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Antioxidants and Anticholinesterase Activities of the Characterized Ethanolic of Ripe Sesoot (*Garcinia picrorrhiza* Miq.) Fruit Extract (GpKar) and Xanthone

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ABSTRACT

Oxidative stress has been known to contribute to Alzheimer's disease. Acetylcholinesterase (AChE) enzyme may lead to Alzheimer's disease as a neurotransmitter. Antioxidants may have protective activities against oxidative damage and Alzheimer's disease. Acetylcholinesterase inhibitors also can be used in the treatment of various neurological disorders for management of Alzheimer's disease. This study aimed to determine antioxidant and anticholinesterase effects of Garcinia picrorrhiza Miq. fruit extract (GpKar) and its compounds, xanthone. Antioxidant activity was measured by H_2O_2 scavenging inhibitory activity, while anticholinesterase activity was measured using modified Ellman method. GpKar has higher H_2O_2 scavenging inhibitory activity (IC_{50}= 967.28 µg/ml) compared to xanthone (IC_{50}= 1198.95 µg/ml). In the anticholinesterase inhibitory activity, GpKar has lower activity (IC_{50}= 70.25 µg/ml) compared to xanthone (11.80 µg/ml). In summary, GpKar has higher antioxidant activity but lower anticholinesterase activity compared to its compounds, xanthone. However, GpKar has potency as antioxidant agent to prevent Alzheimer's disease.

Keywords: *GpKar; xanthone; antioxidant; anticholinesterase*

INTRODUCTION

Alzheimer's is one of the neuro-degenerative diseases that forms dementia and is assumed to be doubled in the next 20 years in that prevalence (Mattson, 2004; Weiner, *et al*., 2013). Hypothesis of Alzheimer's disease is caused by the age-related myelin breakdown in the brain (Kulkarni and Bairagi, 2014). Acetyl-cholinesterase (AChE) has principal biological role that is termination of impulse transmission at cholinergic synapses by rapid hydrolysis acetylcholine (ACh) as a neurotransmitter. AChE inhibitors are utilized in the treatment of various neurological disorders, and are the principal drugs approved by FDA for management of Alzheimer's disease (Dvir, *et al*., 2010).

Alzheimer's disease may be also induced by oxidative stress, but its underlying mechanism remains unclear (Dvir, *et al*., 2010). Other study convinced that oxidative stress may cause damage in pathogenesis Alzheimer's disease. Oxidative stress can damage cell body and thus contributes to many pathological conditions, including cancer, atherosclerosis, neurological disorders, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, and chronic obstructive pulmonary disease. Antioxidant systems for balancing between oxidant and antioxidant to prevent oxidative stress are needed (Birben, *et al*., 2012). Thus, antioxidant agent is expected to prevent and reduce progression of this disease (Gilgun-Sherki, *et al*., 2003).

Medicinal herb has been used as alternative to commercial drug. *G. picrorrhiza* Miq. is one of the species of *Garcinia* (family of *Cluciaceae*) that grows in tropical to temperate climates (Soemiati, 2005). *G. picrorrhiza* is rare plant that apparently has some medicinal properties. *Garcinia* and its compound, xanthone, have potency as source of pharmacology such as antioxidant, antitumor, antiallergic, antiinflammatory, antibacterial,

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antifungal, and antiviral activities (Shan, *et al*., 2011. The fruit of *G. picrorrhiza* Miq. may have antioxidant activity, that induces this study to observe antioxidant and anticholinesterase activities of *G. picrorrhiza* Miq. In the present study, GpKar and xanthone are evaluated as antioxidant and anticholinesterase that is related to Alzheimer's disease.

MATERIALS AND METHODS

Preparation of GpKar

The fruits of sesoot were collected from the Bogor Botanical Garden, West Java. The plants were identified by herbarium staff, Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The ripe fruit ofsesoot (1330 g) were mashed and extracted using distilled ethanol 70% (1000mL). Standards compounds used in this study were xanthone with 99% purity [Sigma, X0626].

Hydrogen Peroxide (H_2O_2) Scavenging Activity Assay

Hydrogen peroxide scavenging activity was measured by the modification method of ferrous ammonium sulphate and phenanthroline (Mukhopadhyay, *et al*., 2016). The reaction of ferrous ammonium sulphate and phenanthroline could form Fe^{2+}-tri-phenanthroline complex with the color of orange, but if H_2O_2 exists in that reaction, Fe^{2+}-tri-phenanthroline complex would not be formed, thus scavenger of H_2O_2 might not form Fe^{2+}-tri-phenanthroline complex. The mixture of ferrous ammonium sulphate 12µL, 1mM, 60µL of sample, H_2O_2 3µL 5mM was incubated at dark room temperature for 5 minutes. Briefly, it was added 1,10-phenanthroline 1mM 75µL, and then incubated for 10min at room temperature.

Absorbance was measured on wavelength of 510nm.

H_2O_2 Scavenging Activity= $\frac{Atest}{Acontrol}$ x 100%

A_{test} = Test sample absorbance; $A_{control}$ = Control absorbance (ferrous ammonium sulphate and 1,10-phenanthroline).

