ANTIOXYDANT ACTIVITY OF LEAVES EXTRACTS FROM *Gnetum gnemon* Linn USING DPPH

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ABSTRACT

Background: *Gnetum gnemon* Linn leaves usually used as a vegetable. It has an antioxidant substances such as polyphenols and flavonoids. Antioxidant are substances that protect other chemicals of the body from oxidation reactions by reacting with free radicals and other reactive oxygen species.

Objective: The aim of this research were to study antioxidant activity of leaves extract from *Gnetum gnemon* Linn using DPPH (1,1-diphenyl picrilhydrazyl 1-2) essay

Methods: Extraction of *Gnetum gnemon* Linn leaves was performed by reflux using gradient polarity solvent. The extracts were vaporated using rotavapor. Chromatogram pattern on each extract of samples were observed by Thin Layer Chromatography (TLC) then sprayed with 0,2% DPPH solution. Antioxidant activity (IC_{50}) of each extracts using DPPH assay and analyzed by spectrophotometry UV-VIS.

Outcome measured : Antioxidant activity (IC_{50}).

Results : The results of the research showed the presence of the antioxidant activity in n-hexane, ethyl acetate and ethanol extract which characterized by yellow spots with a purple backgroud on the TLC plate in visible light. The IC50 value using DPPH essay of n-hexane, ethyl acetate and ethanol extract obtained 755.50 µg/mL, 80.92 µg/mL, 39.10 µg/mL respectively.

Conclusion: The *Gnetum gnemon* Linn leaves has antioxidant activity was given by ethanol extract but it has not stronger than vitamin C.

Keywords: *Gnetum gnemon* Linn leaves, antioxidant activity, DPPH, free radicals

INTRODUCTION

The use of antioxidant compounds is growing both for food and for treatment along with increased knowledge about the activity of free radicals (Boer, 2000). Oxidative stress is a condition that is no balanced between the number of molecules of free radicals and antioxidants in the body (Trilaksani, 2003). Antioxidant compound is an inhibitor that is used to inhibit autooxidation. Effect of phenolic compounds due to oxidation properties played a role in neutralizing free radicals (Panovska, 1982) .

The research on *Gnetum gnemon* Linn plants showed that *Gnetum gnemon* Linn produces an antioxidant compounds. The seed contained polyphenolic compounds (phenols, flavonoids, and tannins), which acts as an antioxidant that can reduce free radicals activity(Santosa, 2010).

The objective of this research were to study antioxidant activity of leaves extracts from *Gnetum gnemon* Linn using DPPH (1,1-Diphenyl picrilhydrazyl 1-2) essay.

METHODS

Materials

Leaves of *Gnetum gnemon* Linn, DPPH (2,2-diphenyl-1 picrylhydrazyl), methanol, ethanol, ethyl acetate, n-hexane, ascorbic acid, amyl alcohol, magnesium powder, kalium bromide, sodium hydroxyde, TLC plate GF254.

Preparation of sample

Leaves of *Gnetum gnemon* L. were collected from Tasikmalaya were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux using increasing gradient polarity solvents. The n-hexane extract was repeated three times. The remaining residue was then extracted three times with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were n-hexane extracts, ethyl acetate extracts and ethanolic extracts.

Phytochemical Screening

Each extracts was screened using appropriate reagents. Phytochemical screening aimed to identify the secondary metabolite content of each extracts of *Gnetum gnemon* Linn leaves.

DPPH assay on TLC

DPPH assay with TLC was used to measure the antioxidant activity of extracts. Method of Bektas was followed ; 1:10 dilution of each extract was made in methanol. Five microlitres of this dilution was applied on the TLC plate. Plate was developed by n-hexane-ethyl acetate in ratio of 9:6 for the n-hexane and ethyl acetate extracts, while the ethanol extract was chloroform-acetone in ratio of 7:3.. Then the plate was sprayed with 0.2% of DPPH reagent in methanol and stayed for 30 minute at room temperature. Purple colour of DPPH reagent bleaching by yellow spots is the indication of positive antioxidant activity.

