fraction and CME, while the lowest activity is BCM (55.37%) (p<0.05). This indicated EACM has potential as antiaging agent due to elastase inhibitory activity compared to ascorbic acid as marker compound.

Table 1. IC$_{50}$ value of elastase inhibitory activities by *C. mangga* extracts, its fractions, and ascorbic acid

| Samples    | Equation               | R$^2$ | Average IC$_{50}$ (µg/mL) |
|------------|------------------------|-------|---------------------------|
| Ascorbic Acid | y = 0.0789x + 48.775 | 0.93  | 15.53                     |
| CME       | y = 0.1708x + 23.792  | 0.91  | 153.44                    |
| WCM       | y = 0.0729x + 23.163  | 0.97  | 368.13                    |
| EACM      | y = 0.1297x + 46.584  | 0.91  | 26.34                     |
| BCM       | y = 0.1022x - 4.4088  | 0.91  | 532.38                    |
| HCM       | y = -0.131x + 35.097  | 0.93  | 113.76                    |

*CME= C. mangga ethanol extracts, WCM= Fraction of C. mangga water, EACM= fraction of C. mangga ethyl acetate, BCM= fraction of C. mangga butanol, HCM= fraction of C. mangga hexane; IC50= The half-maximal inhibitory concentration. IC50 of the samples was calculated.*

Ascorbic acid has the highest activity (15.53 µg/mL) but ethyl acetate fraction of *C. mangga* (EACM) also has high activity (26.34 µg/mL) compared to other fractions and CMEE in elastase inhibitory activity.

EACM has the highest elastase inhibitory activity in the present study but not higher than ascorbic acid (26.34 and 15.53 µg/mL, respectively). *C. aromatica* rhizomes exhibited marked elastase (IC50= 252.7 µg/mL) and hyaluronidase inhibitory activities (95.00% inhibition at 500 µg/mL). Another study with *C. longa* was evaluated by to suppress fMLP/CB-induced superoxide and radical anion (O$_2^-$) and elastase release. Among curcuminoid compounds of the *C. longa* which inhibit O-2 generation and elastase release, curcumin exhibited more effective inhibition [17]. The inhibition of elastase by phenolic compound is known to protect proteins of the extracellular matrix, improve the capillary walls, and activate the reconstruction [18].

4.2. Hyaluronidase Inhibitory Activity

The percentage of hyaluronidase inhibitory activity of *C. mangga* extracts, its fractions, and ascorbic acid can be seen in Figure 2 and the median inhibitory concentration (IC50) of samples towards hyaluronidase inhibitory activity can be seen in Table 2.

![Figure 2. Hyaluronidase inhibitory activity of *C. mangga* extracts, its fractions and ascorbic acid.](image-url)
Based on Figure 2, hyaluronidase inhibitory activity of CMEE has the highest percentage (103.04%) compared to ascorbic acid (98.18%), while the lowest percentage is BCM (50.89%). It is notable that CMEE exhibited good in hyaluronidase inhibitory activity than those fractions and also ascorbic acid (p<0.05).

Table 2. IC50 value of hyaluronidase inhibitory activities by C. mangga extracts, its fractions, and ascorbic acid

| Samples       | Equation            | R²  | Average IC50 (µg/mL) |
|---------------|---------------------|-----|----------------------|
| Ascorbic Acid | y = 0.1151x + 46.815| 0.91| 27.67                |
| CMEE          | y = 0.1389x + 38.883| 0.94| 80.04                |
| WCM           | y = 0.1335x + 17.504| 0.95| 243.42               |
| EACM          | y = 0.101x + 39.102  | 0.93| 107.90               |
| BCM           | y = 0.122x – 7.535   | 0.91| 484.99               |
| HCM           | y = 0.0812x + 34.468 | 0.96| 191.28               |

*CMEE= C. mangga ethanol extracts, WCM= Fraction of C. mangga water, EACM= fraction of C. mangga ethyl acetate, BCM= fraction of C. mangga butanol, HCM= fraction of C. mangga hexane; IC50= The half-maximal inhibitory concentration. IC50 of the samples was calculated.

As shown in Table 2, CMEE has the lowest IC50 value is 80.04 µg/mL compared to other fractions, but not lower than ascorbic acid (27.67µg/mL). This indicated EACM has antiaging activity but not higher than ascorbic acid.

Thus, hyaluronidase inhibitors are useful cosmeceutical ingredients as they have anti-wrinkle and anti-aging effects on the skin [20]. The present study shows that CMEE has the highest inhibitory of hyaluronidase (80.04 µg/mL) compared all C. mangga fractions. C. mangga rhizome known has antiaging activity, based on other studies, A. zerumbet (Zingiberaceae) had stronger inhibitory activity than the other parts on elastase, hyaluronidase and tyrosinase inhibitions, rhizome [19]. The Phytomolecules in C. mangga is curcumin have shown to protect skin from wrinkles, leading to glowing, and healthy younger skin [20]. Thus, these result obtained shows that the CMEE possess promising potential for application in the antiaging products processing industries.

