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Antioxidant Activity of Prenylated Flavonoid Compound from Dichloromethane Extract of *Artocarpus communis* Leaves

(Aktivitas Antioksidan Senyawa Flavonoid Terprenilasi dari Ekstrak Diklorometana Daun *Artocarpus communis*)

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Abstract: This research investigated the antioxidant activity of prenylated flavonoid compound from the ichloromethane extract of *Artocarpus communis* leaves on 1,1-diphenyl-2-picryl hydrazyl free radicals scavenging assay. The dichoromethane extract of *Artocarpus communis* leaves was fractionated by column chromatography techniques on silica gel, and each fraction was assayed for its activity. The active compound, 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl] 1-propanone, showed moderate antioxidant activity with IC_{50} 54.6 µg mL^{-1}.

Keywords: *Artocarpus communis*, prenylated flavonoid, antioxidant activity.

Abstrak: Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan senyawa flavonoid terprenilasi dari ekstrak diklorometana daun *Artocarpus communis* dengan menggunakan uji penangkapan radikal bebas1,1-difenil-2-pikril hidrazil. Ekstrak diklorometana daun *Artocarpus communis* difraksinasi dengan teknik kromatografi kolom pada silika gel, dan setiap fraksi diuji aktivitasnya. Senyawa aktif, 1-(2,4-dihidroksifenil)-3-[8-hidroksi-2-metil-2-(4-metil-3-pentenil)-2H-1-benzopiran-5-il]1-propanon, menunjukkan aktivitas antioksidan yang moderat dengan IC_{50} 54.6 µg mL^{-1}.

Kata kunci: *Artocarpus communis*, flavonoid terprenilasi, aktivitas antioksidan.

INTRODUCTION

AS defined, antioxidants are molecules that terminate oxidative reaction via a wide range of mechanisms. The mechanisms could either be radical scavenging reactions, as in the action of tocopherols; complexation of transition metals, linked in peroxide bond decomposition; and inhibition of lipoperoxidative reactions, as the case of the iron chelator deferoxamine mesylate; stabilization of peroxide derivatives, as in the case of butylated hydroxytoluene (BHT); or energy quenching from singlet oxygen, as in the case of Vitamin A[1]. The antioxidants or radical scavengers may be categorized as (a) natural and synthetic enzymatic antioxidants and (b) natural and synthetic nonenzymatic antioxidants. The former includes superoxide dimutases (SOD), catalases, copper coordination compounds as SOD-like products, selenoderivatives with glutathione-like peroxidase activity, while the later includes, vitamin E and its homologues, ascorbic acid, carotenoids, glutathione and derivatives, flavonoids, lazaroids, captodative olefins[1,2].

The genus *Artocarpus* (*Moraceae*) comprises approximately 50 species and is widely distributed in tropical and subtropical regions, including Indonesia. Some members of this genus have been used medicinally to treat various diseases[3]. These plants are known to produce a variety of isoprenylated flavonoids containing the unique feature of an isoprenyl side chain at C-3, as well as 2',4'-dioxygenation or 2',4',5'-trioxigenation patterns in ring B of the flavone skeleton[4,5]. Many of these compounds exhibit interesting biological properties including cytotoxic[5], anti-inflammatory[5,6], antiplatelet[7], antioxidant[8], antibacterial[9], and anticomplementary effects[10]. They also show activity

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as inhibitor of TNF-α formation[5,6], tyrosinase[11,12,13], 5α-reductase[14], and K+-dependent amino acid transport in Bombyx mori midgut[15].

Artocarpus communis J. R. and G. Forster (synonym *A. altilis* (Parkinson) Fosberg) or breadfruit is locally known as Sukun. It is a large evergreen tree, up to 30-m tall. *A. communis* is a native of tropical Asia and the Pacific, and the center of genetic diversity extends from Indonesia to Papua New Guinea. It has long been an important staple food in Polynesia. Now it is widely distributed throughout the humid tropics, and commonly grown in home gardens for its edible fruit[16]. Previously, we have reported the structure elucidation of prenylated flavonoid compound from the dichloromethane extract of this plant, namely 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl] 1-propanone, which showed significant cytotoxicity against murine P-388 leukemia cells[17]. In continuation of this study, we have undertaken a closer examination of metabolites of this species with the aim of investigating the antioxidant activity from this compound using DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay.

MATERIALS AND METHODS

MATERIALS. Plant Materials. Leaves of *Artocarpus communis* were collected in March 2006, from plantation trees growing in Parung, Bogor, Indonesia. The plant was verified by the staff Biology Laboratory, Bandung nstitute of Technology, West Java, Indonesia, and a voucher specimen has been deposited at the Biology Laboratory.

