

Research Article

Antioxidant properties of the isolated flavonoids from the medicinal plant *Phyllanthus niruri*

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Abstract

The antioxidant activity of the isolated flavonoids compound quercetin from chloroform and methanol extract of *Phyllanthus niruri* (dukung anak) was evaluated by various antioxidant assays such as free radical scavenging (DPPH) and antioxidant capacity (β -carotene bleaching). The antioxidant activities were compared to the standard antioxidant, butylated hydroxytoluene (BHT). Quercetin, gallic acid and methyl dehydrochebulate showed strong antioxidant activity with DPPH assays. However, methyl dehydrochebulate and gallic acid did not show significant activity with β -carotene assay. 1, 2- Benzendicarboxylic acid, bis (2-ethyl hexyl) ester showed no antioxidant activity in all of the experiments. Quercetin showed high inhibition activity of xanthine oxidase. This antioxidant activity is probably concentration dependent.

Keywords: *Phyllanthus niruri*, antioxidants, gallic acid, methyl dehydrochebulate, quercetin

Introduction

Phyllanthus niruri, is a small plant which grows mainly in tropical and subtropical regions in Central and South American countries, India and East Asia. It is one of the most important medicinal plants used by people in these countries for treatment of jaundice, asthma, hepatitis, urolithic disease, fever, malaria, stomachache and tuberculosis [1]. Chemical investigation of this plant has been carried out and several constituents were isolated such as lignans, alkaloids, flavonoids, tannins, phthalic acid, gallic acid and terpenoids [2-3].

Many antioxidant compounds from plant sources have been identified as free radical or active oxygen scavengers. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in food or medicine to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity [4]. Natural antioxidants can protect the human body from free radicals and arrest the progress of many chronic diseases as well as retard lipid oxidative rancidity in food [5-8].

Reactive oxygen species (ROS), including free radicals such as superoxide anion radicals ($O^{\cdot-}$), hydroxyl radicals (OH \cdot), singlet oxygen (1O_2), are various forms of activated oxygen and often generated by oxidation products of biological reactions or exogenous factors [5-8]. ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn the attention in much research work. ROS can cause lipid peroxidation in food, which leads to deterioration of the food [9]. In this paper, we describe the antioxidant activity of isolated compounds from the methanol and chloroform extract of *Phyllanthus niruri* (dukung anak).

Materials and Methods

Chemicals

(Tween-20), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nicotinamide adenine denucleotide (NADH), butylated hydroxytoluene (BHT), β -carotene, linoleic acid, xanthine, xanthine oxidase, rutin and allopurinol were purchased from Sigma Chemical Co. All other chemicals and reagents were analytical reagent grade.

Sample preparation

The isolated compounds were dissolved in methanol or dimethylsulfoxide (DMSO) to prepare the desired concentrations.

β -carotene assay

The method was adapted from Dimitris and John [10], with some modifications. Linoleic acid (0.02 ml) and tween 20 (0.2 ml) were placed in (100 ml) round flask, and (1ml) β -carotene

(0.2mg/ml in chloroform) was added to the flask. The mixture was evaporated to dryness under vacuum in the dark. Fifty milliliters of distilled water was added to mixture and shaken. 3.8 ml of the mixture was then dosed with 0.2 ml of corresponding concentration of tested sample or reference (BHT and rutin) compound to the final concentration (15 µg/ml). Sample without dose was used as control.

Absorbance was measured on spectrophotometer at 470 nm, the samples were then subjected to autoxidation, thermally induced with constant water bath temperature of 45°C for 2h. Absorbance was measured at 15 min intervals to monitor the rate of bleaching of β- carotene. Samples were covered with aluminum foil to protect from light oxidation (triplicate).

Free radical scavenging activity (DPPH Assay)

The scavenging activity of DPPH free radicals of compounds under investigation was done according to the method previously reported by Oktay, *et al* [5], with some modifications. The compounds were dissolved in methanol, 200 µl of each compound was mixed with 4 ml of 0.1 mM DPPH-methanol solution. The final concentrations were (1.25, 2.5, 3.75, 5, 5.5 µg/mL). Methanol was used as blank for this experiment. After 60 min of incubation at room temperature, the reduction of the DPPH free radicals was assessed by measuring the absorbance at 517 nm. The lower absorbance indicated higher free radical scavenging activity. BHT was used as positive control (twice).

