INTRODUCTION

Methyl gallate has been isolated from *Cercis chinensis*, *Acer truncatum Bunge*, *Rosa rugosa*, *Acer barbinerve*, *Toxicodendron sylvestre*, *Toona sinensis/Cedrela sinensis*, *Mangifera indica*, *Pholiota adipose*, *Grevia rotterformis* Griff., and *Galla rhois* [1-3]. The radical scavenging effect of methyl gallate has high activity [4-6]. Methyl gallate is derivative of gallic acid. Methyl gallate possess wide range biological properties that include inhibitor of herpes simplex virus, antioxidant activity, antimicrobial activity, anti-inflammatory, and cancer chemopreventive effect [2,7].

*Archidendron jiringa* (Jack) I. C. Nielsen (Fabaceae:Mimosoideae), the jiringa is known as “jengkol” in Indonesia. Jengkol can growth in tropical area like Indonesia. We also can find jiringa in Malaysia and Thailand [8]. There are so many benefits from this plant the young shoots of jaringa can we eat as a vegetable; seeds can we eat with rice before or after processing such as boiling, frying, or add with seasoning [9,10]. Jiringa is one of the traditional medicine herbs. The leaves of jiringa used to treat skin disease [11,12]. The woods of this plant can be used for handicrafts [13]. The jiringa can growth up to 25 m. The color of its bark is gray and pods are brown or black with red or purple inside. The pods consist of 3–9 beans with diameter 3.5 cm and thickness 2.0 cm [14].

Phenolic compounds are very useful for the treatment of various diseases. This class of compounds has high antioxidant activity [15]. Antioxidant compounds can reduce free radicals that can cause various diseases, such as cancer, atherosclerosis, emphysema, and arthritis [12,16,17].

Phenolic compounds of plant can inhibit oxidation in the human body due to it’s antioxidant potential [18]. This time, there has been interesting to find natural sources of antioxidant in plants, due to their potential health associated with several degenerative and aging-related diseases such as cancer and cardio vascular diseases [18-22]. Synthetic antioxidants may have toxic, carcinogenic, and negative effects to human’s body. Ascorbic acid is one of the sources of natural antioxidants [20-22]. We use it as a standard in this research.

Jiringa’s pods are still a lot of wasted and become garbage. Jiringa’s pods show in Fig. 1. In this study, we tried to isolated phenolic compounds from *A. jiringa* (Jack) I. C. Nielsen pods and test it’s antioxidant activity. Active compounds were identified using data analysis from nuclear magnetic resonance of proton (*¹H NMR*), NMR of carbon (*¹³C NMR*), and mass spectrometry (MS). We use 1,1-diphenyl-2-picrylhydrazyl (DPPH) method to identified antioxidant activity. Therefore, we would like to report isolation and characterization methyl gallate from pods of jiringa (*A. jiringa* (Jack) I. C. Nielsen) in this paper. Methyl gallate has never been reported from pods of jiringa.

METHODS

Plant material

The pods of jiringa were collected from Namorambe village, Deli Serdang, North Sumatra, Indonesia. Identification of plant was done at the Herbarium Bogoriensis, LIPI, Cibinong-Indonesia.

Preparation of the extracts

The pods of jiringa cut small, made powder, and dried at room temperature. In this experiment, we got powder 4,160 g. Further, it’s antioxidant potential [18]. This time, there has been interesting to find natural sources of antioxidant in plants, due to their potential health associated with several degenerative and aging-related diseases such as cancer and cardio vascular diseases [18-22]. Synthetic antioxidants may have toxic, carcinogenic, and negative effects to human’s body. Ascorbic acid is one of the sources of natural antioxidants [20-22]. We use it as a standard in this research.

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RESULTS AND DISCUSSION

Identification of jiringa as a sample in this research was done at the Herbarium Bogoricensis, LIPI, Cibinong Indonesia. The result of plant identification was A. jiringa (Jack) I. C. Nielsen.

The methyl gallate was founded from A. jiringa (Jack) I. C. Nielsen. First, we macerated sample with methanol, further tested using qualitative test (FeCl₃ test). The FeCl₃ test showed that phenolic compounds are existed over there. It probes the color of sample extract is black. Then, we isolated methyl gallate from A. jiringa (Jack) I. C. Nielsen.