4.3. Tyrosinase Inhibitory Activity

The inhibition of tyrosinase was determined by a modification of the dopachrome method using L-DOPA and L-tyrosine as substrate [21]. The percentage of tyrosinase inhibitory activity of CMEE, its fractions, and ascorbic acid can be seen in Figure 3 and the median inhibitory concentration (IC50) of samples towards hyaluronidase inhibitory activity can be seen in Table 3.

Figure 3. Tyrosinase inhibitory activity of C. mangga extracts, its fractions and ascorbic acid.
Based on Figure 3, tyrosinase inhibitory activity of CMEE has the highest percentage (93.82%) compared to ascorbic acid (87.83%), while the lowest percentage is BCM (58.90%). It is notable that CMEE exhibited good in tyrosinase inhibitory activity than those fractions and also ascorbic acid.

Table 3. IC50 value of tyrosinase inhibitory activities by C. mangga extracts, its fractions, and ascorbic acid.

| Samples   | Equation          | R²  | Average IC50 (µg/mL) |
|-----------|-------------------|-----|----------------------|
| Ascorbic Acid | y = 0.1792x + 38.174 | 0.93 | 65.99                |
| CMEE      | y = 0.2148x + 33.685 | 0.94 | 75.95                |
| WCM       | y = 0.1599x + 19.623 | 0.93 | 189.97               |
| EACM      | y = 0.146x + 44.111  | 0.94 | 40.34                |
| BCM       | y = 0.1465x + 17.455 | 0.95 | 222.15               |
| HCM       | y = 0.1448x + 42.674 | 0.95 | 50.59                |

*CMEE= C. mangga ethanol extracts, WCM= Fraction of C. mangga water, EACM= fraction of C. mangga ethyl acetate, BCM= fraction of C. mangga butanol, HCM= fraction of C. mangga hexane; IC50= The half-maximal inhibitory concentration. IC50 of the samples was calculated.

In tyrosinase inhibitory activity, EACM has the highest value (40.34 µg/mL) compared to ascorbic acid (65.99 µg/mL). This shows EACM has antiaging activity through tyrosinase inhibitory activity (Table 3).

In this study, the highest antityrosinase activities were exhibited by EACM which inhibited over 40.34 µg/mL compared to ascorbic acid (IC50= 65.99 µg/mL). Zingiberaceae plant such as ginger, thus leaves and rhizomes has the strongest tyrosinase inhibition activity [21] and also capable to scavengen several types of reactive oxygen species (ROS) [22]. In previous study, using turmeric oil which was fractionated to get three fractions were tested for antioxidant activity [23]. The fraction eluted with benzene showed maximum antioxidant capacity [23]. Based on [24] study, the essential oils of plants have small phenolic compounds could be promising as natural tyrosinase inhibitors [24]. Among the five fractions of C. mangga extract has inhibitory in elastase, hyaluronidase, and tyrosinase activity. And these results suggest that medicinal plants showing biological activities may be potent inhibitors of tyrosinase, elastase, and hyaluronidase, it could be useful for application in antiaging cosmetics.

5. Conclusion
In summary, C. Mangga ethanol extracts (CMEE) have anti-hyaluronidase, while EACM has good antiaging properties due to antielastase and antityrosinase activity. Thus present data suggest that C. mangga ethanolic extract and its fractions can be used as a good source of natural antioxidant and antiaging for health benefits. In future studies, the further isolation of bioactive compounds from C. mangga is required for identifying the unknown compounds to establish their pharmacological properties.

References
[1] Farage M A, Miller K W, Elsner P and Maibach H I 2008 Intrinsic and extrinsic factors in skin ageing: a review Int. J. Cosmet. Sci. 30 87–95
[2] Poljšak B, Dahmane R G and Godić A 2012 Intrinsic skin aging: the role of oxidative stress. Acta dermato-venereologica Alpina, Pannonica, Adriat. 21 33–6
[3] Rinnerthaler M, Bischof J, Streubel M, Trost A and Richter K 2015 Oxidative Stress in Aging Human Skin Biomolecules 5 545–89
[4] Fayad S, Morin P and Nehmé R 2017 Use of chromatographic and electrophoretic tools for assaying elastase, collagenase, hyaluronidase, and tyrosinase activity J. Chromatogr. A 1529 1–28
[5] Indis N A and Kurniawan F 2016 Determination of free radical scavenging activity from aqueous extract of Curcuma mangga by DPPH method J. Phys. Conf. Ser. 710
[6] Baharudin M K A, Hamid S A and Susanti D 2015 Chemical composition and antibacterial activity of essential oils from three aromatic plants of the zingiberaceae family in Malaysia J. Phys. Sci. 26 71–81

