International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 770-772

Received: 22-09-2017
Accepted: 25-10-2017

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Evaluation of DPPH free radical scavenging activity of *Magnolia coco* flowers

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Abstract
The *Magnolia coco* flower which is widely used in traditional medicine and aromatic spa has been extracted. The extraction used solvent of methanol, ethyl acetate, and n-hexane to analyze its antioxidant activity. The research revealed that DPPH free radical scavenging activity of *Magnolia coco* extract varied widely increased with increase of concentration level. The *Magnolia coco* flowers which extracted methanolic had the highest antioxidant activity was 4,138 µg/mL. On the other hand, ethyl acetate and n-hexan extract had no antioxidant activity.

Keywords: Free radical scavenging, *Antioxidant activity, Magnolia coco, Extraction*

Introduction
Reactive oxygen species are necessary for the cell to carry out several biochemical functions as cell signaling, apoptosis or metabolism of xenobiotic [1]. However, free radical are toxic in high quantities during an imbalance of redox state as long as the generation of reactive oxygen species in neuronal disease plays important role in the neuronal cell death and neurological dysfunctions [2]. An antioxidant is required to protect their possible damages to biological molecules and to maintain an optimum balance of free radicals in human body. Epidemiological studies indicated that the antioxidant properties in fruits can use as protectors against certain diseases [3].
Although free radicals are linked to a transition of acute to chronic pain [4], recent studies have demonstrated that the relationship between free radicals and chronic pain with two oxidant like superoxide. DPPH method used to determined antioxidant activity caused DPPH as free radicals could be scavenged by active compound. Magnolia coco knows as "kembang telor" in Indonesia is widely used in traditional medicine and aromatic spa caused contains essential compound and secondary phytochemicals compounds. In addition to flowers as potential resources of antioxidant that have rich of polyphenol [5, 6]. The low incidence of chronic diseases in societies whose diets are rich in fruit, vegetable and derived products has been extensively document [7, 8]. Many studies have determined the antioxidant capacities of flowers extract or aromatic spa. Thus, the aim of this study was to measure antioxidant activity of hexane, ethyl acetate and methanolic extract of Magnolia coco flowers and to evaluate DPPH free radical scavenging activity.

Material and Method
General experimental procedures
Magnolia coco was harvested from local area in Bogor, West Java. The flower of Magnolia coco has to be washed and cutted. Reagent were methanol, ethyl acetate, n-hexane, 1,1-Diphenyl-2-picrylhydrazil (DPPH) were purchased from Merck. All chemicals used were of analytical grade.

Sample Extraction
Sample preparation was conducted by maceration using several organic solvents. A 25 g of fresh flowers of *Magnolia coco* were immersed in 100 mL of n-hexane for 3 days, and then filtered. Filtrate was evaporated until dry sample was obtained, and this step resulted in raw extract of n-hexane. The residue from first immersion was entirely immersed back in 100 mL ethyl acetate for 3 days to obtain raw extract of ethyl acetate.

The solution was then filtered and evaporated, and the residue from this step was immersed in 100 mL methanol for 3 days, resulted in raw methanolic extract. The maceration process was repeated several times to obtain clear extract containing all of expected chemical species.

Antioxidant activity

Antioxidant activity of fresh flowers of Magnolia coco extract was tested by monitoring radical scavenging activity using DPPH method [9]. Briefly, 1 mL of 200 µM DPPH (1,1-diphenyl-2-picrylhydrazil) solution in methanol was pipetted and transferred to vial. The samples were prepared separately to obtain 200µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL and 1000 µg/mL solution in methanol, and transferred to the vials which contain 200 µM DPPH. Each vial was diluted by adding methanol until the total volume of 5 mL. The absorbance of DPPH solution was measured by = 517 nm, every 5 minutesλUV-Vis spectrophotometer at for total of 30 minutes. Antioxidant activity was calculated as a function of absorbance decrease of DPPH solution as a consequence of sample addition.

$$\text{DPPH}_{\text{Scavenging Activity}}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \text{ X } 100$$

Where, $A_{control}$ is absorbance of control and A_{sample} is the absorbance of the sample.

Result and Discussion

DPPH free radical scavenging activity of methanolic extracts of magnolia coco flowers

The DPPH radical scavenging assay is a convenient and fast technique to evaluate antioxidative activity [10]. DPPH is a deep-purple colored stable free radical, color of which changes from violet to yellow in the hydrogen-or electron donation process and becomes a stable diamagnetic molecule [11]. DPPH is nitrogen centered free radical having an odd electron which gives a strong absorption at 517 nm, the DPPH assay results are indicative of the hydrogen donating propensity of a test compound. Likewise, the antioxidant activity of plant extracts is also correlated with their reducing powers, which are generally associated with the presence of reductones. DPPH free radical scavenging activity was evaluated in terms of percent reduction (%) of the initial DPPH absorption, and the results were expressed as relatives activity from methanol, n-hexane and ethyl acetate extract of *Magnolia coco* flowers (Table 1). Free radical scavenging activity widely increased with increase of concentration levels with line linearity (R^2) was 0.9692 to 0.9973 (Table 1). Among several solvent, DPPH free radical scavenging activity (%) of methanol extract was the highest ranging 38.34 to 78.70, followed by ethyl acetate (0.02 to 24.15), and n-hexane (0.00 to 1,21) at concentration 1000 to 10000 µg/mL. The IC_{50} values of methanol extract was exhibited higher activity (4,138µg/mL) in comparison to both the other solvent. The n-hexane and ethyl acetate of *Magnolia coco* flowers showed no antioxidant activity because IC_{50} value was very high. Other research showed the highest of DPPH radical scavenging activity was possibly caused by secondary phytochemicals compounds in them such as phenolic acid and flavonoid compounds [5, 6].

