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Short Communication

The Effect of Different Extraction Methods on the Total Phenolic Content and Antioxidant Activity in Galam Sawdust (*Melaleuca Leucadendron* Linn.)

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ABSTRACT

Sawdust is a waste that can be used as microcrystalline cellulose. Galam (*Melaleuca leucadendron* Linn.) is one of the woods that produce microcrystalline cellulose from its sawdust which is used for construction and furniture manufacturing purposes. Microcrystalline cellulose sawdust extraction can produce residual extracts that can be utilized for certain potential activities. This study aimed to determine the difference between reflux extraction and maceration method towards antioxidant activity and total phenolic content of Galam sawdust. Extraction of Galam sawdust was carried out using n-hexane: ethanol (1:2) consisting of two treatment groups: maceration and reflux extraction methods. Total phenolic contents were determined spectrophotometrically using Folin-Ciocalteu reagent with gallic acid as a standard. Measurement of antioxidant activity was done by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method. The total phenolic contents of the reflux extract (93.555 ± 0.872 mg GAE/g extract) were found significantly higher as compared to the macerated extract (40.735 ± 0.903 mg GAE/g extract). The reflux extract showed higher DPPH scavenging activity (IC_{50} = 259.431 ppm) than the macerated extract (IC_{50} = 389.698 ppm) but very weak antioxidant activity when compared with the positive control, Gallic acid (IC_{50} = 2.256 ppm). The results of the study showed that the reflux extract from Galam sawdust produces better antioxidant activity compared to the macerated extract, even though very weak antioxidant activity, the antioxidant result correlated with their phenolic contents.

Keywords: Galam, Sawdust, Reflux, Maceration, Antioxidant, Phenolic.

Introduction

Aromatic, medicinal plants and tropical timbers produce certain bioactive molecules from extracts compounds that react to many surrounding agents or pathogens either by inhibiting their activities or being toxic to the host cells. These biological effects have been reported due to the phenolic compounds which can be extracted from wide plant kingdoms. Phenolic compounds are common in plants (free or combined with esters or glycosides and are abundant in all plant parts, including wood, bark, stems, leaves, fruits, flowers, and seeds. A study on extracts from the bark and heartwood showed that both had strong antioxidant activities mainly due to the polyphenolic substances (polyphenols and tannins). In addition, many natural compounds isolated from woody plants performed significant pharmacological activities which could be harnessed as drug formulation.[1]

Wood is an essential and renewable natural resource. It is commonly used in many areas, such as in furniture and wood-frame houses, as it consists mostly of cellulose (40-50%), hemicellulose (20-30%), lignin (20-30%), has small water content, and amounts of inorganic ingredients.[2] Cellulose was an organic polymer known to occur in a wide variety of living species from the world of plants, bacteria, and animals. Cellulose structure consists of a linear homopolymer of *β*-D-glucopyranose units linked together by (1/4)-glycosidic bonds.

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It was important to emphasize that cellulose has the advantage of having an abundance of hydroxyl groups at its surface; thus, chemical modifications of these functional sites can be performed while functionalities that are not mechanical can be added to the polymer. Bioactive materials in polymer matrices can provide both high mechanical and biological potential. The attachment of antioxidant molecules onto polymers has already been explored. The use of antioxidants as nutrients has been proposed due to the potential benefits for human health and food conservation. Polyphenols, for example, have numerous biological activities, in particular as antimicrobials and antioxidants.[3]

Plant-derived antioxidants, especially phenolics, have gained considerable importance due to their potential health benefits. Epidemiological studies have shown that the consumption of plant foods containing antioxidants is beneficial to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardiovascular diseases. Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. For example, soluble phenolics are present in higher concentrations in the outer tissues (epidermal and sub-epidermal layers) of fruits and grains than in the inner tissues (mesocarp and pulp).[4]