Superoxide anions scavenging activity

Superoxid anions were estimated by the spectrophotometric measurement of the product of the reduction of nitro blue tetrazolium NBT, [5, 11]. Superoxide anions were generated in a non-enzymic system (phenazine methosulphate-NADH). The non-enzymic generation of superoxide anions was measured in samples which contained 10µM phenazine methosulphate, 78 µM NADH and 25µM NBT in 0.1M phosphate buffer pH 7.4. After 5min of incubation at room temperature the colour was measured at 560 nm against blank sample, which contained no phenazine methosulphate. The inhibition ratio (%) was calculated from the following equation;

$$\text{Inhibition \%} = \frac{(\text{Absorbance control} - \text{Absorbance of test sample})}{(\text{Absorbance control})} \times 100 \quad (1)$$

Xanthine oxidase inhibition

Xanthine oxidase (EC 1.1.3.22) inhibition activity was evaluated by spectrophotometric measurement of the formation of uric acid from xanthine reported by Jadwiga, *et al*. [11]. 100 µM solution of xanthine in 0.1M phosphate buffer pH 7.8 with 0.1 units/ml of xanthine oxidase was incubated for 10 min at room temperature and measured at 295 nm against a blank sample which did not contain the enzyme. Various concentrations of tested compounds and chemical

inhibitor allopurinol were added to samples before the enzyme had been instilled and their effect on the generation of uric acid was calculated from the equation;

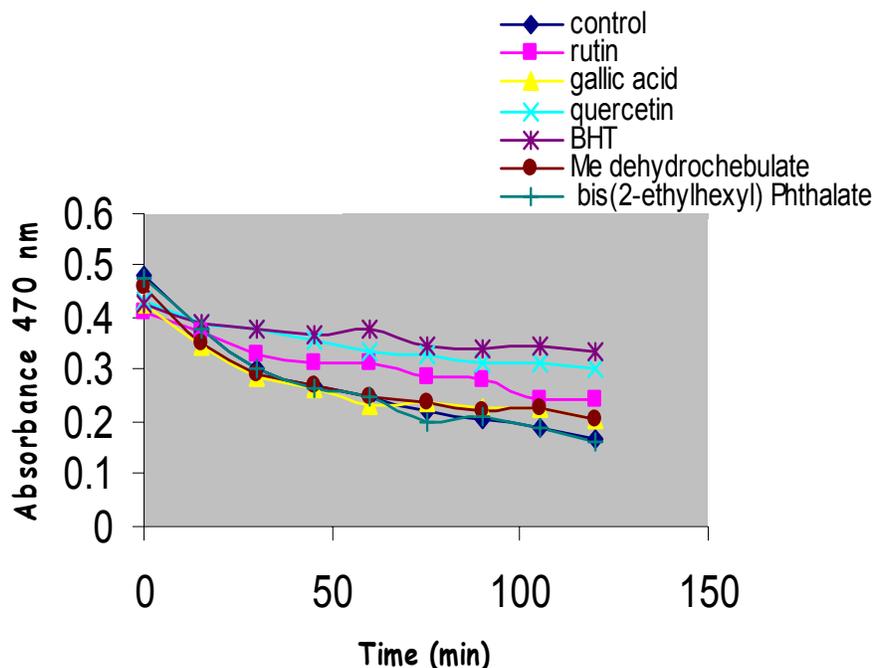
$$\text{Inhibition\%} = (1 - \text{As}/\text{Ac}) \times 100 \quad (2)$$

Statistical Analysis

Experimental results were means \pm S.D of two or three measurements. Differences between means were analyzed BY ANOVA one-way program. P values <0.05 were regarded as significant and P values <0.01 very significant.

Results and Discussion

β -Carotene bleaching activity used to assess potency of isolated compounds as antioxidants is a well-established model system based on β -carotene assay coupled reaction with autoxidized linoleic acid. There is a gradual decrease in 470 nm with β - carotene bleaching. Based on testing with the reference compound BHT, the concentration produces measurable inhibition in β -carotene bleaching. On the basis of the bleaching rates, three parameters relative to control were calculated in order to compare the antioxidant abilities of the compounds. The calculations of all three parameters were judged necessary for comparison because similar data are available in the literature. Figure 1 shows the results of antioxidant activity. The three parameters relative to control (A_A , ROR, and CAA) were calculated in order to compare the antioxidant abilities of the compounds [10]. BHT was the most effective antioxidant of the set and the most efficient compound with respect to all parameters ($p < 0.05$), as it gave the highest A_A and C_{AA} , and the lowest ROR. Quercetin was in the second order with respect to the parameters and gave significantly different values ($P < 0.05$) of A_A and R_{OR} and C_{AA} . Gallic acid and Methyl dehydrochebulate showed low antioxidant activity with respect to all parameters. It is generally assumed that the hydrogen donor ability and inhibition of oxidation are enhanced by increasing the number of hydroxyl groups in the phenol. However, gallic acid and me-dehydrochebulate having three hydroxyle groups did not exhibit exceptional antioxidant activity; this may be related to the hydrophilic character of gallic acid [12]. It is found that quercetin glucoside (Rutin) exhibited significantly different antioxidant activity compared to the control sample, but it was lower than that of aglycone (quercetin). This may be because the sugar moiety reduces the antioxidant efficiency of adjacent hydroxyl groups due to steric hindrance [13]. Whereas 1, 2-Benzenedicarboxylic acid Bis (2-ethylhexyl) ester showed no activity. Free radical scavenging activity of isolated compounds were assessed by DPPH assay. The isolated compounds exhibited antioxidant activity comparable to the references (BHT and Rutin). Quercetin, gallic acid and



