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Secondary Metabolites and Antioxidant Activity of Methanol Extract of *Castanopsis costata* Leaves

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Abstract

Castanopsis costata (C.costata) leaves were empirically used by people in North Sumatera, Indonesia, as an antioxidant dietary supplement. However, its pharmacological effect has not been scientifically explored. The purpose of this study was to identify the secondary metabolites and antioxidant activity of *C. costata.* The leaves were cold extracted with methanol. The secondary metabolites were determined using thin layer chromatography. Its antioxidant activity was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The results showed that methanol extract of *C. costata* leaves consisted of alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins and triterpenoids. The methanol extract of *C. costata l*eaves showed comparable antioxidant activity with vitamin C, IC_{50} 35.56 µg/ml and 14.17 µg/ml, respectively.

Keywords: *Castanopsis costata* leaves, methanol extract, antioxidant, DPPH, vitamin C.

Introduction

Free radicals reactive oxygen species are responsible for development of degenerative diseases such as atherosclerosis, coronary heart disease, stroke, cancer, and kidney failure. They are derived either from normal essential metabolic processes or from external sources such as exposure to x-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. [1-3]

Endogoneous antioxidants, such as catalase enzymes bound to Fe, glutathione peroxidase, glutathione S-transferase binding to Se, superoxide dismutase binding to Cu, Zn and Mn are responsible to diminish free radical activities. However, an imbalance between free radical production and antioxidant can result in oxidative stress. Exogenous antioxidants are necessary to prevent harm caused by oxidative stress.[4]

Antioxidant compounds can be either from synthetic or natural compounds. Several studies showed that synthethic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl

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gallate (PG), and tert-butylhydroquinone (TBHQ) can cause several side effects.[5] It encourages investigation to find new natural compounds as natural antioxidants which are safer for human health.

Natural compunds can be used as new sources of antioxidant. Major plant antioxidants were resulted from shikimic acid pathway and phenyl-propanoid metabolism. One of the plant that is empirically used as dietary antioxidant supplement is *Castanopsis costata* (*C costata*) with the local name *cep-cepan* plant. However, its pharmacological effect has not been scientifically explored yet. Thefore, this study was conducted to identify the secondary metabolites and antioxidant activity of *C. Costata.*

Methods

The materials used in this study were fresh *C. Costata.* leaves, 1,1-Diphenyl-2-picryl hydrazyl (DPPH), methanol, and destilled water. The chemicals used included alpha-naphthol, ammonia, acetic acid anhydride, glacial acetic acid, concentrated hydrochloric acid, nitric acid, concentrated sulfuric acid, acetone, benzene, iron (III) chloride, bismuth (III) nitrate, iodine, isopropanol, potassium hydroxide, potassium iodide, whatmann paper no.1, chloroform, cuprisulfate, magnesium powder, mercury (II) chloride, sodium hydroxide, zinc powder, lead (II) acetate. Instrument used in this study was spectrophotometer ultraviolet-visible (Shimadzu).

Plant determination

Identification and determination of *C. Costata.* were conducted at Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Extraction

Cold extraction was obtained by immersing the sample in an methanol at room temperature

Table 1. Phytochemical screening of *C. costata* leaves methanol extract

No	Secondary metabolite	Reagent	Observation	Result
1	Alkaloid	Dragendorff Bouchardat Mayer	(+) Light brown (+) Dark brown (+) Muddy and white sediment	(+) Alkaloid
2	Flavonoid	Zn + HCl (p) Mg + HCl (p)	(+) Red	(+) Flavonoid
3	a. Glycoside b. Non Sugar	1. Molish 2. Fehling A+B Anhydride acetic acid + sulfuric acid	(+) Purple ring (+) Brown-red sediment (+) Brown-purple	
4	Saponins	Hot water + HCl	(-) Bubble	(-) Saponins
5	Glycoside Antraquinon	NaOH	(+) Purple-red in NaOH layer	(+) Glycoside Antraquinon
6	Tannin	$FeCl_3$ 1%	(+) Yellow	(+) Tannin
7.	Triterpenoid	Anhydride acetic acid + sulfuric acid	(+) Purple-brown	(+) Triterpenoid

Table 2. DPPH absorbance of *C. costata* and vitamin C.

No	Extract	DPPH	Extract with various concentration (µg/ml)				
			10	20	30	40	50
1	*C. costata*	0.69974	0.37671	0.35863	0.36457	0.34773	0.31025
		0.69951	0.37697	0.35872	0.36416	0.34735	0.31472
		0.69927	0.37965	0.35879	0.36445	0.34769	0.31799
2	Vitamin C	0.69974	0.19834	0.18540	0.17831	0.15271	0.11756
		0.69951	0.19832	0.18564	0.17743	0.15432	0.11773
		0.69927	0.19837	0.18553	0.17361	0.15925	0.11732

for 5 x 24 hours. Separation of residue and filtrate were done every 1 x 24 hours.The filtrate was collected and concentrated using a rotary evaporator at 58^{0}C.[6,7]

Phytochemical screening

Phytochemical screening was performed using thin layer chromatography (TLC) and chemical reagents to prove the presence of bioactive compounds.

