ORIGINAL ARTICLE

In vitro studies on the cytotoxicity, elastase, and tyrosinase inhibitory activities of tomato (*Solanum lycopersicum* Mill.) extract

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ABSTRACT

Tomatoes (*Solanum lycopersicum* Mill.), a common vegetable in Indonesia, contain high levels of lycopene, which is good for the body. This research further investigates the activity of polar and nonpolar fractions of tomatoes as elastase and tyrosinase inhibitory, and cytotoxic agents. The extraction procedure used is maceration, fractionation through liquid-liquid fractionation, purification of phytochemical substances is achieved through the application of thin layer chromatography. Elastase and tyrosinase inhibitory activity was analyzed using spectrophotometry and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cytotoxic assay. The result showed that the extract yield was 0.004%. The percentage of polar fraction from the extract was 2.58%, while the nonpolar fraction was 0.69%. The elastase inhibitory activity of polar and nonpolar fractions of tomato extract is 87.21% ± 7.57% and 73.12% ± 7.44%, respectively, The elastase inhibitory activity of polar and nonpolar fractions of tomato extract is 87.21% ± 7.57% and 73.12% ± 7.44%, respectively. The fractions had higher the anti-elastase activity than the positive control quercetin (65.97% ± 3.00%). The tyrosinase inhibitory activity of polar and nonpolar fractions of tomato extract is 23.71% ± 7.91% and 41.16% ± 5.41% (kojic acid as standard is 65.07% ± 0.86%), respectively. The IC_{50} of the cytotoxic assay to NIH 3T3 mouse embryonic fibroblast cells of the polar and nonpolar fraction of tomato extract is 1820.90 μg/mL and 1643.86 μg/mL, respectively.

Key words: Cytotoxicity, elastase inhibitory activity, tomato (*Solanum lycopersicum* Mill.), tyrosinase inhibitory activity

INTRODUCTION

Tomato is a Solanaceae family from America, primarily North and South America Territories. The nutrition content of this fruit consists of Vitamin A, B, C, and E, phytosterol, folic acid, antioxidants, lycopene, alpha- and beta-carotene, potassium, carbohydrate, fat, protein, calcium, phosphor, and zinc.[1] Tomato fruit is the source of lycopene, which can trigger the occurrence of cancer cells. Besides containing lycopene, tomato fruit also contains pro-Vitamin A, Vitamin E, and other flavonoids.[2]

Lycopene is one of the plant pigments, including the hydrocarbon carotenoid.[3] One activity of lycopene is

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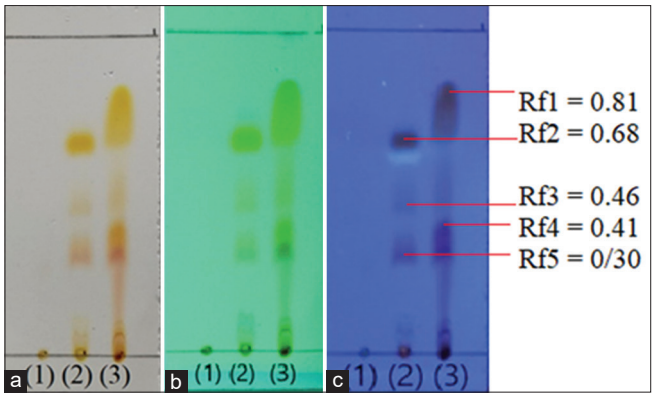

Figure 1: TLC separations of the tomato extract (1), nonpolar fraction (2), and polar fraction (3) developed in (a) UV lamp at 366 nm (long-wave UV), (b) visible light, (c) UV lamp at 254 nm (short-wave UV). TLC: Thin-layer chromatography, UV: Ultraviolet

to inhibit the work of the elastase enzyme on the breath of people with asthma.[4] It has been researched that extracts of apple tomato and ordinary tomato by ethanol solvent and water have a great potential for inhibiting photo-oxidation of linoleic acid caused by oxygen singlets.[5]