Results are means \pm SD (n=3)

me-dehydrochebulate gave significant difference ($P < 0.05$) and higher inhibition than rutin and BHT. The results (Figure.2) indicated that the three compounds have noticeable effect on scavenging free radicals. However, compound 1, 2-Benzenedicarboxylic acids; Bis(2-ethylhexyl) ester did not show any degree of inhibition. Free radical scavenging activity was also increased with concentration.

There was no significant difference between the activity of quercetin and gallic acid. However, the difference in activity of quercetin and Rutin was significant ($P < 0.05$) due to steric hindrance [13].

In the PMS/NADH-NBT system (non-enzymic method), superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 556 nm with antioxidants thus indicated the consumption of superoxide anion in the reaction mixture (antioxidant). In this experiment all the compounds except 1, 2-benzene carboxylic acid bis(2-ethylhexyl) ester inhibited the reduction of NBT, (Fig. 3). Quercetin exhibited inhibition of

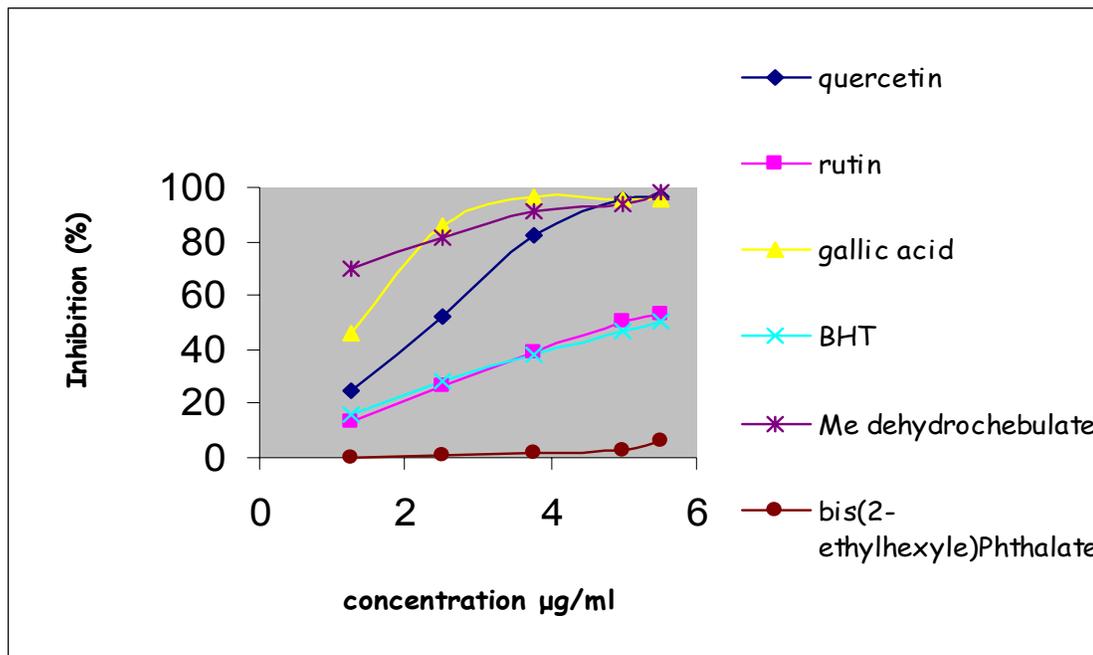


Figure 2. Free radical scavenging activity of isolated compounds (DPPH assay).