Acetylcholinesterase Enzyme Inhibitory Activity (AChE)

AchE inhibitory activity was done by modified Ellman method (Owokotomo, *et al*., 2015). This method was done by adding 5µL, 0.5 U/mL AchE enzymes type VI-S from electric eel (in 0.1M of Tris-HCl, pH 8 in 0.1% BSA), sample (2µL), and 83µL buffer Tris-HCl to well-plate. The mixed solution was incubated at room temperature for 15min. Subsequently, 1.83 mM of AChI (in aquades) (15µL) and 95µL of DTNB (in 50mM buffer Tris-HCl pH 8 in NaCl 0.1 M and $MgCl_2$ 0.02 M) were added, and incubated for 30min at room temperature. Absorbance was measured in 405 nm wavelength. The percentage inhibition was calculated using formula as follows:

Inhibitory activity (%) =

$$1- \frac{A_{sample}}{A_{control}} \times 100\%$$

A_{sample} = Sample absorbance; $A_{control}$ = Control absorbance

RESULTS AND DISCUSSION

H_2O_2 Scavenging Inhibitory Activity

Hydrogen peroxide is one of the reactive oxygen species that has positive roles in energy production *in vivo* systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (Packer, *et al*., 2008). The percentage H_2O_2 scavenging activity of GpKar and xanthone can be seen in Figure 1 and the median inhibitory concentration (IC_{50}) of samples toward H_2O_2 radical scavenging activity can be (Table I).

Figure 1 shows the percentage of H_2O_2 scavenging activity of GpKar and xanthone. In the highest concentration, GpKar has higher value (157.42±3.57%) than xanthone does (30.69±2.26%).

Table I shows the result that GpKar has lower IC_{50} (967.28 µg/ml) compared to xanthone (1198.95 µg/mL). This indicates that GpKar has higher H_2O_2 scavenging activity than xanthone does.

Anti-cholinesterase Activity

AChE inhibitors or anti-cholinesterases inhibit the cholinesterase enzyme from breaking down ACh, increasing both the level and the duration of the neurotransmitter action, that is required for Alzheimer's disease treatment (Colovic, *et al*., 2013).

Based on figure 2, acetylcholinesterase inhibitory activity of GpKar has higher percentage (57.90 ± 3.95%) compared to xanthone (45.70 ± 1.32%) in highest concentration (100 µg/mL).

IC_{50} value of GpKar has higher value compared to xanthone (Table II), this shows that GpKar has lower inhibitory activity of acetylcholinesterase compared to xanthone. Each sample has IC_{50} value of 70.25µg/mL (GpKar) and 11.80µg/mL (xanthone). This indicates that xanthone has activity to inhibit acethylcholinesterase enzymes.

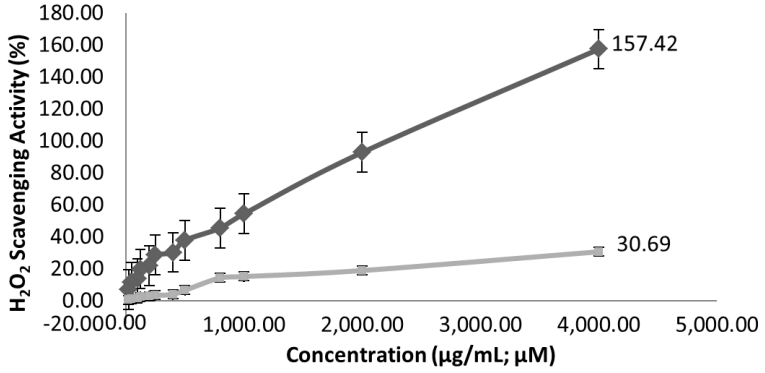

Figure 1. H_2O_2 scavenging activity of GpKar and xanthone. GpKar and xanthone were diluted using DMSO to reach the final concentration of 25.00; 50.00; 100.00; 125.00; 200.00; 250.00; 400.00; 500.00; 800.00; 1000.00; 2000.00; 4000.00 (µg/mL; µM).

Table I. IC_{50} value of H_2O_2 scavenging activity of GpKar and xanthone

Sample	Linear Regression	r^2	IC_{50} (µM)	IC_{50} (µg/mL)
GpKar	y = 0.04x ± 14.40	0.99	-	967.28
Xanthone	y = 0.01x ± 2.34	0.91	6110.82	1198.95

*The data was presented as mean ± standard deviation.

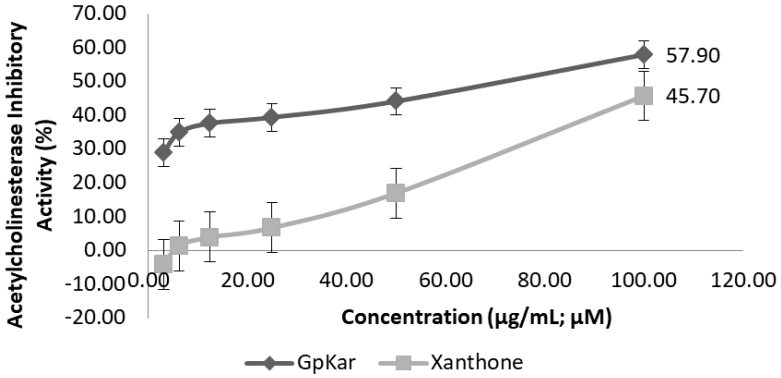

Figure 2. Acetylcholinesterase inhibitory activity of GpKar and xanthone (%). GpKar and xanthone were diluted using DMSO to reach the final concentration of 3.125; 6.25; 12.50; 25.00; 50.00; 100.00 (µg/mL; µM).

