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Research Article

METHYL GALLATE FROM JIRINGA (*ARCHIDENDRON JIRINGA*) AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: This research was study about phytochemical active phenolic compound from pods of jiringa *(Archidendron jiringa* (Jack) I. C. Nielsen) and it's antioxidant.

Methods: Pods of jiringa were air dried and macerated with methanol. Extract was evaporated using rotary evaporator, and then, crude extract dissolved with water and partitioned with ethyl acetate. Extracts were evaporated and partitioned again with methanol and n-hexane. Column and preparative chromatography used to separate pure compound. Pure compound as methyl gallate was identificated with data from nuclear magnetic resonances of proton H (^{1}H NMR), NMR of carbon (^{13}C NMR), and mass spectrometry (MS). Antioxidant activity of pure compound tested by 1,1-diphenyl-2-picrylhydrazyl method.

Results: We found pure phenolic compound from pods of jiringa as methyl gallate that exists on fraction III-2, and it has high antioxidant activity with inhibition concentration 50 was 3.7576 µg/ml.

Conclusions: Pods of jiringa contain of active phenolic compound as methyl gallate that has high antioxidant activity. Therefore, it can be used as a source of natural antioxidant.

Keywords: Antioxidant, *Archidendron jiringa*, Methyl gallate, Nuclear magnetic resonance.

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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, Givotia rottleriformis* 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 possesses 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 jiringa 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 H (^{1}H NMR), NMR of carbon (^{13}C NMR), and mass spectrometry (MS). We use 1,1-diphenyl-2-picrylhydrazyl (DPPH) method to identificated 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,

Fig. 1: Pods of Jiringa

powder macerated with methanol 16 l. We got methanol extract 140 g. Then, extract was dissolved with water for many times up to 16 l of water. After filtration, the aqueous solution was partitioned with ethyl acetate. Fraction ethyl acetate concentrated in rotary evaporator, same with procedure concentrated methanol extract. We got 37.88 g ethyl acetate extract, dissolved with methanol solvent and partition with n-hexane for many times until n-hexane solvent clearly. We got total phenolic 13.87 g. We use $FeCl_3$ to test phenolic compounds [23].

Separation of compounds

Total phenolic was subjected to column chromatography silica gel (only 10 g was subjected) and eluted with chloroform:methanol (chloroform 100%; 9:1; 8:2; 7:3; 6:4; and methanol 100%) to got six fractions.

Purification

Fraction III (only 200 mg) purification by preparative chromatography used eluent $CHCl_3$:ethyl acetate (6:4) and eluted with methanol:ethyl acetate (1:1) with volume 200 ml. From preparative chromatography, we obtained six zones. Zone III-2 cristalizated with aseton and n-hexane to got pure compound.

Identification of pure compound

Structure pure compound from sample was determined by data from NMR of proton H (^{1}H NMR), NMR of carbon (^{13}C NMR), and MS.

Antioxidant activity test

We use DPPH to test antioxidant activity of pure compound from sample. The DPPH solution was made from 7.9 mg DPPH (BM 394.32) weighed then dissolved with methanol proanalysis up to 50 ml and placed in a dark bottle. Subsequently, the blank solution was prepared from 1 ml of 0.4 mM DPPH solution into 5 ml test tube then added methanol proanalysis until the mark and homogenized. The tube was covered with aluminum foil. A total of 5 mg sample were weighed and then dissolved into 10 ml methanol proanalysis (500 µg/ml), this solution was the mother liquor. A total of 50, 100, 150, 200, and 250 µl of mother liquor were piped into 5 ml tapered test tube to obtain concentrations of 5, 10, 15, 20, and 25 µg/ml. Into each tube was added 1 ml of 1 mM DPPH solution and added with methanol proanalysis up to 5 ml, then homogenized. The tube was covered with aluminum foil. Vitamin C solution made from 5 mg of Vitamin C and then dissolved into 10 ml methanol proanalysis (500 µg/ml), this solution was the mother liquor. A total of 50, 100, 150, 200, and 250 µl of mother liquor were piped into 5 ml tapered test tube to obtain concentrations of 5, 10, 15, 20, and 25 µg/ml. Into each tube was added 1 ml of 1 mM DPPH solution and added with methanol proanalysis up to 5 ml, then homogenized. The tube is covered with aluminum foil. The test solution with some concentration was incubated in a 37°C water bath for 30 min. The absorption of the solution was measured at a maximum absorption wavelength of 515 nm using ultraviolet-visible.