Galam (*Melaleuca leucadendron* Linn.) is a plant indigenous to the peatlands of South Kalimantan. Reports show that the ethanol extract of Galam bark contains alkaloids, flavonoids, polyphenols, and quinones, which can act as antioxidants.[5] The total concentration of flavonoids in the Galam bark extract is 0.2826 mg QE (Quercetin Equivalent)/ g extract and the total phenol content of 30.47814 mg GAE (Galic Acid Equivalent)/ g. The IC_{50} value for antioxidant activity of the stem bark extract is 44.4888 ppm. Based on the range of antioxidant strength, it is known that the antioxidant intensity of Galam bark extract is very strong because the IC_{50} value obtained is less than 50 ppm.[5]

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Sawdust is a waste from wood that can be used as microcrystalline cellulose.[2,6] Galam is one of the Myrtaceae family woods that produce microcrystalline cellulose from its sawdust which is used for construction and furniture manufacturing purposes. Microcrystalline cellulose is a derivative of cellulose which can also be used as additives in pharmaceutical, food, cosmetics, and other industries.[7] Microcrystalline cellulose sawdust extraction can produce residual extracts that can be utilized for certain potential activities because several studies showed the stems of many Myrtaceae families possess strong chemical and biological properties due to their components, some of which have useful medicinal properties such as antioxidant bioactivity. Galam sawdust extract may provide potential alternatives to antioxidant agents because it constitutes a rich source of bioactive chemicals.[8] This research is a preliminary study to compare the phenolic contents and antioxidant activity between the reflux and maceration methods of extraction.

Materials and Methods

Chemicals and instruments

Analytical grade reagents and solvents such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, along with sodium carbonate, ferric chloride ($FeCl_3$), n-hexane, and ethanol were obtained from Merck KGaA (Germany), except standard Gallic acid, which was obtained from Sigma Aldrich Co (Germany). All spectrophotometric measurements were made with a PG Instruments-T60 UV-Vis spectrophotometer.

Collection and extraction of plant material

Galam sawdusts were obtained from Liang Anggang, Banjarbaru, South Kalimantan on February 5th, 2019. Specimens of the plant were identified in the Basic Laboratory, Faculty of Mathematics and Natural Sciences *(FMIPA)*, Lambung Mangkurat University as *Melaleuca leucadendron* Linn. Two types of extraction methods were utilized to find the best extraction method. Galam sawdust was extracted with n-hexane: ethanol (1:2) consisting of two treatment methods: maceration and reflux extraction methods.[7] Extraction using the reflux method was carried out using 500 g of dry pulverized Galam wood for 6 hours at a steady-state above. Another 500 g of dry pulverized Galam was macerated with 2 L of the solvent mixture for five days at room temperature.[9]

Phytochemical screening for phenolics

The extracts were tested for the presence of phenolic compounds with ferric chloride as reported by Roghini and Vijayalakshmi.[10]

Total phenolic content assay

Total phenolic content (TPC) was determined using a colorimetric assay (Folin-Ciocalteu reagent) along with analytical grade gallic acid as the standard. TPC was ascertained as mg of Gallic acid equivalent per gram of dry extract by using the calibration curve. Briefly, 0.6 mL of each crude extract (in ethanol solvent) were mixed with 1 mL of Folin Ciocalteau's phenol reagent (previously diluted with water 1:10). After 5 min, 2 mL of 10% sodium carbonate (Na_2CO_3) was added to the reaction mixture and allowed to stand in the dark for 70 min. The absorbance was measured at 734 nm, and TPC was obtained from a calibration curve using gallic acid (5-25 ppm) as a standard reference. Estimation of TPC was carried out in triplicate. The results were expressed as mean values ± standard deviations and expressed as mg gallic acid per g samples. All procedures were carefully carried out with minimum exposure to light.[11]

DPPH free radical scavenging assay

The quantitative antioxidant activity using DPPH assay for the two Galam sawdust extracts was carefully carried out at concentrations of 150, 200, 250, 300, and 350 ppm in 96% ethanol. Gallic acid standards were also made into concentrations of 1, 2, 3, 4, and 5 ppm. One mL of DPPH (0.4 mm) solution was added to each of the 4 mL sample solutions and placed in a dark room for 30 minutes after which the absorbance was measured at wavelength of 516 nm. The percentage inhibition of DPPH was calculated.[12] The results were expressed in terms of IC_{50} value (ppm).[13]

Statistical analysis

Each experiment was conducted in triplicate. Data were expressed as mean ± standard deviation. Statistical analysis was carried out using statistical package for social sciences (SPSS) version 18.0 software for Windows. Data analysis was carried out by comparing extraction methods using the Independent t-test method on the TPC and IC_{50} value. Statistical significance was evaluated at the significance level of $p \leq 0.05$.

