



# Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit as antiaging

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## ABSTRACT

**Introduction:** Nitric oxide (NO) is a free radical that belongs to reactive nitrogen species (RNS). The excess amount of NO in body generates physical changing on skin as a consequence of alteration in connective tissue through formation of lipid peroxides, cell content, and enzymes. These free radicals induce damage to extracellular matrix (ECM) and are responsible in reducing skin elasticity. Antioxidants possess significant role in delaying aging process by scavenging free radicals and preventing collagenase and elastase enzymes activities. This study aimed to evaluate antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit (GpKar) as antiaging remedy.

**Methods:** Antioxidant activity was performed by NO scavenging activity assay, while anti-aging activity was performed through inhibitory effects of collagenase and elastase activities.

**Results:** In antioxidant activity, GpKar had lower NO scavenging activity ( $IC_{50}$ =1530.34  $\mu$ g/mL) compared to xanthone ( $IC_{50}$ =85.40  $\mu$ g/mL). In collagenase inhibitory activity, GpKar also had lower inhibition collagenase activity ( $IC_{50}$ = 1169.31  $\mu$ g/mL) compared to xanthone ( $IC_{50}$ = 286.32  $\mu$ g/mL). In elastase inhibitory activity, GpKar had lower inhibition elastase activity ( $IC_{50}$ = 152.93  $\mu$ g/mL) compared to xanthone ( $IC_{50}$ = 21.26  $\mu$ g/mL).

**Conclusion:** In summary, GpKar and its compounds possess antioxidant, anticollagenase, and antielastase activities for antiaging, and might be beneficial in these subjects.

### Implication for health policy/practice/research/medical education:

Extract of *G. picrorrhiza* has potential to increase antioxidant, anticollagenase, and antielastase activities. Therefore, more investigations need for identification of the active compounds in *G. picrorrhiza* extract involved in antioxidant and antiaging activities.

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## Introduction

Skin aging process is divided into two categories i.e. intrinsic aging and extrinsic aging (1,2). Intrinsic aging is naturally caused by the alteration of skin elasticity as time goes by, whereas extrinsic aging generally is caused by accumulation of free radicals (1,3). Nitric oxide (NO) is a free radical belonging to reactive nitrogen species

(RNS). NO is produced essentially as bio-regulatory molecule in several physiological processes such as neural signal transmission, immune response, vasodilatation, and blood pressure regulation. On certain conditions, the concentration of NO can be increased as a result of photo radiation or chemical exposures (4,5).

In addition, the excessive NO in body may result in premature

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aging. The excessive NO leads to structural changes of cells and stimulates matrix metalloproteinases (MMPs) enzymes such as collagenase and elastase that may induce collagen and elastin loss (6-8). Collagenase is an enzyme that plays an important role in degradation of collagen. Collagen is the main component with percentage of 70%-80% of the total skin weight. The increasing degradation of collagen is significant in aging (9).

Aging can be prevented by scavenging free radicals. The excessive free radicals like NO can be reduced by escalating antioxidant intake through food or supplements. Furthermore, the other way to retard aging is inhibiting collagenase and elastase activities. As collagenase and elastase increase significantly with age, inhibiting their activities may retard skin aging without interfering their abilities to breakdown damaged skin components. In other words, the use of inhibiting agents helps restore the balance that the skin possessed when it was younger.

Nowadays, the use of natural substances to prevent premature aging is more preferable in food, cosmetic, and therapeutic industry. These would be promising alternatives for synthetic antioxidants in respect of low cost, high compatibility with dietary intake and low harmful effects inside the human body. Many compounds in plants have been identified as free radical or active oxygen scavengers (10). It has been investigated that *Garcinia picrorrhiza* fruit contains secondary metabolites that act as antioxidant such as xanthone, biflavonoids, and benzophenones and their derivatives. Based on a primary research, fruit extract of *G. picrorrhiza* contains camboginol or garcinol, a derivative of benzophenones. In the present study, NO scavenging activities of GpKar and its compounds as well as their inhibitory activities on collagenase and elastase were evaluated.