RESULTS AND DISCUSSION

Identification of jiringa as a sample in this research was done at the Herbarium Bogoriensis, 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 ($FeCl_3$ test). The $FeCl_3$ 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 creamy white crystal. Data NMR of proton H pure compound is shown in Fig. 2. The peak at δ7.04 shown two protons (H) at position 2 and 6, δ3.81 shown H bounded with OCH_3. 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 δ7.04 shown two protons (H) at position 2 and 6, δ3.79 shown H bounded with OCH_3 [3]. Methyl gallate was isolated from seed coats of *G. rottleriformis* Griff. shown data NMR of proton H at peak δ7.22 shown two protons (H) at position 2 and 6 [4]. Data NMR of proton H isolated methyl gallate from *T. sylvestre* was shown peak at δ7.1 and δ3.4. This peak identification protons H at position 2 and 6, H bounded with OCH_3 [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 δ7.02 and δ3.79. This peak also identification protons H at position 2 and 6, H bounded with OCH_3 [24].

Further, Data NMR of carbon shown in Fig. 3. The peaks at δ169.1 identificated carbon at position -COOH, δ146.6 (position carbon at 3 and 5), δ139.87 (position carbon at 4), δ121.49 (position carbon at 1), δ110.09 (position carbon at 2 and 6), and δ49.27 (-OCH_3) were strong evidence the methyl gallate was found, according to data NMR of carbon methyl gallate had isolated from mushroom (*P. adiposa*), seed coats of *G. rottleriformis* Griff., *T. sylvestre,* and Chinese toon. Data NMR of carbon methyl gallate had isolated from mushroom (*P. adiposa*) was peaks at δ168.99 identificated carbon at position -COOH, δ146.34 (position carbon at 3 and 5), δ139.66 (position carbon at 4), δ121.38 (position carbon at 1), δ110.00 (position carbon at 2 and 6), and δ52., (-OCH_3). Data NMR of carbon methyl gallate had isolated from seed coats *G. rottleriformis* Griff. was peaks at δ167.01 identificated carbon at position -COOH, δ143.98 (position carbon at 3 and 5), δ136.61 (position carbon at 4), δ119.87 (position carbon at 1), and δ108.14 (position carbon at 2 and 6). Data NMR of carbon methyl gallate had isolated from seed coats *T. sylvestre* was peaks at δ167.3 identificated carbon at position -COOH, δ146.1 (position carbon at 3 and 5), δ138.8 (position carbon at 4), δ121.9 (position carbon at 1), δ109.9 (position carbon at 2 and 6), and δ52.0 (-OCH_3). Data NMR of carbon methyl gallate had isolated from seed coats Chinese toon was peaks at δ167.8 identificated carbon at position -COOH, δ145.3 (position carbon at 3 and 5), δ138.6 (position carbon at 4), δ120.2 (position carbon at 1), δ108.8 (position carbon at 2 and 6), and δ51.1 (-OCH_3) [3,4,6,24].

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 $C_8H_8O_5$ [4,25].

Based on data NMR of proton H (^{1}H NMR), NMR of carbon (^{13}C 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.