[7] Pujimulyani D, Raharjo S, Marsono Y, Santoso U and Materials A 2013 The Phenolic Substances and Antioxidant Activity of White Saffron (Curcuma mangga Val.) as Affected by Blanching Methods Int. J. Biol. Vet. Agric. Food Eng. 7 10–3

[8] Hong G W, Hong S L, Lee G S, Yaacob H and Malek S N A 2016 Non-aqueous extracts of Curcuma mangga rhizomes induced cell death in human colorectal adenocarcinoma cell line (HT29) via induction of apoptosis and cell cycle arrest at G0/G1 phase Asian Pac. J. Trop. Med. 9 8–18

[9] Widowati W, Fauziah N, Herdiman H, Afni M, Afifah E, Kusuma H S W, Nufus H, Arumwardana S and Rihibiha D D 2016 Antioxidant and anti-aging assays of Oryza sativa extracts, Vanillin and Coumaric Acid J. Nat. Remedies 16 88

[10] Utami S, Sachrowardi Q R, Damayanti N A, Wardhana A, Syarif I, Nafik S, Arrahman B C, Kusuma H S W and Widowati W 2018 Antioxidants, anticollegenase and antielastase potentials of ethanolic extract of ripe sesoot (Garcinia picrorrhiza Miq.) fruit as anti-aging J. HerbMed Pharmacol. 7 88–93

[11] Widowati W, Jasaputra D K, Sumitro S B, Widodo M A, Afifah E, Rizal R, Rhihiha D D, Kusuma H S W and Widowati W 2011 The Comparison of Antioxidative and Proliferation Inhibitor Properties of Piper betle L., Catharanthus roseus [L.] G. Don, Dendroptoe petandra L., Curcuma mangga Val. Extracts on T47D Cancer Cell Line 1 22–8

[12] Pujimulyani D, Yulianto W A, Setyowati A, Arumwardana S and Rizal R 2018 Antidiabetic and antioxidant potential of Curcuma mangga Val extract and fractions Asian J. Agric. Biol. 6 162–8

[13] Thring T S A, Hili P and Naughton D P 2009 Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants BMC Complement. Altern. Med. 9 1–11

[14] Tu P T B and Tawata S 2015 Anti-oxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of Alpinia zerumbet Molecules 20 16723–40

[15] Fais A, Corda M, Era B, Fadda M B, Matos M J, Quezada E, Santana L, Picciau C, Podda G and Delogu G 2009 Tyrosinase inhibitor activity of coumarin-resveratrol hybrids Molecules 14 2514–20

[16] Chen J-J, Tsai C-S, Hwang T-L, Shieh P-C, Chen J-F and Sung P-J 2010 Sesquiterpenes from the rhizome of Curcuma longa with inhibitory activity on superoxide generation and elastase release by neutrophils Food Chem. 119 974–80

[17] Ivana B, Viktor L, Milanka L, Jelena M and Dusan S 2013 Skin Ageing: Natural Weapons and Strategies Evidence-Based Complement. Altern. Med. 2013

[18] Chompoo J, Upadhyay A, Fukuta M and Tawata S 2012 Effect of Alpinia zerumbet components on antioxidant and skin diseases-related enzymes BMC Complement. Altern. Med. 12 1058

[19] Mukherjee P K, Maity N, Nema N K and Sarkar B K 2011 Bioactive compounds from natural resources against skin aging Phytotherapy 19 64–73

[20] Chan E W C, Lim Y Y, Wong L F, Lianto F S, Wong S K, Lim K K, Joe C E and Lim T Y 2008 Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species Food Chem. 109 477–83

[21] Somman A and Siwarungson N 2015 Comparison of antioxidant activity and tyrosinase inhibition in fresh and processed white radish, garlic and ginger J. Food Meas. Charact. 9 369–74

[22] Jayaprakasha G K, Jagan Mohan Rao L and Sakariah K K 2005 Chemistry and biological activities of C. longa Trends Food Sci. Technol. 16 533–48
[24] da Silva A P, Silva N de F, Andrade E H A, Gratieri T, Setzer W N, Maia J G S and da Silva J K R 2017 Tyrosinase inhibitory activity, molecular docking studies and antioxidant potential of chemotypes of Lippia origanoides (Verbenaceae) essential oils ed J Chamani PLoS One 12 e0175598

Acknowledgment
We gratefully acknowledge the financial support of Hibah Fundamental 2017 from Directorate General of Higher Education, Ministry of Research, Technology and Higher Education of the Republic of Indonesia. This study was supported by Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia for the research grant, laboratory facilities and research methodology. We are thankful to Rizal Rizal, Ni Luh Wisma EY, Yukko Arinta, Fajar Sukmaperdana, and Annissa Arlisyah from Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia for their valuable assistance.