Results are mean \pm SD (n=3)

86.8% of xanthine oxidase while other compounds did not show any measurable inhibition (Fig. 4). The compounds isolated from methanol extract of *Phyllanthus niruri* (dukung anak), showed strong antioxidant activity power in β -carotene bleaching, DPPH radical and superoxide anion scavenging, when compared to standards. Quercetine exhibited inhibition of xanthine oxidase and uric acid formation which is considered as one of the factors responsible for kidney stone formation.

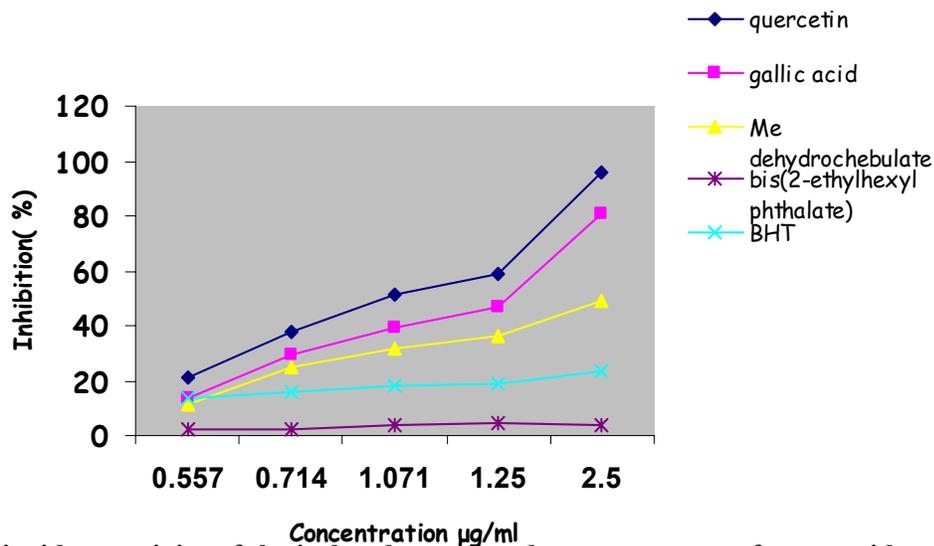


Figure 3. Antioxidant activity of the isolated compounds as scavengers of superoxide anions. Results are mean \pm SD (n=3)

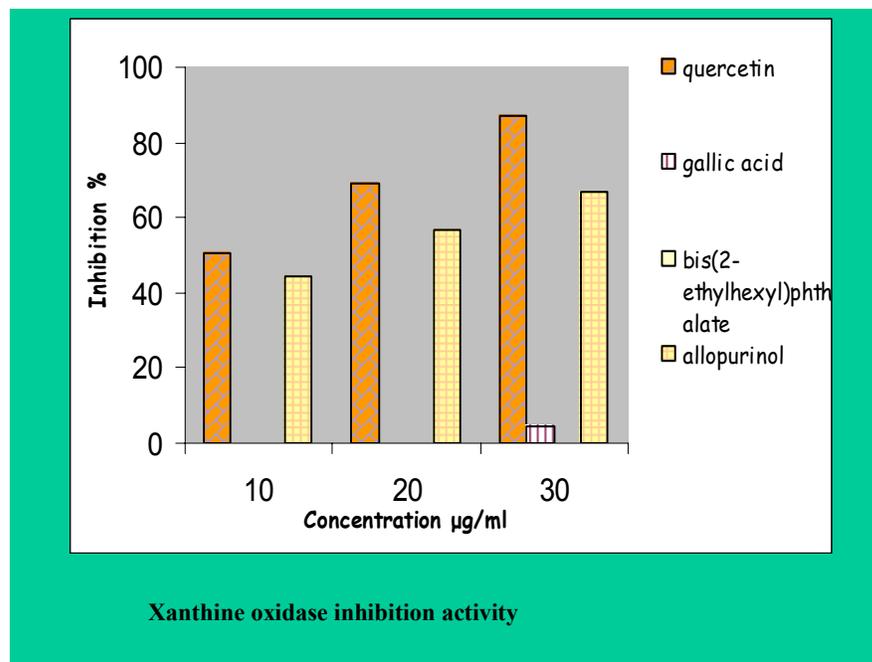


Figure 4. Xanthine oxidase inhibition activity of the isolated compounds. Results are means \pm SD (n=3)

Conclusion

The result of this study shows that the polar extract of *Phyllanthus niruri* (dukung anak) can be used as an easily accessible source of natural antioxidants for use as a food supplement or in pharmaceutical industries. Therefore, it is suggested that further work could be performed on the isolation and identification of antioxidants in this herb.

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