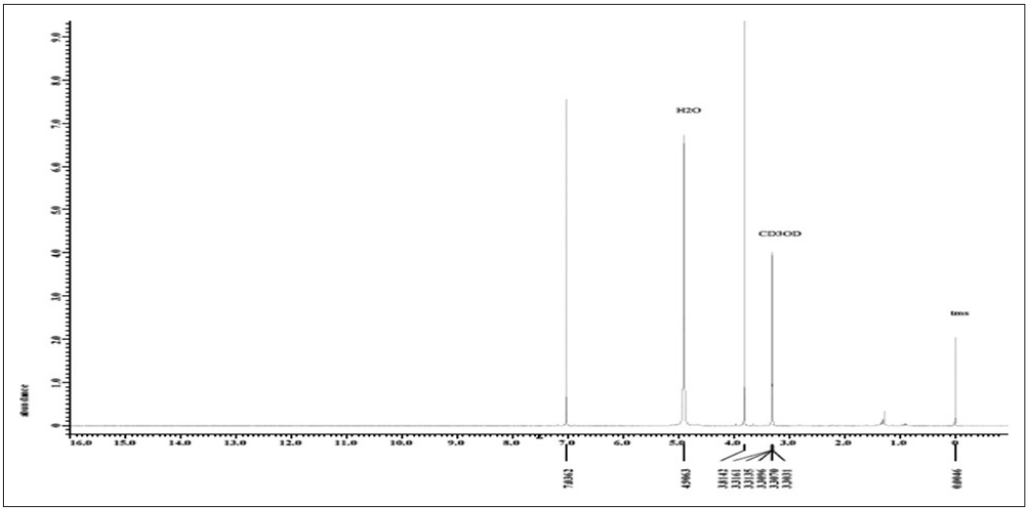

Fig. 2: Data nuclear magnetic resonance of proton H (^{1}H NMR) from pure compound

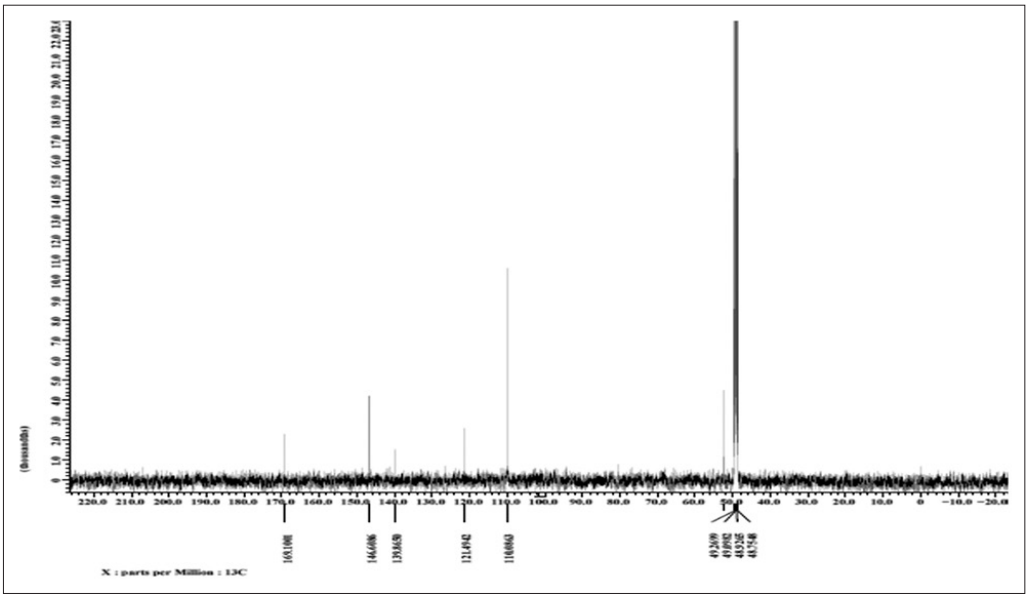

Fig. 3: Data nuclear magnetic resonance of carbon (^{13}C NMR) from pure compound

