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Antioxidant activity of luteolin extracted from nutshell waste *Arachis Hypogea*

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Abstract
Luteolin is a plant flavonoid and pharmacologically active agent that has been isolated from several plant species. In the present study, fractionation using methanol, petroleum ether, and ethyl acetate was performed to extract luteolin from the nutshell waste, *Arachis hypogea.* Its antioxidant activity was then examined with DPPH radical scavenger method. Our results show that luteolin obtained from the nutshell waste has strong antioxidant activity (IC_{50} = 8.772 ppm). Thus it would be useful to agro-solid waste management as well as pharmaceutical purposes.

Keywords: antioxidant, *arachis hypogea*, luteolin, nutshell waste management

Introduction

Excess free radicals could cause damage to cellular protein, membrane lipid, and nucleic acid, and also cause cell apoptosis. An antioxidant is required to protect these biological molecules and to maintain an optimum balance of free radicals in human body [1]. Epidemiological studies indicated that secondary constituent in plants such as phenolics, flavonoids, and terpenes can be used as antioxidant agent. Luteolin, a 3', 4', 5,7-tetrahydroxyflavone, is one of plant flavonoid and pharmacologically active agent that has been isolated from several plant species [2-6].

Peanut (*Arachis hypogea*) seeds were widely used as food or processed food while its shells were used as animal food or just thrown as waste. A study conducted in Korea shows that nutshell has high luteolin content compared to other plants, reaching 4485 mg/kg [2]. Thus nutshell is a potential source of luteolin to be developed. The objective of this study was to extract luteolin from nutshell waste and evaluate its antioxidant activity, so that it can be used as a source of potent antioxidants to maintain health.

Material and Methods

General experimental procedures

All chemical reagents were of analytical grade and used without further purification unless otherwise stated. Nutshell waste was obtained from peanut home industries in Bogor. Luteolin standard, ethanol, DPPH (1, 1 *diphenyl picryl hidrazin*), methanol, petroleum ether, ethyl acetate, and acetic acid glacial were purchased from Merck (Germany). All solutions used in high performance liquid chromatography analysis (HPLC LC-20AD, Shimadzu, Japan) were filtered and degassed using a 0.22 µm membrane filter with a filtration system.

Luteolin extraction

The nutshell of *Arachis hypogea* (3 kg) was air-dried, powdered and then macerated with 95% methanol (3 × 400 mL, 2 h each). The combined methanol solution was filtered and evaporated under reduced pressure to yield a crude extract which was dissolved in 80% ethanol (400 mL), and extracted with petroleum ether (60~90 °C, 3 × 400 mL, 2 h each). The petroleum ether (PE) layer was removed. Then, evaporation of the aqueous layer under reduced pressure yielded a brown residue which was dissolved in water (800 mL), and then extracted with ethyl acetate (5 × 800 mL, 4 h each) to form an ethyl acetate extract.

To identify luteolin, the ethyl acetate extract was filtered through a 0.22 µm milipore membrane. The filtered sample was separated using a high performance liquid chromatography (HPLC) equipped with Diamonsil ODS C_{18} reversed-phase column. The optimum separation of HPLC was carried out with a mobile phase composed of methanol and 0.3% acetic acid solution (60:40, v/v) at flow rate of 0.8 mL/min. Elution of luteolin was detected by UV detector set at 350 nm. The luteolin content of peanut shells was calculated by comparisons with known concentrations of their standards.

Antioxidant activity

Antioxidant activity of luteolin extracted from nutshell was determined using radical scavenging DPPH method. A sum of luteolin extracted from nutshell was weighed and dissolved in 95% ethanol then diluted to obtained sample solution series with concentration ranged from 1-30 ppm. Sample solutions then filtered, resulting a clear yellow-green solution.

DPPH scavenging activity then performed. Briefly, 1 mL of DPPH (1,1-diphenyl-2-picrylhidrazyl) solution (400 µM in methanol) was pipetted into reaction tube then added by 3 mL 95% ethanol and 0.1 mL of each sample prepared before. The mixtures then vortexed and the absorbances were measured at 0, 5, 10, 15, and 20 minutes using UV-Vis spectrophotometer (λ 517 nm). The antioxidant activity was calculated as a function of absorbance decrease of DPPH solution as a consequence of sample addition. All procedures were carried out in triplicate. The antioxidant activity of luteolin extracted from nutshell was then compared to that of vitamin C as standard.

Result and Discussion
Luteolin Extraction
Luteolin was extracted from nutshell waste using methanol, petroleum ether, and ethyl acetate as solvent in sequence, followed by RP-HPLC analysis to identify and quantify luteolin content [6, 7]. In this study, we have successfully obtained 15.4 grams ethyl acetate fraction from 3 kg of nutshell. The ethyl acetate fraction was then analyzed and quantified using HPLC. The result showed it had similar time retention with luteolin standard (Fig.1), thus it can be deduced that we successfully extracted luteolin from nutshell waste, with luteolin content 83% (reaching 4260.67 mg/kg).
Our result is similar to those of Radhakrishnan's that obtained 546.8–4485.0 mg/kg luteolin from varied Korean peanut cultivars. In an earlier study, Miean and Mohamed (2001) detected the luteolin content only in broccoli (74.5 mg/kg), green chili (33.0 mg/kg), bird chili (1035.0 mg/kg), onion leaves (391.0 mg/kg), belimbi fruit (202.0 mg/kg), belimbi leaves (464.5 mg/kg), French bean (11.0 mg/kg), carrot (37.5 mg/kg), white radish (9.0 mg/kg), local celery (80.5 mg/kg), limau purut leaves (30.5 mg/kg), and dried asam gelugur (107.5 mg/kg), among the 62 edible tropical plants [2]. In addition, luteolin was also isolated (133 mg/27 kg) from *Elsholtzia rugulosa* [6]. The results obtained from the current study suggest that nutshells have maximum amount of luteolin content than other plants. This result suggests that the abundant quantity of nutshell waste is a rich source of luteolin compared to others.