Elastase enzymes are proteins that regulate the solution of the extracellular matrix elastin.[6] Elastin is a protein that helps the skin stay chewy and tight. When the skin is drawn, elastin will return the skin to normal conditions. Wrinkles are a combination of intrinsic aging (natural aging) and extrinsic aging. Intrinsic skin aging or natural aging is caused by changes in skin elasticity from time to time, while extrinsic skin aging is more due to exposure to solar radiation. Free radicals are formed due to oxidative stress and are the main causes of intrinsic and extrinsic aging.[7]

Tyrosinase is a multifunction enzyme with a substrate of tyrosine that can make melanine. Excessive melanin can cause hyperpigmentation, and tyrosinase enzyme inhibitory activities can be used for skin lightening.[8]

Lycopene has also been investigated as being capable of reducing the risk of various cancer typologies.[9] Cancer is the main cause of death, coming in second after cardiovascular disease. Diet and environmental factors have a major influence on the emergence of cancer.[10]

This research aimed to investigate polar and nonpolar fraction biological activities of tomato extract in elastase, tyrosinase, and cytotoxic activity.

MATERIALS AND METHODS

Extraction and fractionation process

The tomato was peeled, and the flesh was dried using an oven[11] and macerated using n-hexane: methanol: acetone (2:1:1) for 24 h.[12] The tomato residue was re-macerated until the last remaining bioactive.[13-15] The filtrate was vaporized using a water bath at 40°C to obtain the thick extract.[15,16] The tomato extract was then fractionated using liquid–liquid fractionation to separate the thick extract with distilled water and n-hexane to obtain polar and nonpolar fractions.

Extract and fraction identification

The tomato extract, polar fraction, and nonpolar fraction were identified using thin-layer chromatography using lycopene as a positive control.[11]

Cytotoxic test in fibroblast cell NIH 3T3 (3-day transfer)

This 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for this testing. In a nutshell, 20 µl of the cell suspension was placed in a microtube, followed by 20 µL Trypan blue solution, and homogenized. The wells of 100 µL each transfer the cell suspension, and three wells are emptied (not filled in cells). Cells were incubated using a 5% CO_2 incubator at 37°C so that cells would attach to the bottom of the well (±24 h) under observation using a microscope. After a normal cell was immediately added, 100 µL of samples with a serial concentration of 1000, 500, 250, 125, 62.5, and 31.25 ppm, all concentrations were carried out by triple in the well and incubated in the 5% CO_2 incubator at 37°C for 24 h. After that, the cells were washed with phosphate-buffered saline once, and the MTT reagent (0.5 mg/ml) 100 µl was added to each well. A 10% sodium dodecyl sulfate stopper was added if the formazan crystal was formed. The plate was wrapped using aluminum foil and incubated in the dark (room temperature). An enzyme-linked immunosorbent assay (ELISA) reader measured each absorbance at 570 nm.

In vitro elastase inhibitory assay

An elastase inhibitory assay was performed using spectrophotometry at 405 nm. In brief, The 20 L of elastase enzyme solution was put into a 96-well microtiter then the 20 L of the 100 ppm extract/fraction/positive control solution was added to each well, added 140 L of 0.2 M Tris-HCl buffer solution (pH 8.0) into the wells, and incubated for 15 minutes at room temperature then added 20 L of substrate SANA (-Succ-(Ala)-3-p-nitroanilide). The blank has used all components except the enzyme.[17] Epigallocatechin gallate was used as a positive control. Each assay was conducted in duplicate.[18] Finally, elastase inhibition was calculated in percentage using Equation (1):

$$\text{Percentage inhibition of elastase (\%)} = \frac{\text{OD Control} - (\text{OD Sample} - \text{OD blank})}{\text{OD Control}} \times \text{X100\%}$$

The result was written as the mean ± standard deviation (SD) from several experiments. The concentration of extract and

percentage of inhibitory activity were plotted to obtain the IC_{50} value.[17]