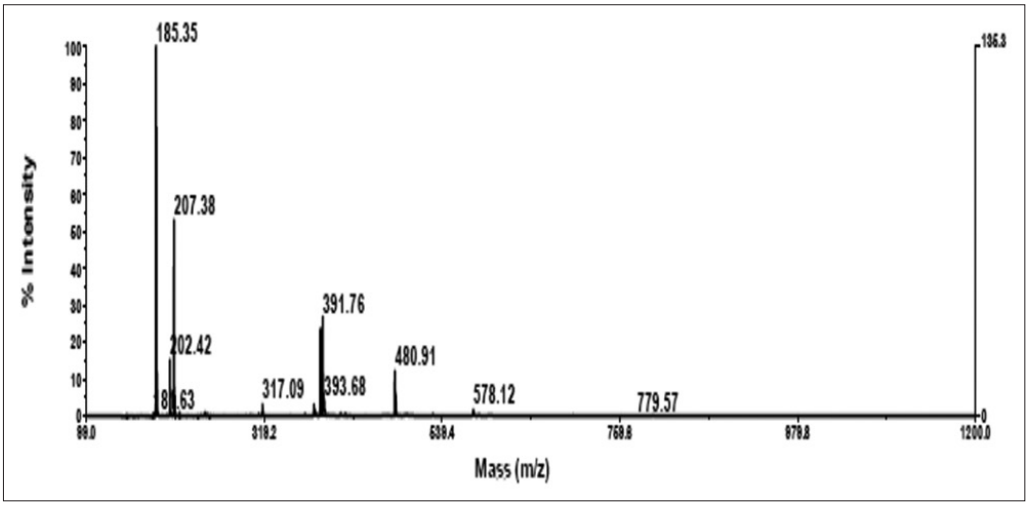

Fig. 4: Mass spectrometry data of pure compound

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	59.87±0.000212
	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 (Vitamin C)		0.4366	0.4924	50.98±0.039457
	10	0.2980	0.2521	70.97±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 mean±SD, SD: Standard deviation

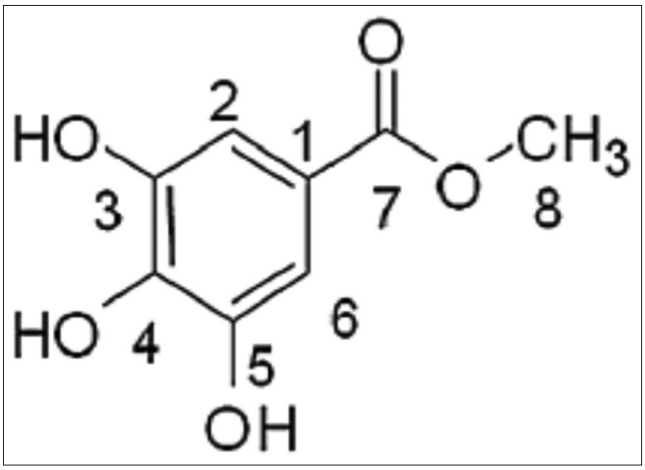

Fig. 5: Structure pure compound as methyl gallate

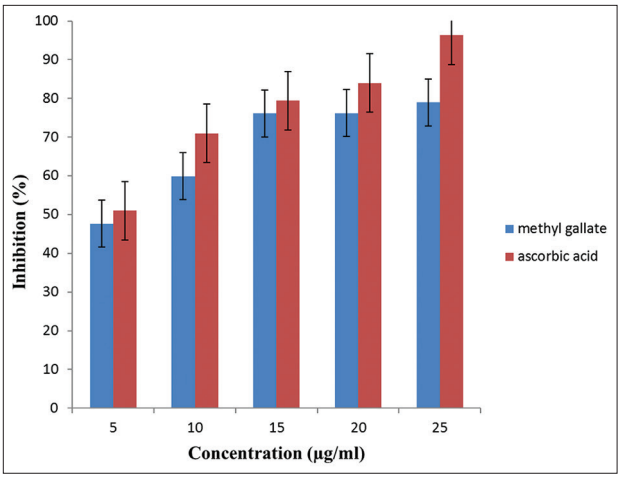

Fig. 6: Inhibition (%) from various concentrations of methyl gallate in comparison with ascorbic acid

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.