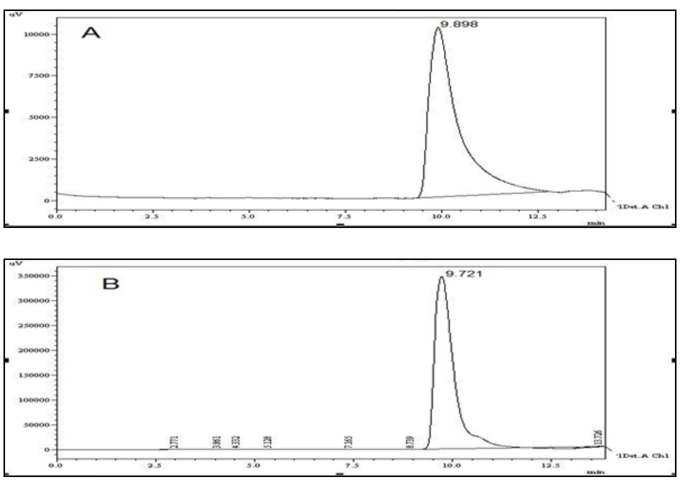

Fig 1: Chromatogram of (A) luteolin standard; (B) luteolin extracted from nutshell waste

Antioxidant activity
DPPH is nitrogen centered free radical having an odd electron which gives a strong absorption at 517 nm. Its color changes from purple to yellow when DPPH* odd electron paired off in the presence of radical scavenger to form the reduced DPPH-H. The DPPH radical scavenging activity is a good way to determine the antioxidant capacities in the samples [1]. In this present study, we monitored DPPH free radical scavenging activity of luteolin extracted from nutshell waste and compared to that of vitamin C as antioxidant standard. The activity was increased with the increasing of concentration level for both compounds, leading to graphics as can be seen in Figure 2, with each regression equation (y) and level of confidence (r^2) displayed on Table 1. As can be seen in Figure 2, luteolin extracted from nutshell waste showed a slightly weaker inhibition activity compared to that of vitamin C.
The IC_{50} for both nutshell luteolin and vitamin C was calculated from each regression equation (see Table 1). The IC_{50} value of luteolin extracted from nutshell waste was 8.772 ppm while vitamin C has 2.728 ppm IC_{50} value. Several references stated that the strength of antioxidant activity was classified (based on IC_{50} value) as strong when the value <100 ppm, moderate when the value is in the range of 100-500 ppm, and weak when the value is in the range of 500-1000 ppm [8]. Thus, our results show that luteolin extracted from nutshell waste has a strong antioxidant activity. These antioxidant properties are contributed by the presence of aromatic and oxygen rings present in the luteolin structure. Since the DPPH assay results are indication of the hydrogen-donating prospensity of a test compound and the antioxidant activity of plant extracts is also correlated with their reducing power, the ortho-dihydroxy structure of the B ring and the C2-C3 double bond conjugation with the 4-oxo functional group on C ring provide good antioxidant capacity of luteolin. Luteolin can also form chelates with metal ions, but not oxidized during the chelation process. This explains why luteolin does not experience redox cycles such as quercetin and other flavonols. The redox cycle is a process that increases the potential of the compound to be pro-oxidant with the possibility of a deleterious effect [1, 4].

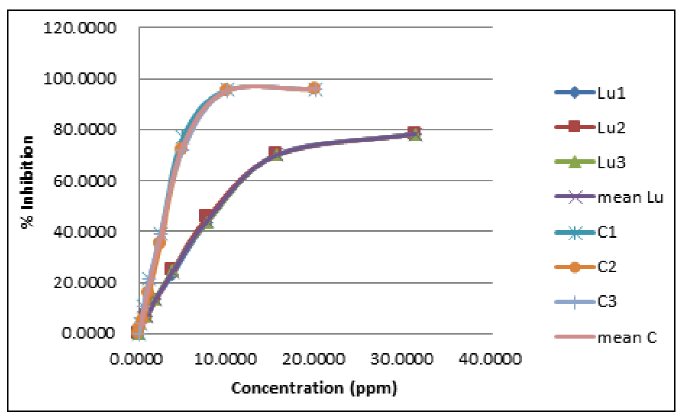

Fig 2: Antioxidant activity of luteolin extracted from nutshell waste compared to Vitamin C (Lu = nutshell luteolin, C = vitamin C, all procedures in triplicate)

Table 1: Antioxidant activity of Nutshell Luteolin and Vitamin C

Samples	Regression Equation	r^2	IC_{50} (ppm)
Nutshell luteolin	Y = 22.55ln(x) + 1.031	0.963	8.772
Vitamin C	Y = 25.98ln(x) + 23.92	0.944	2.728

Conclusion
Luteolin extracted from nutshell waste, *Arachis hypogea*, has a strong antioxidant activity with IC_{50} 8.772 ppm. Further research is needed for the commercialization of purification of

luteolin from nutshell waste as a potent antioxidant agent. Thus it would be useful to agro-solid waste management as well as pharmaceutical purposes.

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