Table II. IC_{50} value acetylcholinesterase inhibitory activity of GpKar and xanthone

Sample	Linier Regression	r^2	IC_{50} (µM)	IC_{50} (µg/mL)
GpKar	y = 0.43x + 7.96	0.91	-	70.25
Xanthone	y = 0.67x + 9.89	0.93	60.16	11.80

Garcinia plants belong to the family of Clusiaceae that are distributed throughout tropical regions of the world. *Garcinia* has been known to possess potential source of antioxidants. *G. picrorrhiza* Miq. stem bark has two triterpenoids (Soemiati, 2005). In this study, antioxidant activity of GpKar was shown in H_2O_2-scavenging activity which was higher than xanthone. Previous phytochemical studies on *Garcinia* plants have reported that some of their constituents possess antioxidant activity. The ethyl acetate fraction from the leaf of *G. xanthochymus* showed a potent antioxidant activity which was similar to that of the standard antioxidant BHT (Meng, *et al*., 2012). *Garcinia* has

important natural source of xanthone that has pharmacological properties such as anticancer, antiinflammatory and antimicrobial effects (Aisha, *et al*., 2013). Xanthones in *G. mangostana* rind have various kinds that have strong antioxidant activity included alpha mangostin (Jung, *et al*., 2006). In the other study, *G. mangostana* rind has potential antioxidant properties (Tjahjani, *et al*., 2014).

Oxidative stress is correlated with many diseases, such as Alzheimer's diseases, as indicated by a progressive loss of memory and deterioration of higher cognitive functions (Kar, *et al*., 2004). The consumption of antioxidants is highly correlated with lower incidences of Alzheimer's diseases (Howes, *et al*., 2003; Houghton, *et al*., 2007). As a result, the use of natural compounds with high levels of antioxidants has been proposed as an effective therapeutic approach for Alzheimer's diseases.[19] In this study, GpKar had the higher H_2O_2 scavenging activity compared to xanthone. Xanthones from the pericarp, whole fruit, heartwood, and leaf of mangosteen (*G. mangostana* Linn.), are known to possess a wide spectrum of pharmacologic properties, including antioxidant, antitumor, antiallergic, antiinflammatory, antibacterial, antifungal, and antiviral activities (Shan, *et al*., 2011; Yang, *et al*., 2009). *G. schomburgkiana* has high phenolic, flavonoid and xanthone contents that have antioxidant capacities and radical scavenging activities than vitamin C and Trolox (Meechai, *et al*., 2016). The hepatoprotective effects of *G. indica* extracts in ethanol-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation in liver (Panda, *et al*., 2012). Based on the other study, it is suggested that the free radical scavenging activities of methanolic pericarp of *G. xanthochymus* and *G. lanceaefolia* extract was directly correlated to both phenolic and flavonoids contents (Gogoi, *et al*., 2015). Aqueous extract of *G. indica* has been reported to possess potent antioxidant, free radical scavenging and antilipid peroxidative activities (Mishra, *et al*., 2006).

Recently, a number of treatments are used against Alzheimer's disease as well as to counter the effect of oxidative stress which including acetylcholinesterase inhibitors (AChEIs) (Syad, *et al*., 2012). The inhibition of AChE prevents the hydrolysis of ACh thereby maintaining normal memory function (Howes, *et al*., 2003; Houghton, *et al*., 2007). In the present study, xanthone has higher value compared to GpKar in acethylcholinesterase inhibitory activity (IC_{50} =11.80μg/mL). This result was supported with some study, xanthones have a potent cholinesterase (ChE) inhibitory activity, which may have important roles in the treatment of Alzheimer's diseases (Brühlmann, *et al*., 2004; Urbain, *et al*., 2004; Louh, *et al*., 2008). Xanthones has activity in inhibition of MOA/AchE with IC_{50} 7.34μg/mL in MAO-A, while in MAO-B has IC_{50} value is 12.85μg/mL (Brühlmann, *et al*., 2004). Water extract of *G. cambogia* has lower anticholinesterase activity compared to neostigmine (standard drug), however total phenolic content, anticholinesterase, and antioxidant activity in compounds extract also has potential as antioxidant and anticholinesterase (Subhashini, *et al*., 2011). In the other study, xanthones showed weak inhibitory activity against AchE compared to tacrine as standard drugs (Chen, *et al*., 2011).

CONCLUSION

GpKar has higher antioxidant activity through H_2O_2 inhibitory scavenging activity, while that antiaging activity by anticholinesterase activity was lower than xanthone. This indicates that GpKar has potential as antioxidant activity that may has capability to decrease Alzheimer's disease progression.

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