Subsequently, we identified methyl gallate with data from NMR of proton H and NMR of carbon, support with MS. We found six fractions in this experiment. From fraction III-2, we found methyl gallate as a white crystal. Data NMR of proton H pure compound is shown in Fig. 2. The peak at 8.77 showed two protons (H) at position 2 and 6, 8.381 shown H bounded with OCH₃. This data NMR of proton H was compared with data NMR of proton H methyl gallate was isolated from mushroom (Pholiota adiposa). The peaks of proton H methyl gallate from mushroom (P. adiposa) were at 8.78 shown two protons (H) at position 2 and 6, 8.381 shown H bounded with OCH₃ [3]. Methyl gallate was isolated from seed coat of G. rottleriiformis Griff. shown data NMR of proton H at peak 8.77 showed two protons (H) at position 2 and 6 [4]. Data NMR of proton H isolated methyl gallate from T. sylvestre was shown peak at 8.71 and 8.34. This peak identification protons H at position 2 and 6, H bounded with OCH₃ [6]. This data also agreement with Cheng et al. had been isolated methyl gallate from Chinese toon. Data NMR of proton H from methyl gallate isolated from Chinese toon shown peak at 8.72 and 8.79. This peak also identification protons H at position 2 and 6, H bounded with OCH₃ [2,4].

Further, Data NMR of carbon shown in Fig. 3. The peaks at 169.41 identified carbon at position -COOH, 146.16 (position carbon at 3 and 5), 141.64 (position carbon at 1), and Δ110.00 (position carbon at 2 and 6), and 85.8 (–OCH₃). Data NMR of carbon methyl gallate had isolated from mushroom (P. adiposa) seed coats of G. rottleriiformis Griff., T. sylvestre, and Chinese toon. Data NMR of carbon methyl gallate had isolated from mushroom (P. adiposa) was peaks at 168.99 identified carbon at position -COOH, 146.34 (position carbon at 3 and 5), 139.66 (position carbon at 4) and 111.08 (position carbon at 2 and 6), and 52.1 (–OCH₃). Data NMR of carbon methyl gallate had isolated from seed coats T. sylvestre was peaks at 167.33 identified carbon at position -COOH, 146.16 (position carbon at 3 and 5), 138.8 (position carbon at 4), 121.38 (position carbon at 1), 110.09 (position carbon at 2 and 6), and 85.8 (–OCH₃). Data NMR of carbon methyl gallate had isolated from Chinese toon was peaks at 167.89 identified carbon at position -COOH, 146.34 (position carbon at 3 and 5), 139.66 (position carbon at 4) and 121.38 (position carbon at 1), 110.00 (position carbon at 2 and 6), and 85.8 (–OCH₃). Data NMR of carbon methyl gallate had isolated from seed coats G. rottleriiformis Griff. was peaks at 167.01 identified carbon at position -COOH, 146.34 (position carbon at 3 and 5), 139.66 (position carbon at 4) and 121.38 (position carbon at 1), 110.00 (position carbon at 2 and 6), and 85.8 (–OCH₃). Data NMR of carbon methyl gallate had isolated from Chinese toon was peaks at 167.89 identified carbon at position -COOH, 146.34 (position carbon at 3 and 5), 139.66 (position carbon at 4) and 121.38 (position carbon at 1), 110.00 (position carbon at 2 and 6), and 85.8 (–OCH₃) [3,6,24].

Strong evidence of fraction III-2 was methyl gallate also confirmed by Mass Spectrometry data in Fig. 4 shown 185.35 [M+H]. Molecular weight of methyl gallate is 184 g/mol and molecular formula is C₁₃H₁₈O₅ [4,25].