In vitro tyrosinase inhibitory assay

The inhibitory effect on tyrosinase activity was evaluated using a 3,4-dihydroxy-L-phenylalanine (L-DOPA) substrate (Sigma-Aldrich, USA) and lyophilized mushroom tyrosinase (Sigma-Aldrich, USA).[19] Tyrosinase and L-DOPA were diluted in phosphate buffer solution (0.1 M, pH 6.8) into optimum concentration previously conducted of 18.488 mM for L-DOPA and 250 U/mL for tyrosinase, respectively.[20] In brief, an enzyme reaction mixture containing 40 µL of diluted extract solution in dimethyl sulfoxide (DMSO) (100 ppm), 40 µL of L-DOPA, 40 µL of tyrosinase, and 80 µL of 0.1 M phosphate buffer (pH 6.8) was incubated at 37°C for 10 min.[21] Kojic acid (Thornhill, Canada) was used as the positive control.[21,22] Each sample was made the control sample and control blank (without enzyme); the blank was made using phosphate buffer with DMSO.[23,24] Tyrosinase inhibition activity was quantified triplicate by measuring absorbance at 490 nm using a microplate reader (VersaMax ELISA Microplate Reader, USA).[25] The tyrosinase inhibitory activity (%) counted as[8]: inhibition (%) = (1-B/A) ×100%, where A is the absorbance of the blank and B is the absorbance of the sample.

Statistical analysis

All tests were conducted in triplicate, and the result was the average and stated as mean ± SD. Comparison between two samples was used *t*-test using Minitab 18th version software and if $P < 0.05$, then it is different significantly.[26]

RESULTS

Extraction

Ten kilogram of fresh tomatoes yielded 265.03 g of dried tomatoes. Maceration using n-hexane: methanol: acetone (2:1:1, v/v) resulted in 0.01 g extract. The result showed that the extract yield was 0.004%.

Fractionation

The tomato extract was diluted in distilled water and separated using liquid–liquid fractionation in n-hexane. Polar and nonpolar fractions yielded 6.83 g and 1.83 g, respectively. The percentage of polar fraction from the extract was 2.58%, while the nonpolar fraction was 0.69%.

Extract and fraction identification

The extract and fraction of tomato were identified by thin-layer chromatography with eluent n-hexane: acetone (8:2, v/v). The result showed that all extracts and fractions contained lycopene as can be seen in Figure 1.

Cytotoxic assay

The cytotoxicity assay result of tomato extract (polar and nonpolar fractions) showed in Tables 1 and 2.

Table 1: Cytotoxic assay of polar fraction of tomato to fibroblast cell NIH 3T3

Sample (µg/mL)	Proliferation inhibitory±SD	
	Polar fraction	Nonpolar fraction
31.25	1.857±1.883	−0.575±0.314
62.5	3.818±3.060	1.491±0.045
125	5.230±1.569	6.878±1.804
250	9.074±1.255	5.387±0.314
500	11.585±2.118	18.253±0.392
1000	29.315 ± 5.021	29.341 ± 0.432

SD: Standard deviation

Table 2: IC^{50} of cytotoxic assay to fibroblast cell NIH 3T3

The fraction of tomato extract	IC^{50} (µg/mL)
Polar fraction	1820.90
Nonpolar fraction	1643.86

Table 3: Elastase and tyrosinase inhibitory activities of fractions and standard

Sample	Elastase inhibitory activity (%)	Tyrosinase inhibitory activity (%)
Polar fraction	87.21±7.57	23.71±7.91
Nonpolar fraction	73.12±7.44	41.16±5.41
Quercetin	65.97±3.00	-
Kojic acid	-	65.07 ± 0.86

Elastase and tyrosinase inhibitory activities

Elastase and tyrosinase inhibitory activities of tomato extract (polar and nonpolar fractions) are shown in Table 3.

DISCUSSION

A spot on the thin-layer chromatography test can indicate the presence of a compound in the extract. From the results of thin-layer chromatography, it can be seen that the skinless tomato fruit extract has no spots. This can be due to the very small content of bioactive compounds so that they are not detected. Meanwhile, three spots were obtained from the nonpolar fraction of tomato fruit extract on thin-layer chromatography with Rf values of 0.30, 0.46, and 0.68. Furthermore, the Rf values of the spot obtained by thin-layer chromatography of the polar fraction of tomato fruit extract were 0.30, 0.41, and 0.81. Spots with an Rf value of 0.30 were found in the nonpolar and polar fractions. This indicates that the compound is present in both polar and nonpolar fractions. The mobile phase used in this thin-layer chromatography is n-hexane and acetone in a ratio of 8:2, and the stationary phase used in this thin-layer chromatography is silica gel. Silica gel has polar properties, while the eluent has nonpolar properties, so it

is suitable for separating lycopene, a secondary metabolite compound that is nonpolar. The result of Rf points out that the separation method from the secondary metabolic compound from the extract of polar and nonpolar extract of this tomato fruit has been optimal because the value of Rf is in the range between 0.3 and 0.8.