## Materials and Methods

### Preparation of GpKar

Sesoot fruits were collected from Bogor Botanical Garden, Bogor, West Java. The plant was authenticated by herbarium staff of Bogor Botanical Garden. Sesoot fruits were mashed (500 g) and extracted using ethanol 70% by maceration method. Every 24 hours the ethanol was filtered and the wastes were re-macerated in triplicate. The ethanol filtrate was collected and condensed in 50 °C using rotary evaporator (Hitachi) to obtain GpKar. The extract in paste form was stored at -20 °C and used for the assay. Standard compound used in this study was xanthone (Sigma X0626, USA) (11,12).

### Nitric oxygen scavenging activity assay

Sodium nitroprusside is soluble in aqueous solution at physiological pH of 7.2 producing NO. NO reacts with oxygen to produce stable products (nitrate and nitrite) under aerobic conditions. Scavengers of NO compete with oxygen leading to reduced production on nitrite ions.

Griess reagent was used in NO assay (13).

In this study the NO scavenging activity was estimated using Griess Illosvoy reaction based on the method that was performed by Parul et al with minor modifications (14). Sodium nitroprusside (10 mM) (Merck 106541, Germany) in phosphate buffered saline (PBS) (Gibco 1740576, Canada) was mixed with different concentrations of GpKar and xanthone (20.83–666.67 µg/mL; µM). The mixtures were incubated for 2 hours in 25 °C. After the incubation period, Griess reagent (1% sulfanilamide [Merck 111799, Germany], 2% H<sub>3</sub>PO<sub>4</sub> [Merck 100573, Germany] and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) (Sigma 222488, USA) were added. The absorbance of the chromophore that was formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was read at 546 nm with microplate reader (Thermo Scientific Multiscan GO). The same reaction mixture without the extract yet equivalent in amount of ethanol served as the control.

### Collagenase inhibitory activity assay as antiaging potential

Inhibition of collagenase activity was measured based on the method that was elaborated by Sigma Aldrich and Thring et al with some modifications. The assay was performed by dissolving 10 µL collagenase from *Clostridium histolyticum* (Sigma C8051, USA) (0.01 U/mL in cold distilled water), 60 µL buffer Tricine (50 mM, pH 7.5, contains 10 mM CaCl<sub>2</sub> and 400 mM NaCl), 30 µL sample (0-250 µg/mL in DMSO). The mixtures were incubated for 20 minutes at 37 °C. After incubation time, 20 µL substrate N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (Sigma F5135, USA) (1 mM in buffer Tricine) was added. Absorbance at 335 nm was measured immediately after adding the substrate (11,15,16).

$$\% \text{ Collagenase inhibition} = (1 - B/A) \times 100\%$$

A = Sample absorbance

B = Control absorbance

### Elastase inhibitory activity assay

Elastase inhibitory activity was measured by modified method of Sigma Aldrich and Thring et al with some modifications by Widowati et al (11,15). A mixture of 10 µL of various levels of samples (4.17–133.33 µg/mL), 5 µL elastase 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 minutes at 25 °C. Mix solution was added N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate [Sigma 54760, USA] and then incubated for 15 minutes at 25 °C. Absorbance was measured immediately after incubation time with 410 nm wavelength.

$$\% \text{ Elastase inhibition} = (1 - B/A) \times 100\%$$

A = Sample absorbance

B = Control absorbance

**Results**

**The effect of GpKar on nitric oxide scavenging activity**

Inhibition potentials of nitrite formation by GpKar and the standard antioxidant xanthone were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentration of each extract and standard antioxidant. IC<sub>50</sub> which is an inhibitory concentration of each extract required to reduce 50% of the NO formation was determined (14).