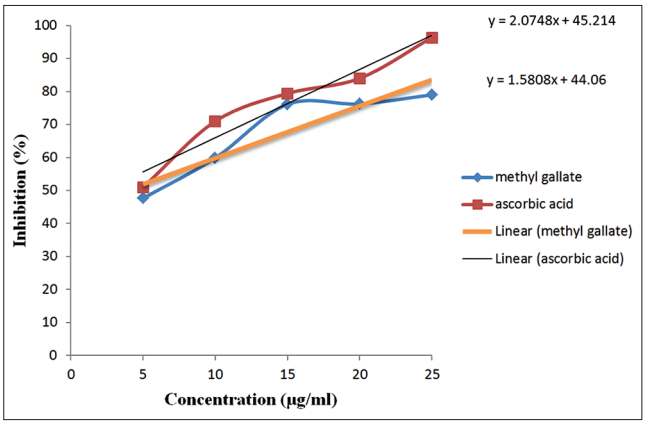

Fig. 7: Linear regression of methyl gallate and ascorbic acid to obtain the value of inhibition concentration (IC_{50})

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]

$$\% \text{ inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\% \quad (1)$$

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 x denotes the value of IC_{50}. The IC_{50} of methyl gallate calculated from linear regression equation (y = 1.5808x + 44.06) 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].

CONCLUSIONS

Based on data spectral NMR 1 dimension proton H and carbon (^{1}H NMR and ^{13}C NMR), supported by MS data, pure compound from fraction III-2 was determined as methyl gallate and shows very high antioxidant activity with IC_{50} 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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REFERENCES