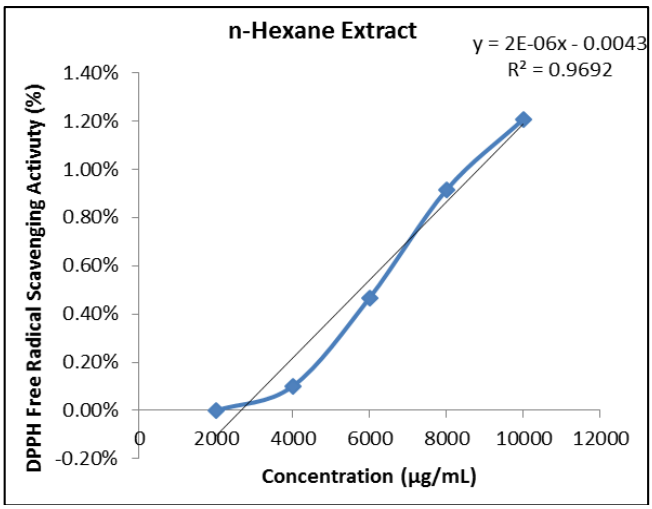
Table 1: DPPH Free Radical Scavenging Activity (%) of *Magnolia coco* Flowers

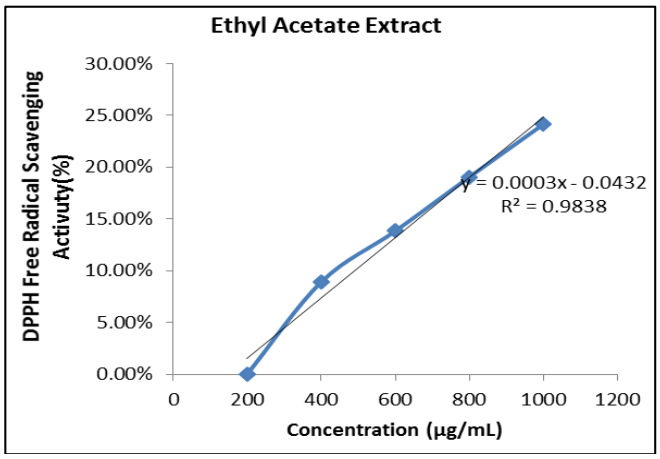
Solvent	DPPH Free Radical Scavenging Activity (%) of *Magnolia coco* Flowers						
	2000 µg/mL	4000 µg/mL	6000 µg/mL	8000 µg/mL	10000 µg/mL	Regression equal	IC-50 (µg/mL)
Methanol	38.34	50.16	60.06	69.76	78.70	y = 0,005x + 29,308 R² = 0.9973	4138
Ethyl Acetate	0.02	8.92	13.84	19.05	24.15	y = 0,0003x - 0,0432 R² = 0.9838	NA
n-Hexane	0.00	0.10	0.47	0.92	1.21	y = 2E-06x - 0,0043 R² = 0.9692	NA

NA = Non Activity

Based on Table 1 showed the differences of IC_{50} values of DPPH scavenging radical activity might be caused by differences solvent used. The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity [12]. Since the numerous investigation of qualitative composition of plant extract revealed the presence of high concentrations of phenols in the extracts obtained using polar [13], and the other hand ethyl acetate was semi polar solvent and n-hexane was non polar solvent.

Extraction of *Magnolia coco* flowers using different solvent has been resulted graphic of increasing of DPPH free radical scavenging activity. Graphical representative of DPPH free radical scavenging activity (% inhibition) of n-hexane, ethyl acetate and methanolic extract of *Magnolia coco* flowers at different concentration levels is given Fig 1.





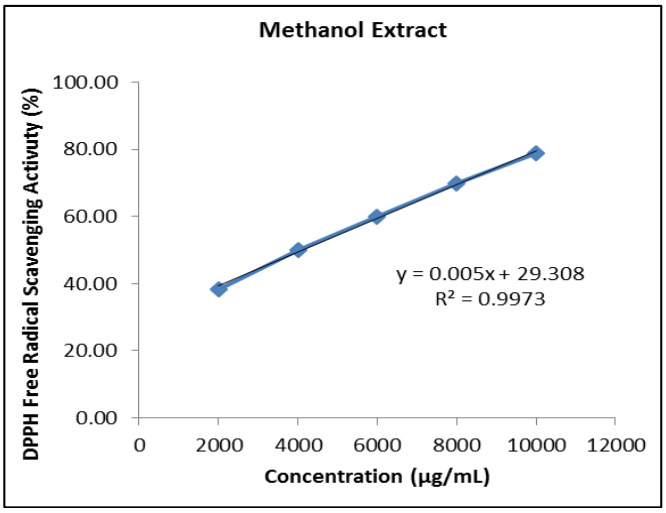


Fig 1: DPPH Free Radical Scavenging Activity of n-hexane, Ethyl acetate and Methanolic Extract of *Magnolia coco* Flowers

Conclusion

The present study revealed DPPH free radical scavenging activity of Magnolia coco Flowers which extracted with methanol, ethyl acetate, and n-hexan widely increased with increase of concentration level. The *Magnolia coco* flowers which extracted methanolic had the highest antioxidant activity was 4,138 µg/mL. On the other hand, ethyl acetate and n-hexan extract had no antioxidant activity.

Acknowledgements

This work was financially supported by Polytechnic of AKA Bogor. The authors are also grateful to the institution for providing several apparatus and instrumentation.

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