Figure 1 shows that the NO scavenging activity was concentration dependent. At the highest concentration (666.67 µg/mL) NO scavenging activity of GpKar (20.26 ± 0.47%) was higher than xanthone (63.38 ± 0.12%). Furthermore, IC<sub>50</sub> value of GpKar (1530.34 µg/mL) was higher than xanthone (85.40 µg/mL) (Table 1). These results indicate that GpKar has lower NO scavenging activity compared to xanthone.

**The effect of GpKar on collagenase inhibitory activity**

A spectrophotometric method was performed to find out collagenase inhibitory activity. The collagenase inhibitory activity of GpKar and xanthone can be seen in Figure 2 and Table 2. Figure 2 shows that collagenase inhibitory activities of GpKar and xanthone are concentration-dependent. At the highest concentration (2500 µg/mL) collagenase inhibition activity of GpKar (68.08 ± 4.18%) was lower than xanthone (68.91 ± 1.72%). Furthermore, IC<sub>50</sub> value of GpKar (1169.31 µg/mL) was higher than xanthone (286.32 µg/mL) (Table 2). These results indicate

that GpKar has lower collagenase inhibitory activity compared to xanthone.

**The effect of GpKar on elastase inhibitory activity**

The percentage of elastase inhibitory activities of GpKar and xanthone can be seen in Figure 3. Figure 3 shows that the elastase inhibitory activities of GpKar and xanthone are concentration-dependent. At the highest concentration (133.33 µg/mL), the elastase inhibitory activity of GpKar (40.37 ± 3.11%) was lower than xanthone (59.55 ± 0.48%). Furthermore, IC<sub>50</sub> value of GpKar (152.93 µg/mL) was higher than xanthone (21.26 µg/mL) (Table 3). These results indicate that GpKar has lower elastase inhibitory activity compared to xanthone.

**Discussion**

The natural substances from plants have been widely used for treating aging. It has been investigated that the investigated plant contains active antioxidants that are related to its phytochemical compounds (17,18). In several studies, some plants have shown the ability to reduce free radicals having anti-aging potential through their components such as polyphenols from blackberry, curcumin from *C. longa* (19), procyanidins from *V. vinifera* (20) and garcinol from *G. indica* (21). Sesoot belongs to genus of *Garcinia*. Recent studies have shown that sesoot contains high levels of xanthone, bioflavonoids, and prenylated benzophenones. Previous studies have shown that the extract of sesoot fruit

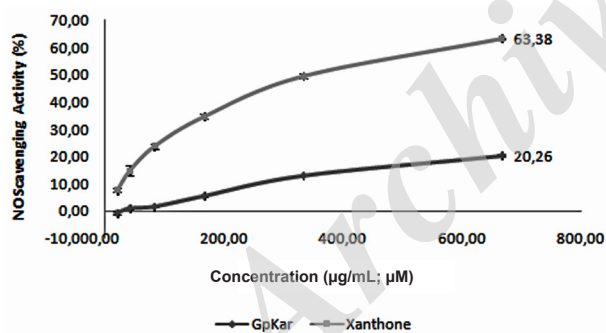


Figure 1. Nitric Oxide (NO) scavenging activity of GpKar and xanthone.

\*GpKar and xanthone were diluted using DMSO to reach the final concentration of 20.83; 41.67; 83.33; 166.67; 333.33; 666.67 (µg/mL; µM).

Table 1. IC<sub>50</sub> values of nitric oxide scavenging activities by GpKar and xanthone

Samples	Equation	r <sup>2</sup>	IC <sub>50</sub> (µM)	IC <sub>50</sub> (µg/mL)
GpKar	y = 0.03x - 0.51	0.97	-	1530.34
Xanthone	y = 0.08x + 14.48	0.90	435.28	85.40

\*GpKar= *G. picrorrhiza* extracts, IC50= The half maximal inhibitory concentration. Linear equations, coefficient of regression (r2) and IC50 of the samples were calculated. IC50 of GpKar in µg/mL xanthone was presented in µM and µg/mL.