Based on data NMR of proton H (H NMR), NMR of carbon (¹³C NMR), and MS, pure compound isolated from pods of jiringa was determined as methyl gallate. Structure pure compound isolated from pods of jiringa shown in Fig. 5. This study was reported methyl gallate from pods of jiringa for the first time.
Fig. 2: Data nuclear magnetic resonance of proton H ($^1$H NMR) from pure compound

Fig. 3: Data nuclear magnetic resonance of carbon ($^{13}$C NMR) from pure compound

Fig. 4: Mass spectrometry data of pure compound
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Pure compound as methyl gallate isolated from pods of jiringa exhibited high DPPH activity (Table 1). Methyl gallate is one of the phenolic compounds. Phenolic compounds are the main antioxidant constituents of jiringa’s pods. The phenolic compounds are known as powerful chain-breaking antioxidant, and it is very important plant constituents due to their scavenging ability due to their hydroxyl group and may contribute directly to antioxidant action.

Various concentrations of pure compound and ascorbic acid were added into DPPH solution to initiate the reaction.

Analysis was performed in duplicate for each concentration of pure compound and ascorbic acid. Comparing % inhibition methyl gallate and ascorbic acid shown in Fig. 6.

Antioxidant activity of pure compound from jiringa’s pods was relatively high when compared to ascorbic acid. The antioxidant activity can be obtained by calculating the value of % inhibition from sample and blank absorbance.

The percentage of inhibition is calculated by the following formula 1 below: [26,27]

\[
\text{% inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%
\]

The value of IC_{50} is the concentration of antioxidant (µg/ml) that can inhibit 50% free radicals. We used linear regression equation in Fig. 7 to obtain the IC_{50} value. The value of IC_{50} is obtained from the intersection of line between 50% barrier power with concentration axis, then substituted value of y=50 to linear regression equation y = ax + b. The value of IC_{50} is the value of x which denotes the IC_{50}. The IC_{50} of methyl gallate calculated from linear regression equation (y = 2.0748x + 45.214) was obtained 3.7576 µg/ml. In the other hand, the IC_{50} of ascorbic acid as a standard in this research, calculated from linear regression equation (2.0748x + 45.214) was obtained 2.3067 µg/ml.

According to standard value IC_{50}, sample with IC_{50}<50 µg/ml it had very strong antioxidant. Sample with 50 µg/ml <IC_{50}<100 µg/ml it had strong antioxidant. Sample with 101 µg/ml <IC_{50}<150 µg/ml it had medium antioxidant. Sample with IC_{50}>150 µg/ml it had weak antioxidant [15,28].

Table 1: DPPH scavenging capacities

| Sample           | Concentration (µg/ml) | Absorbance of the first measurement | Absorbance of the second measurement | Inhibition (%) |
|------------------|-----------------------|-------------------------------------|-------------------------------------|----------------|
| Methyl gallate   | 5                     | 0.4960                              | 0.4960                              | 47.65±0        |
|                  | 10                    | 0.3801                              | 0.3804                              | 59.67±0.00212  |
|                  | 15                    | 0.2254                              | 0.2271                              | 76.12±0.001202 |
|                  | 20                    | 0.2243                              | 0.2261                              | 76.23±0.001273 |
|                  | 25                    | 0.1980                              | 0.2001                              | 78.99±0.001485 |
| Ascorbic acid    |                       | 0.4366                              | 0.4924                              | 50.98±0.039457 |
| (Vitamin C)      | 10                    | 0.2980                              | 0.2521                              | 70.07±0.032456 |
|                  | 15                    | 0.2767                              | 0.1137                              | 79.40±0.115258 |
|                  | 20                    | 0.1030                              | 0.2004                              | 83.99±0.068872 |
|                  | 25                    | 0.0120                              | 0.0574                              | 96.34±0.032103 |

Values are expressed as means±SD, SD: Standard deviation

Fig. 5: Structure pure compound as methyl gallate

Fig. 6: Inhibition (%) from various concentrations of methyl gallate in comparison with ascorbic acid

Fig. 7: Linear regression of methyl gallate and ascorbic acid to obtain the value of inhibition concentration (IC_{50})
CONCLUSIONS

Based on data spectral NMR 1 dimension proton H and carbon (1H NMR and 13C NMR), supported by MS data, pure compound from fraction III-2 was determined as methyl gallate and shows very high antioxidant activity with IC50 3.7576 μg/ml. This study demonstrated that jiringa's pods are a good source of natural antioxidant. Pure compound showed strong activity in the DPPH assay. The plant has a potential source for bioactive substances that supports several pharmaceutical uses and therapeutic value.

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