Antioxidant activity

Determination of antioxidant activity was performed using DPPH.[8] DPPH 200 µg/ml stock solution was made by dissolving 20 mg of DPPH to 100 ml methanol. Sample solution was obtained by dissolving 25 mg of viscous extract with 25 ml of methanol. It was then diluted into 10, 20, 30, 40, and 50 µg/ml. It was mixed until homogeneous and incubated at 37°C for 30 minutes. The antioxidant activity was carried out using spectrophotometry UV-Vis at a wavelength of 515.50 nm. The analysis was replicated three times. The inhibition concentration (IC_{50}) of DPPH was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}}$$

Results and Discussion

Plant determination

Plant used in this study was determined as *C. costata* with the following identity:

Kingdom : *Plantae*
Division : *Tracheophyta*
Class : *Magnoliopsida*
Ordo : *Fagales*
Family : *Fagaceae*
Genus : *Castanopsis*
Species : *Castanopsis costata.*

The extraction and phytochemical screening

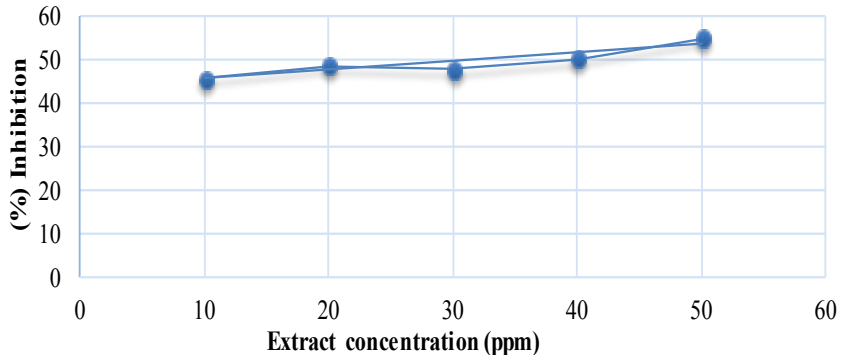

Figure 1. Inhibition activity of methanol extract of *C. costata* on DPPH

Table 3. Antioxidant activity of *C. costata* and vitamin C

Sample	IC_{50} (ppm)	Value	Information on IC_{50} (ppm)
Methanol extract	35.56	Very strong	< 50 (very strong), 50-100 (strong), 100-150 (average), 150-200 (weak)
Vitamin C	14.17	Very srong	

The maceration resulted 4.0 g of viscous methanol extract of *C. Costata*. Phytochemical screening showed that this extract consisted of alkaloid, flavonoid, glycoside, antraquinon, tannin,and triterpenoid. The result is presented in the Table 1.

Antioxidant activity

Quantitative antioxidant activity determined by DPPH method was expressed as IC*50*, *i.e.,* the concentration required to inhibit 50% of DPPH free radicals.[9] Antioxidant activity of *C. Costata* methanol extract was performed at different concentrations. We found that the higher the concentration of the extract showed the higher of the percentage value of the inhibition. We observed a decrease in the absorbance of DPPH by the addition of combination of *C. Costata* and vitamin C. The higher concentration of the extract yielded the greater reduction of absorbance (Table 2, Figure 1).

We found that antioxidant activity of *C. Costata* leaves was comparable with that of vitamin C, which was in the category of very strong activity (Table 3). Polar substances such as polyphenol (flavonoid), cinnamic acid derivatives, coumarin, and tocopherol in *C. Costata* might contribute in its antioxidant activity.[9,10]

Polar substances are more easily dissolve in polar solvent such as methanol, ethanol, and water. Methanol has a polarity index of 5.1. Most polar compunds can be extracted using methanol and certain group of non polar compounds are fairly soluble in methanol. Therefore, methanol is generally preferred for the extraction of bioactive compounds. Beside, it is relatively inexpensive and easily evaporated.[10-14]

This is the first study investigating *C. costata* leaves antioxidant activity. This analysis could provide insight into the variations in the antioxidant profiles between different concentration of *C. Costata*. Further research might be necessary to examine the mechanism of its antioxidant activity.

Conclusion

The methanol extract of *C. costata* leaves had comparable antioxidant activity with vitamin C, which showed the IC_{50} value of 35.56 ppm and 14.17 ppm, respectively. Secondary metabolites of *C. Costata* leaves consisted of alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins and triterpenoids.

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