Water–ethanol extract of tomato fruit contains polyphenolic and carotenoid compounds.[26] Tomatoes also contain lycopene (a terpenoid compound), chlorophyll, flavonoids, ascorbic acid, folic acid, and beta-carotene.[27] Carotene is soluble in nonpolar solvents such as hexane and toluene, while xanthophylls are soluble in polar solvents such as ethanol and pyridine. Maceration extraction using a mixed solvent n-hexane-acetone-methanol (1:2:1) can extract lycopene, and a small portion of other hydrocarbon carotenoids will be extracted into nonpolar solvents (n-hexane-acetone), while xanthine compounds and polar compounds others will be extracted into a polar solvent (methanol). The ratio of the solvent mixture is the most optimal solvent ratio in extracting lycopene compounds.[27,28]

The test results of the proliferation inhibition activity of NIH 3T3 fibroblast cells from the polar fraction of tomato extract had an IC_{50} of 1820.90 g/mL, while the IC_{50} of the nonpolar fraction of tomato extract was 1643.86 g/mL. Cytotoxicity test and determination of fibroblast cell proliferation kinetics, NIH 3T3, were performed using the MTT method. Abnormal proliferation of fibroblast cells can lead to keloids, scars that appear in incomplete wound healing. The IC_{50} value of inhibition of proliferation between 1500 and 2000 g/mL indicates that the polar and nonpolar fractions of tomato fruit extract have the potential as anti-keloid, although weak.

The elastase enzyme inhibition test points out the highest result, where the inhibitory activity of this enzyme exceeds the activity of the positive control, quercetin. The results of the *t*-test showed that the inhibitory activity of the elastase enzyme from the polar and nonpolar fractions of tomato fruit extract was not significantly different, with $P = 0.051$ (>0.05). The tyrosinase inhibitory activity of the nonpolar fraction of tomato extract was also not significantly different from kojic acid, with $P = 0.263$ (>0.050). Meanwhile, the inhibitory activity of the polar fraction of the elastase enzyme from tomato extract was significantly different from that of quercetin, with $P = 0.046$ (<0.050). This *t*-test showed that the inhibitory activity of the polar fraction of the elastase enzyme of tomato fruit extract was better than that of quercetin. The higher value of elastase enzyme inhibitory activity than quercetin activity indicates that the polar fraction of tomato fruit extract has a very high potential as a cosmetic ingredient, especially as an antiaging agent.

The result of tyrosinase inhibition assay points out more minor results than the positive control activity, kojic acid. The results of the *t*-test showed that the tyrosinase inhibitory activity of the polar and nonpolar fractions of tomato fruit extract was not significantly different, with $P = 0.105$ (>0.05). The inhibitory activity of the nonpolar fraction of the elastase enzyme from tomato fruit extract was significantly different from that of kojic acid, with $P = 0.017$ (<0.050). The inhibitory activity of the polar fraction elastase enzyme from tomato fruit extract was also significantly different from that of kojic acid, with $P = 0.012$ (<0.050). This *t*-test showed that the tyrosinase inhibitory activity of the polar and nonpolar fractions of tomato extract was lower than that of kojic acid. This indicates that the polar and nonpolar fractions of tomato extract have potential as skin lightening agents, although their activity is lower than kojic acid.

CONCLUSION

Polar and nonpolar fractions from tomato extract had a tyrosinase inhibitory activity of 87.21% ± 7.57% and 73.12% ± 7.44%, elastase inhibitory activity of 23.71% ± 7.91% and 41.16% ± 5.41%, and cytotoxic activity of IC_{50} 1820.90 µg/mL and 1643.86 µg/mL, respectively. Both fractions showed potential use in cosmetic ingredients as lightening, antiaging, and anti-keloid agents. Further study is needed to identify the active compound from that fraction.

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Conflicts of interest

There are no conflicts of interest.

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