DPPH radical scavenging activity

DPPH radical scavenging activity of each extracts was determined according to the method of Blois. Briefly, 50 µg/mL of extract was added to 50 µg/mL of DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution (in methanol) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbuc acid was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula: DPPH Radical Scavenging Activity (%) = [(A0 – A1 /A0) ×100], where A0 is the absorbance of the control, and A1 is the absorbance of extract or standard sample. To determine an IC50 value, prepare each extracts in various concentrations as well as standard compound (ascorbic acid). take 2 mL of the sample as well as the standard then mixed with 2 mL of DPPH solution (volume ratio 1:1) and incubated for 30 minute. The absorbance was taken after 30 minute at 517 nm using methanol as blank on UV-visible spectrometer. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50}

value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

RESULTS AND DISCUSSION

Medicinal plants are rich sources for natural occurring antioxidants. Among these substances, the phenolic compounds have the ability to scavenge free radicals, super oxide and hydroxyl radicals through single-electron transfer reactions (4,5). In this study, we identified antioxidant substances of each extracts of *Gnetum gnemon*. The phenolic and flavonoid substances of each extracts was identified in screening phytochemical results (Table 1).

Table 1. Phytochemical screening of each extracts leaves from *Gnetum gnemon* Linn

substances	Extract		
	n-heksane	ethyl acetate	Ethanol
Alkaloid	-	-	-
Flavonoid	+	+	+
Tannin	-	-	+
Phenolic	+	+	+
Steroids and triterpenoids	-	-	-
Kuinon	-	-	+
Saponin	-	-	-

DPPH assay on TLC

Thin layer chromatography was conducted to determine the separation of compounds in the extracts. The results showed purple colour of DPPH reagent was bleached by yellow spots, it means the indication of positive antioxidant activity. The presence showed on TLC for each extracts (figure 1).

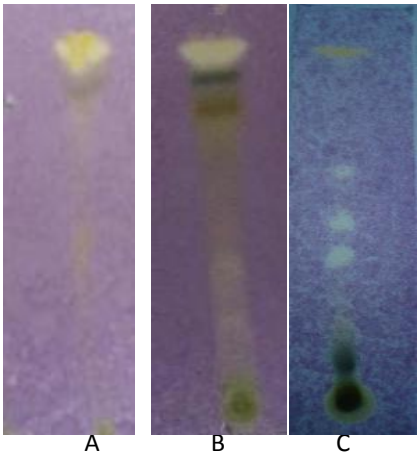

Figure 1. The presence of antioxidant activity of leaves extract on TLC A. n-hexane extract, B. Ethyl acetate extract, C. Ethanol extract

DPPH radical scavenging activity

The ability of each extracts to scavenge the DPPH radical measured as IC_{50} varied significantly. The Ethanol extract showed high antioxidant activity with their IC_{50} 38,83 µg/mL, in moderate antioxidant activity were ethyl acetate extract (IC_{50} 80,83µg/mL) and in low levels were n-hexane extract (IC_{50} 755,5 µg/mL). While the IC_{50} of ascorbic acid were 4,21 µg/mL.(table 2)

Table 2. IC_{50} value of each extracts from melinjo leaves and ascorbic acid with DPPH essay

materials	IC_{50} value (µg/mL)	antioxidants intensity[9]	Range of IC_{50} value[9]
n-hexane	755,5	weak	> 150 µg/mL
ethyl acetate	80,83	strong	50-100 µg/mL
ethanol	38,83	Very strong	< 50 µg/mL
ascorbic acid	4,21	Very strong	< 50 µg/mL

CONCLUSION

In our research, showed that the *Gnetum gnemon* Linn leaves was good sources of dietary antioxidants as determined by the chemical DPPH radical scavenging assay. The ethanol extract of melinjo leaves showed a high antioxidant activity but not as high as ascorbic acid.. However, the ability of these plant to protect cell components from oxidative damage remains to be investigated.

DISCLOSURE : -

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