METHODS. Extraction and Isolation. The dried leaves (4.95 kg) of *A. communis* were extracted exhaustively using macerator with ethanol 70%. The ethanol extracts (250 g) were concentrated using vacuum rotary evaporator and then partitioned with hexane-water (1:4). The water extract was extracted with dichloromethane and further partition with ethyl acetate and butanol. The dichloromethane extract was fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate, ratio from 100:0 to 0:100) to produce 80 fractions. Each fraction was identified using Thin Layer Chromatography, and the fraction which has the same spot was combined. Fraction 6-17 were combined and recrystallization to give yellow crystal (Compound F-1). All fractions were examined for their antioxidant activity using the DPPH free radical scavenging assay.

DPPH free radical scavenger activity assay [18,19]. Four mg of sample was dissolved in 4 mL DMSO to obtain 1000 µg/mL as mother solution of test sample. These test samples were diluted with ethanol to concentrations of 10, 40, 200 and 1000 µg/mL for analysis and four mg of crystallized compound was diluted to 10, 20, 50, 100, and 200 µg/mL, for pure compounds, respectively.The test samples were mixed with the ethanol solution of 300 µM DPPH (Sigma) in 90-well micro-titer plate and incubated at 37oC for 30 minutes. The absorption were measured at 515 nm. Inhibition (%) of the test samples were compared to that of control (DMSO). There were three positive control test i.e solutions of t-Butyl Hydroxy Anisole (BHA), t-Butyl Hydroxy Toluene (BHT), and ascorbic acid (Vit C). Free radical scavenging activity as calculated by the equation: [1-(B/A)] x 100%; whereas A is absorbance in the absence of sample and B is absorbance in the presence of sample. IC_{50} value denotes the concentration of sample (µg mL^{-1}) required to scavenge 50% of DPPH free radical.

RESULTS AND DISCUSSIONS

Isolation of *A. communis* leaves yielded 5.05% of ethanol extract. Further partition of ethanol extract produced 4 fractions of hexane, dichloromethane, ethyl acetate, and butanol and residue, with yield to the ethanol extract is 2.41%, 19.43%, 4.84%, 2.05%, 44.70%. The DPPH free radical scavenging assay of these extracts shown in Table 1.

Table 1. Yield fractionation and antioxidant activity (DPPH free radical scavenging activity) from *A.communis* extracts.

Sample	Weight (g)	IC_{50} (µg mL^{-1})*
Ethanol extract	250	38.98
Hexane extract	6.03	298.50
Dichloromethane extract	48.58	59.20
Ethyl acetate extract	12.11	42.48
Butanol extract	5.12	115.47
Residue	111.76	635.49

Note: Extract consider active if IC_{50} value is < 100 µg mL^{-1} [19]

Table 1 showed that the dichloromethane extract has significant antioxidant activity against DPPH. Based on the antioxidant activity above, the dichloromethane extract was selected for further antioxidant study. The dichloromethane extract was further fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate, ratio from 100:0 to 0:100), were resulted 80 fractions. Each fraction was evaluated for its activity against DPPH. Compound F-1 is the most active fraction with IC_{50} of 54.6 µg mL^{-1} (Table 2) than other fractions (IC_{50} > 100 µg mL-1). In the earlier study[17], F-1 as the potential compound, was identified as prenylated flavonoid, 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone) (Figure 2).

Table 2 shows that the antioxidant activity of

compound F-1 was lower than positive controls BHA and ascorbic acid, but similar with BHT. This result indicated that compound F-1 has potent as an antioxidant.

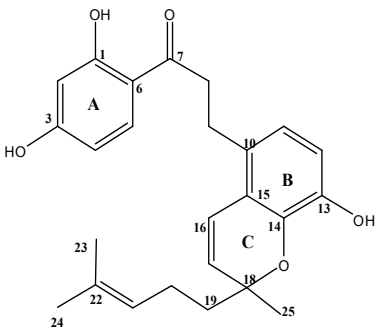

Figure 2. Molecular Structure Compound F-1 [1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1- benzopyran-5-yl]-1-propanone][17].

Table 2. DPPH free radical scavenging activity of F-1 compound.

Sample	IC_{50} (μg mL^{-1})
F-1	54.6
BHA	11
BHT	49
Ascorbic Acid	11

BHA, BHT, Ascorbic acid: positive control

CONCLUSION

1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (F-1) compound isolated from *A. communis* leaves, has moderate antioxidant activity with IC_{50} of 54.6 μg mL^{-1}.

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