1. Nithitanakool S, Pithayanukul P, Bavovada R. Antioxidant and hepatoprotective activities of thai mango seed kernel extract. Planta Med 2009;75:1118-23.
2. Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Takayasu J, Okuda M, *et al.* Cancer chemopreventive effects of a Brazilian folk medicine, Juca, on *in vivo* two-stage skin carcinogenesis. J Ethnopharmacol 2002;81:135-7.
3. Wang CR, Zhou R, Ng TB, Wong JH, Qiao WT, Liu F, *et al.* First report on isolation of methyl gallate with antioxidant, anti-HIV-1 and HIV-1 enzyme inhibitory activities from a mushroom (*Pholiota adiposa*). Environ Toxicol Pharmacol 2014;37:626-37.
4. Kamatham S, Kumar N, Gudipalli P. Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rottleriformis* griff. And their anti-proliferative effect on human epidermis carcinoma A431 cells. Toxicol Rep 2015;2:520-9.
5. Chae HS, Kang OH, Choi JG, Oh YC, Lee YS, Brice OO, *et al.* Methyl gallate inhibits the production of interleukin-6 and nitric oxide via down-regulation of extracellular-signal regulated protein kinase in RAW 264.7 cells. Am J Chin Med 2010;38:973-83.
6. Yuan GQ, Li QQ, Qin J, Ye YF, Lin W. Isolation of methyl gallate from *Toxicodendron* sylvestre and its effect on tomato bacterial wilt. Plant Dis 2012;96:1143-7.
7. Lee SH, Kim JK, Kim DW, Hwang HS, Eum WS, Park J, *et al*. Antitumor activity of methyl gallate by inhibition of focal adhesion formation and akt phosphorylation in glioma cells. Biochim Biophys Acta 2013;1830:4017-29.
8. Lim TK. Edible Medicinal and Non-Medicinal Plants. Vol. 2. Heidelberg, London, New York: Springer Dordrecht; 2012. p. 544-8.
9. Ashuwini S, Alias A, Karim, Rajeev B. *Pithecellobium jiringa* legume flour for potential food applications: Studies on their physico-chemical and functional properties. Food Chem 2012;130:528-35.
10. Ruzilawati AB, Imran A, Shaida FS. Effect of *Pithecellobium jiringa* as antimicrobial agent. Bangladesh J Pharmacol 2012;7:131-4.
11. Mohammad AC, Nik NN, Setianto WB, Mohammad OA. Supercritical Carbon Dioxide Extraction of Constituents of *Pithecellobium jiringa* Seeds and Their Identification Using Time of Flight Gas Spectrometry. Proceedings of the 1st International Conference on Natural Resources Engineering and Technology 24-25th July 2006; Putrajaya, Malaysia 2006. p. 616-25.
12. Fonseca JC, Barbosa MA, Silva IC, Duarte-Almeida JM, Castro AH, Santos LA. Antioxidant and allelopathic activities of *Smilax brasiliensis* Sprengel (*Smilacaceae*). South Afr J Bot 2017;111:336-40.
13. Charungchitrak S, Petsom A, Sangvanich P, Karnchanatat A. Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. Food Chem 2011;126:1025-32.
14. Muslim N, Abdul MA. *Pithecellobium jiringa*: A traditional medicinal herb. Webmed Central Complementary Medicine (WMC001371) 2010;1(12):1-4.
15. Irda F, Evelyne N, Komar RW. *In vitro* antioxidant activities, total flavonoid, phenolic and carotenoid content from various extracts of four species *Asteraceae* Herb. Int J Pharm Pharm Sci 2017;7:192-7.
16. Sethupandian G, Kokkaiah I, Palanichamy M. Evaluation of antioxidant and free radical scavenging activities of different solvent extracts of leaves of *Piper umbellatum*. Asian J Pharm Clin Res 2017;10:274-6.
17. Saswati B, Sukhendu M, Debarati P, Alok KH, Madhubrata C. HPLC phenolic compounds, antioxidant and antimicrobial activity of bulbs from three ornitogalum species available in India. Int J Pharm Pharm Sci 2016;8:187-92.
18. Amarowicz R, Shahidi F. Antioxidant activity of broad bean seed extract and its phenolic composition. J Funct Foods 2017;38:656-662.
19. Bharti B, Neha S, Rita K. Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. R Soc Chem 2015;5:27540-57.
20. Baydar NG, Ozkanb G, Yasar S. Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control 2007;18:1131-6.
21. Tomás-Barberán FA, Andrés-Lacueva C. Polyphenols and health: Current state and progress. J Agric Food Chem 2012;60:8773-5.
22. Andreata RH. New species of *Smilax* and a key to all species from Minas Gerais, Brazil. Syst Bot 2009;34:28-31.
23. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. Molecules 2013;18:2328-75.
24. Cheng KW, Yang RY, Tsou SC, Clive SC, Lo HC, Lee TC, *et al*. Analysis of antioxidant activity and antioxidant constituents of Chinese toon. J Funct Food 2009;1:253-9.
25. Lim YA, Mei MC, Kusumoto IT, Miyashiro H, Hattori M, Gupta MP, *et al*. HIV-1 reverse transcriptase inhibitory principles from *Chamaesyce hyssopifolia*. Phytother Res 1997;11:22-7.
26. Saranya D, Sekar J, Adaikala RG. Assessment of antioxidant activities, phenol and flavonoid contents of different extracts of leaves, bark and root from the *Abutilon indicum* (L.) sweet. Asian J Pharm Clin Res 2017;10:88-94.
27. Manal, Mortady H, Mona A, Motagally M, Wafaa SA. Antioxidant potential, antitumor activity and phenolic profile of *Phoenix dactylivera* Linn. Int J Pharm Pharm Sci 2017;9:130-6.
28. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181:1199-2000.