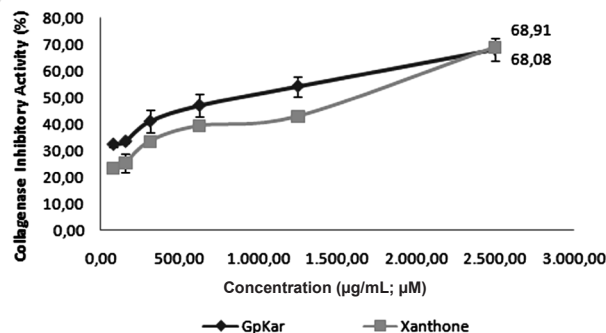


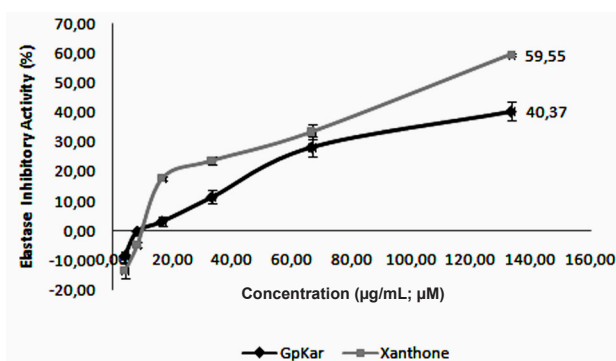
Figure 2. Collagenase inhibitory activity of GpKar and Xanthone.

\*GpKar and Xanthone were diluted using DMSO to reach the final concentration of 78.125; 156.25; 312.5; 625.00; 1250.00; 2500.00 (µg/mL; µM).

Table 2. IC<sub>50</sub> values of of collagenase inhibitory activities by GpKar and xanthone

Samples	Equation	r <sup>2</sup>	IC <sub>50</sub> (µM)	IC <sub>50</sub> (µg/mL)
GpKar	y = 0.01x + 34.28	0.92	-	1169.31
Xanthone	y = 0.02x + 24.69	0.96	1459.35	286.32

\*GpKar= *G. picrorrhiza* extracts, IC50= The half maximal inhibitory concentration. Linear equations, coefficient of regression (r2) and IC50 of the samples were calculated. IC50 of GpKar in µg/mL xanthone was presented in µM and µg/mL.



**Figure 3.** Elastase inhibitory activity of GpKar and Xanthone.

\*GpKar and xanthone were diluted using DMSO to reach the final concentration of 4.17; 8.33; 16.67; 33.33; 66.67; 133.33 (µg/mL; µM).

**Table 3.** IC<sub>50</sub> values of of elastase inhibitory activities by GpKar and xanthone

Samples	Equation	r <sup>2</sup>	IC <sub>50</sub> (µM)	IC <sub>50</sub> (µg/mL)
GpKar	y = 0.33x - 0.25	0.94	-	152.93
Xanthone	y = 0.36x + 11.28	0.99	108.38	21.26

\*GpKar= *G. picrorrhiza* extracts, IC<sub>50</sub>= The half maximal inhibitory concentration. Linear equations, coefficient of regression (r<sup>2</sup>) and IC<sub>50</sub> of the samples were calculated. IC<sub>50</sub> of GpKar in µg/mL xanthone was presented in µM and µg/mL.

has camboginol or garcinol compound, a derivate of benzophenones that has various therapeutic activities. Xanthone compounds in sesoot are oxygenated xanthenes and prenylated ones. Due to the xanthone itself that has phenolic functional group on linear tri-cyclic ring, xanthone has a wide range of biological activities such as antioxidant property (22).

