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Research Article

TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF ETHANOL FRACTION OF *PICRIA FEL-TERRAE* (LOUR.) HERBS

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ABSTRACT

Objective: To evaluate total phenolic, flavonoid content (TFC and TPC) andantioxidant activity of ethyl acetate fraction (ETF) of *Picria fel-terrae* Lour. herbs.

Methods: TPC and TFC in ETF were determined by Folin–Ciocalteu and colorimetric methods, and antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

Results: ETF was found to contain low levels of phenolic (17.71±1.26 mg gallic acid equivalent/g), total flavonoid (14.43±0.03 mg quercetin equivalents/g). Antioxidant activity from DPPH assay was measured as inhibitory concentration 25.72±0.13 µg/mL.

Conclusions: The results reveal that ETF of *P. fel-terrae* Lour. herbs has strong antioxidant activity. Our further study is to the isolation of compounds which responsible for antioxidant components.

Keywords: Phenolic, Flavonoid, Antioxidant, *P. fel-terrae* Lour., Ethanol, Herbs.

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INTRODUCTION

Free radicals are arising from metabolism process or environmental sources which interact simultaneously with the biological system. Reactive species are molecules or atoms that have an electronic stability and most reactive. Reactive oxygen species (ROS) are main sources of a primary catalyst which initiate the process of oxidation *in vivo* and *in vitro* and create oxidative stress. Oxidative stress products when reactive forms of oxygen are produced faster than they could be safely neutralized by antioxidant mechanisms and/or from a decrease in antioxidant defense. The uncontrolled production of oxygen free radicals and the undateables system of antioxidant protection results in the cause of many diseases, such as cancer, diabetes, heart diseases, Alzheimer's, and aging [1-6].

Antioxidants are the material when them present in low concentrations compared to those of an oxidizable substrate significantly defers or avoids oxidation of that substances ethnopharmacological studies conducted and expressed that a large number of indigenous plant species are being used as a source of herbal therapies [7,8].

Poguntano (*Picria fel-terrae* Lour.) have been used as a drug of colic, malaria, diuretic, fever, and skin disease [9]. Modern pharmacological investigations indicated that the extract of *P. fel-terrae* Lour. exerts diuretic, antipyretic, hepatoprotective, cardioprotective, antidiabetic, antioxidant, anti-inflammatory, anthelmintic, anti–asthma, and analgesic activities [10-19]. Moreover, *P. fel-terrae* inhibit hepatitis B (HB) e-antigen excreted by HepG2 2215 cell lines, suggesting to have anti-HB virus activity [20]. It can be developed as a co-chemotherapeutic regimen for breast cancer by inducing apoptosis and cell cycle arrest and suppressing cyclin D1 and Bcl-2 expression based on the recent studies [21,22]. In *in silico* analysis Picfeltarraenin IA and IB as potential PI3K and epidermal growth factor receptor inhibitor [23]. The aim of this study was to determine total phenolic and flavonoid content (TPC and TFC) and antioxidant activity of ethanol fraction of *P. fel-terrae* Lour. herbs.

METHODS

Plant and chemicals material

Fresh herbs of *P. fel-terrae* Lour. were collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. Chemicals used were $AlCl_3.6H_2O$ (Merck), distilled water, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), Folin–Ciocalteu (Sigma), gallic acid (Sigma), Quercetin (Sigma), sodium acetate (Merck), and sodium bicarbonate (Merck).

Preparation of ethyl acetate fraction (ETF)

The air-dried and powdered herbs of *P. fel-terrae* Lour. (1 kg) were repeatedly fractionated by maceration with n-hexane (3×3 day, 7.5 L), the powder was dried in the air and fractionated with ethyl acetate (3×3 day, 7.5 L), the powder was dried in the air and fractionated with ethanol (3×3 d, 7.5 L) at 25-30°C with periodical stirring. The filtrate was collected, and then evaporated to obtain a viscous fraction and then freeze dried to dry [7,10,21,24].

Determination of total phenol content (TPC)

The TPC of the sample was determined using Folin reagent. Briefly, 100 µL of ETF (500 µg/mL) was mixed with 7.9 mL of distilled water and 0.5 mL of Folin–Ciocalteu's reagent (1:10 v/v) and mixed using vortex for 1 minute. After mixing, 1.5 mL of 20% sodium bicarbonate solution was added, and the mixture was incubated for 90 minutes with intermittent shaking. The absorbance was determined at 775 nm with a spectrophotometer. Total phenolic concentration is interpreted as gallic acid equivalent (GAE) in mg/gram of extract. The methanol solution was to use a blank control [2,5,10,25]. The equation to determine total phenolic concentration:

$$C(GAE)=\frac{c\times V}{M}\times F$$

C(GAE): Content of phenolic as GAE, c: Concentration determined from standard curve (µg/mL), V: Volume which used in the assay (mL), M: Mass of the sample which used in the assay (g), and F: Dilution factor.

Determination of TFC

The amount of total flavonoids in the extracts was determined spectrophotometrically as previously reported. Briefly, 2 mL of ETF in methanol was mixed with 0.10 mL of 10% aluminum chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M) and 2.80 mL of distilled water. After incubation along 40 minutes, absorbance was measured at 432 nm with a spectrophotometer. To determine the content of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid content is expressed as quercetin equivalents (QE) in mg/gram of extract [2,26]. The equation to determine total flavonoid concentration:

$$C(QE)=\frac{c\times V}{M}\times F$$

C(QE): Content of flavonoid as QE, c: Concentration determined from standard curve (µg/mL), V: Volume which used in the assay (mL), M: Mass of the sample which used in the assay (g), and F: Dilution factor.

Free radical scavenging activity test

The DPPH assay was carried out according to the previous study with some modifications [27]. 0.2 mM solution of DPPH• in methanol was prepared, and 100 µl of this solution was added to various concentrations of ETF at the concentrations of 12.5, 25, 50, and 100 µg/ml. After 60 minutes, absorbance was measured at 516 nm. Quercetin was used as the reference control, and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples [2,5].

$$\text{Percentage of inhibition}=\frac{\text{Abscontrol-Abstest}}{\text{Abscontrol}}\times 100\%$$

Statistical analysis

Data were expressed as mean±standard deviation, which were analyzed using the SPSS 20 software.

RESULTS AND DISCUSSION

TPC and TFC

TPC was determined by the Folin–Ciocalteau method [28]. The ETF of *P. fel-terrae* Lour. herbs was found to contain low levels of phenolic content 17.71±1.26 mg GAE/g. Phenolic compounds are known as an antioxidant [29], and they are very important plant constituents because of their free radical scavenging ability due to their hydroxyl groups [30].

In the case of TFC, the ETF was given flavonoid content 14.43±0.03 mg QE/g. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, anti-ulcer, anti-allergic, antiviral, and anti-cancer activities [31]. They are capable of effectively scavenging the ROS because of their phenolic hydroxyl groups, and so they are potent antioxidants also [32].

Antiradical activity

Antiradical power of the plant samples was measured in term of hydrogen donating ability using DPPH which is a stable, nitrogen-centered free radical and produces deep purple color in methanol solution [33]. DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action [34] and has been largely used as a quick, reliable and reproducible at *in vitro* antioxidant activity assay [35]. The reducing capacity of compounds could serve as an indicator of potential antioxidant property [36-39]. Inhibitory concentration for ETF and Quercetin in DPPH assay was 25.72±0.13 µg/mL and 4.94±0.05 µg/mL, respectively.

CONCLUSION

The result of this study showed that ethanol fraction of *P. fel-terrae* Lour. possess antioxidant activity.

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