Results and Discussion

Extraction and phenolic identification

Galam sawdust was extracted sequentially by two methods, namely maceration, and reflux, using n-hexane and ethanol solvents at a ratio of 2: 1. Hexane: ethanol mixture has been used to remove wax from Galam wood.[14] The results showed that the yield of Galam extract using the maceration method was 0.27%, while for the reflux method, the yield was 0.55%.

Total phenolic content

The antioxidant activity of plants is often attributed to the phenolic compounds; therefore, the phenol content of the Galam sawdust extracts has been determined. The phytochemical screening of phenol in both groups of Galam sawdust extracts using ferric chloride reaction showed the presence of phenolic compounds. Determination of total phenol content was carried out by the Follin-Ciocalteu method at wavelengths 734 nm. The result is shown in Table 1.

The total phenolic content was expressed as percent w/w gallic acid equivalent (% w/w GAE). The phenolic compound level of the extract were measured using calibration curves of gallic acid y = 0,0195x + 0,3169, R^2 = 0.9935. As can be seen in Table I, the highest phenolic content was present in the reflux extract. The TPC of the reflux extract group (93.555 ± 0.872 mg GAE/g extract) was found significantly higher as compared to the maceration extract group (40.735 ± 0.903 mg GAE/g extract). The difference between both extraction results could be caused by temperature. The higher the phenol content in the extract, the higher the contribution to the antioxidant activity of the extract.[15] The extract obtained from the reflux method gave a greater amount of extract than the maceration method, and it was comparable to higher levels of the phenolic content of the reflux extract. These results also show a better value than ethanol extract of Galam skin bark which showed the TPC of 30.478 mg GAE/g extract[5], but the leaves and fruits extract of Galam gave much higher TPC.[16]

The reflux is a hot extraction, and heating can increase the amount of extracted compounds.[17] Apart from these factors, the extraction time can also affect the phenol content of the extract. The phenol content in the maceration extraction method was low because the extraction time was very long. In this study, the maceration process was carried out for five days. According to previous research,[18] after 8 hours of extraction, the system has already gained the maximum total phenolic content, yet the phenolic content slightly decreased afterward. The diffusion rate of phenolic compounds from the surface of solid to solvent is equal to the diffusion rate from solvent to solid surface so that the concentration of the phenolic compound in a solvent is at equilibrium. Consequently, further maceration time after 8 hours should be stable. This is likely due to the addition of solvent volume at each sampling, which leads to the dilution of the total maximum phenolic content in the extract leading to a decrease in phenolic levels.

DPPH scavenging activity of galam sawdust extracts

In the present research, antioxidant activities by the DPPH method were represented by IC_{50} of DPPH. IC_{50} means the concentration that can scavenge 50% of DPPH free radical. IC_{50} of DPPH can be calculated using the linear regression equation of the calibration curve of the sample. For calculating IC_{50} of DPPH, many concentrations of extract were used, and this showed a linear decrease in absorbance of DPPH. After determining the linear regression equation, the value of IC_{50} of DPPH scavenging activity was calculated in addition to that of

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Table 1: Yield, phenolic identification, and TPC of Galam sawdust extracts

Sample	% Yield	Ferric chloride reaction	TPC (mg GAE/g)
Maceration extract group (MEG)	0.27	+	40.735 ± 0.903
Reflux extract group (REG)	0.55	+	93.555 ± 0.872

Values of TPC are expressed as mean ± standard deviation (SD) (n = 3)