In this study, GpKar was tested to find out its potential in NO scavenging potential, as anticollagenase and antielastase agent. In NO scavenging assay, GpKar has higher IC<sub>50</sub> value compared to xanthone. It means that GpKar has lower NO scavenging activity than xanthone does. Even though GpKar has lower IC<sub>50</sub> than xanthone but GpKar still indicates antioxidant potential. The *Garcinia* contains camboginol that structurally is similar to a well-known antioxidant, curcumin, which contains both phenolic hydroxyl groups and β-diketone moiety. Several studies have demonstrated that garcinol exhibits significantly the antioxidative property and possesses inhibitory activity on lipid peroxidation (23,24).

NO is an important chemical mediator generated by endothelial cells, macrophages and neurons which is involved in regulation of physiological processes (14). NO is generated in biological tissues by a specific NO synthase, which metabolizes arginine to citrulline with the formation of NO via five electrons of oxidative reaction (25). Nevertheless, the excess amount of NO in body may result in aging acceleration or even degenerative chronic ailments (6). In aging-acceleration process, NO does not interact directly with DNA or proteins. However, under

aerobic conditions, NO is very unstable and reacts with oxygen to produce NO<sub>2</sub>, N<sub>2</sub>O<sub>4</sub>, N<sub>3</sub>O<sub>4</sub>, the stable products of nitrate and nitrite and peroxyxynitrite when reacted with superoxide. These progenitors are highly genotoxic. Besides, NO may affect enzymatic activities that lead to mutagenesis (26). NO is responsible for altering the structural and functional behavior of many cellular components. Antioxidants have the ability to reduce NO by donating their electron. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with NO, thereby inhibiting the production of nitrite (14).

Skin aging is a natural process that occurs in organisms with characteristic of losing skin elasticity implicated in formation of wrinkle, uneven pigmentation, brown spots, laxity and leathery appearance (11). Though aging will occur naturally but at some conditions it can be accelerated by some factors like free radicals. Aging can be characterized by its physical and psychosocial changing. Physical changing in visual can lessen skin-elasticity, causing wrinkle, uneven pigmentation, brown spots, laxity and leathery appearance (16). The excess of free radicals in body may overturn natural cellular antioxidant defense and lead to oxidation and further contributing to cellular functional impairment (27).

During aging process, there is also imbalance between collagen production and degradation. Collagen production decreases whereas level of collagenase increases. Exact mechanism of wrinkles formation is still unknown but it has been observed that free radicals cause enhancement in the expression of MMPs which is collagenase and elastase, which in turn causes repetitive breakdown of collagen and elastine (28). However, undesirable aging can also be retarded by scavenging free radical with antioxidant and inhibiting collagenase and elastase activities. Other than that, in collagenase and elastase inhibition activities, GpKar has lower inhibitory activity compared with xanthone. In collagenase inhibitory activity assay, the IC<sub>50</sub> value of collagenase inhibitory activity of GpKar was higher than xanthone (Table 2) and in elastase inhibitory activity IC<sub>50</sub> value of GpKar was higher than Xanthone (Table 3). These results indicate that GpKar has lower collagenase and elastase inhibitory activity compared to xanthone. Collagen and elastin are the main components of skin that possess important role in maintaining skin structure. Collagenase and elastase enzymes cause repetitive collagen breakdown and are responsible for structural defect in dermis and wrinkle development (29). Collagenase and elastase contribute to production and degradation of collagen and elastin (30).

## Conclusion

In summary, GpKar and its compounds possess antioxidant and antiaging activities and might be used to prevent aging. However, xanthone has higher antioxidant

power through NO scavenging activity and has higher collagenase and elastase inhibitory activities than GpKar.

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### Authors' contributions

Author contribution of this study in literature search were QRS, NAD, AW, and IS. SU, QRS, NAD, AW, and IS developed the theory and designed the study. The data collection and data analysis were through SU, HSWK, and WW. The data interpretation and writing were through SU, BCA, SN, HSWK and WW.

### Conflict of interests

All contributing authors declare no conflicts of interest.

### Ethical considerations

All procedures were approved by the ethics committee of YARSI University (0415/K3/KM/2017) and considered in all aspects of the work.

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