Table 2: The DPPH scavenging activity test result of gallic acid, MEG, and REG

Sample	Concentration (ppm)	% of Inhibition ± SD	IC_{50} (ppm)
Gallic acid	1	34.695 ± 4.851	2.256
	2	45.657 ± 0.111	
	3	57.201 ± 1.053	
	4	74.612 ± 1.524	
	5	89.065 ± 0.910	
Maceration extract group (MEG)	150	24.425 ± 0.571	389.698
	200	28.953 ± 1.559	
	250	34.960 ± 0.989	
	300	39.866 ± 1.640	
	350	46.065 ± 0.340	
Reflux extract group (REG)	150	35.793 ± 0.511	259.431
	200	41.922 ± 1.055	
	250	47.884 ± 0.730	
	300	56.981 ± 0.227	
	350	61.247 ± 1.579	

Values are mean ± standard deviation (SD) of triplicate analyses; IC_{50} = inhibitory concentration at which 50% radicals are scavenged.

gallic acid, the standard antioxidant used in this study (Table 2). Based on the explanation above, it can be seen that the percentage of DPPH scavenging activities does not represent the real antioxidant activity; the real antioxidant activity is revealed by the IC_{50} value of DPPH.[19]
Based on the IC_{50} of Galam extract as shown in Table 2. The reflux extract showed higher DPPH scavenging activity (IC_{50} = 259.431 ppm) than the macerated extract (IC_{50} = 389.698 ppm), but very weak antioxidant activity when compared with the positive control; gallic acid (IC_{50} = 2,256 ppm). The higher antioxidant activity in the reflux extract is directly related to their content of phenolic compounds. The results showed that both the extraction yield, phenolic content, and antioxidant activity of extracts were strongly dependent on the solvent and extraction methods.
The extract with the higher TPC value usually showed a higher IC_{50} value of DPPH radical scavenging activity.[20] Wardhani *et al*. stated that the Galam fruits ethanol extract has very strong antioxidant activity with IC_{50} of 37.3752 ppm; meanwhile, the Galam leaves ethanol extract has strong antioxidant activity (IC_{50} = 56.4906 ppm), and it is correlated with their high TPC value.[16] According to the results, the MEG and REG of Galam sawdust have shown the lowest DPPH scavenging activity compared to the fruits, leaves, and skin bark ethanol extract (IC_{50} = 44.4888 ppm).[5,16] However, this study gave a better IC_{50} of antioxidant activity than essential oils from *Melaleuca leucadendron* Linn. leaves.[8] Comprehensive analyses in Melaleuca species showed that the DPPH radical scavenging capacity of *M. bracteata*,[20] *M. diosmifolia*,[21] *M. alternifolia*,[22] and *M. cajuputi*[23] are better than the sawdust of Galam (*M. leucadendron* Linn.), but the TPC of Galam sawdust showed higher value than the bark of *M. diosmifolia* (39.0 mg GAE/g dry weight).[21]
The results of the t-test showed that there is a significant difference in TPC and DPPH radical scavenging activity using maceration and reflux methods ($p < 0.05$). The results indicated that high TPC value and antioxidant capacity are associated with high-temperature extraction methods. The amount of the antioxidant components that can be extracted from plant material is mainly affected by the strength of the extraction procedure, which may probably vary from sample to sample. Amongst other contributing factors, the efficiency of the extracting solvent to dissolve endogenous compounds might also be very important. For the effectiveness of the extracting technique, the results showed that yields of the extract were better when extraction was done under reflux, regardless of the plant material and solvent used. This indicates that hot solvent systems under reflux state are more efficient for the recovery of antioxidant components, thus offering higher extract yields.[24]

Conclusion

The total phenolic contents of the extracts are affected by the extraction methods and operating conditions utilized. Reflux extraction gave the extract a better IC_{50} of antioxidant activity and a higher TPC. The results of the study showed that the reflux extraction of Galam sawdust produces higher antioxidant activity compared to the maceration method, even though both have very weak antioxidant activity compared to gallic acid. The results correlated with their phenolic contents.

Conflict of Interest

The authors declare no conflicts of interest.

Authors Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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