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Original Article

Radical Scavenging Activity of Kemenyan Resin Produced by an Indonesian Native Plant, *Styrax sumatrana*[1]

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ABSTRACT

Kemenyan resin from *Styrax sumatrana* is a unique non-timber forest product (NTFP) native from Sumatera Island, Indonesia. It possesses a wide range of applications in the pharmaceutical, perfume, and cosmetics industries. In this paper, six kemenyan resin samples were investigated to evaluate their free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent. The kemenyan resin samples, which originated from North Tapanuli, Pakpak Bharat, and Humbang Hasundutan, showed high antioxidant activity with IC_{50} < 16 mg/L. The antioxidant activity of common kemenyan resin constituents, i.e., cinnamic acid, ethyl cinnamate, gallic acid, and vanillin was also investigated as positive control, although they exhibited lower antioxidant activity (IC_{50} < 1000 mg/L), except for gallic acid (IC_{50} = 5,23 mg/L). The total phenolic and flavonoid contents (TPC and TFC) for all samples were 44-66 mg gallic acid equivalents (GAE)/g sample and 143-160 mg quarcetin equivalents (QE)/g sample. The results revealed that kemenyan resin has high potency as an antioxidant and could be used as a natural antioxidant resource.

Keywords: *Stryax sumatrana*, kemenyan resin, antioxidant, total phenolic content, total flavonoid content

1. INTRODUCTION

Styrax sumatrana is one of the 130 tree species that belong to genus *Styrax* in *Styracaceae*. Among the genera of *Styracaceae*, *Styrax* stands out for the production of resinous material, commonly called as benzoid resin or kemenyan resin (Pauletti *et al*., 2006). *Styrax sumatrana* (*S. sumatrana*) is endemic from Indonesia and widely distributed in North of Sumatera. In 2012, *S. sumatrana* was cultivated in around 34,000 ha, and the largest area was located at Northern Tapanuli regency (Rahmawati, 2012). The production of kemenyan resin in Indonesia, mainly from six central areas, i.e., North Tapanuli, South Tapanuli, Humbang Hasundutan, Papak Bharat, Toba Samosir, and Dairi Regency - North Sumatera province, is about 4,000 ton/year (Silalahi *et al*., 2013). Previous studies about kemenyan resin from North Sumatera were conducted by Susilowati *et al*. (2017a,b), Rahmat *et al*. (2017), and Iswanto *et al*. (2016). Kemenyan resin is a non-timber forest product (NTFP) with high economical value for the local forest community. The price of resin

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in global market depends on its quality, which is usually determined by factors such as size, color, odor, and fragility properties (Iswanto *et al*., 2015).

This resin has been widely used in many applications such as ritual ceremonies, perfumery, cosmetics, and medicinal fields (Hamm *et al*., 2004, Hovaneissian *et al*., 2008). Among its 130 compounds, the main ones are cinnamic acid, ethyl cinnamate, and vanillin. The resinous material of kemeyan resin from Indonesia is characterized by having a larger proportion of free cinnamic acid and cinnamate derivatives compared with other resins (Hovaneissian *et al*., 2008). Cinnamic acid derivatives bearing hydroxyl groups have been shown to exhibit strong free radical scavenging or antioxidants properties (Pontiki *et al*., 2014). In general, compounds having antioxidant activity are commonly added to foods and cosmetics as preservatives. The antioxidant activity is highly correlated with the content of phenolic/polyphenols, proteins/peptides, Vitamins C and E, and also that of several metals (Sing *et al*., 2017). The hydroxyl groups of phenolic compounds play an important role in the antioxidant activity as scavengers of free radicals and reactive oxygen species (ROS), which are closely related with various diseases and the aging of skin tissues (Epstein, 2009; Park *et al*., 2004). Since kemenyan resin contains many saponin, lignin, triterpenes, and phenolic compounds (Pauletti *et al*., 2006), the investigation of its application as preservative, as well as a medicinal natural product, is of high interest.

The extracts from *S. benzoin* and *S. formosanum* have been previously evaluated, to find that they exhibit potential antioxidant activity (Teissedre and Waterhause., 2000; Hou *et al*., 2003). Moreover, other biological activities including anti-fungal, anti-bacterial, anti-inflammatory, and anti-cancer properties were studied (Pauletti *et al*., 2000; Kim *et al*., 2004a,b; Jung *et al*., 2003; Moon *et al*., 2005). In contrast, the plant and resinous extracts from S. *sumatrana* has been less studied. Therefore, the aim of this study was to investigate the phytochemical properties, and phenolic and flavonoid contents of kemenyan resin originated from North Sumatera, as well as the potential antioxidant activity of its main constituents, i.e., cinnamic acid, ethyl cinnamate, and vanillin.

2. MATERIALS and METHODS

2.1. Sample collection

Samples were collected at North Tapanuli regency, Pakpak Bharat regency, and Humbang Hasundutan regency, North Sumatra, Indonesia. All samples used in this study were kemenyan resin with class quality premium grade (I) and lowest grade (II). Kemenyan resin was diluted with methanol 99% solvent, and the solution was stored in refrigerator prior to use.

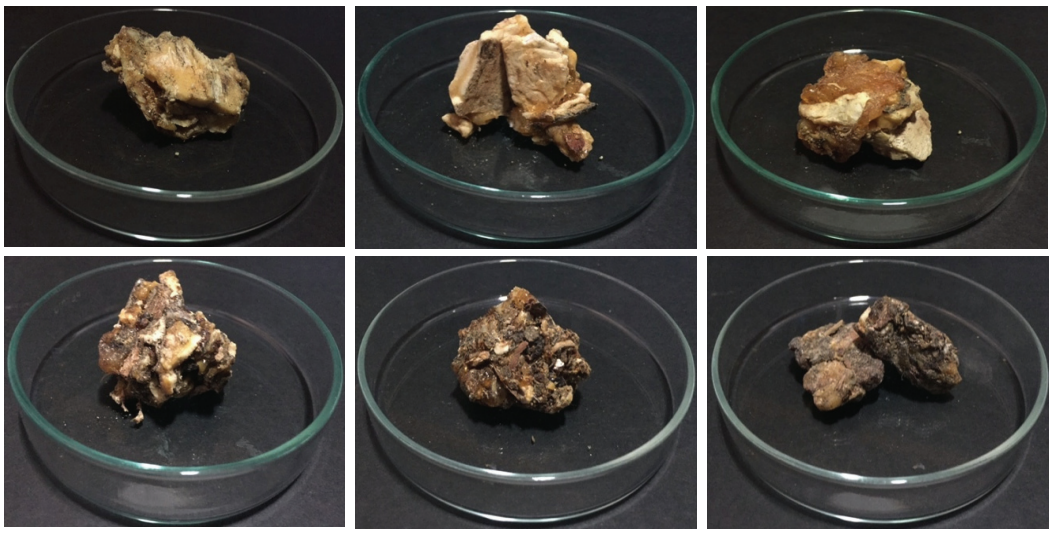
2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals. Cinnamic acid was purchased from Aldrich Chemicals. Vanillin, gallic acid, ethyl cinnamate, and sodium carbonate were purchased from Himedia Chemicals. Folin-Ciocalteu (FC) reagent was purchased from Merck Chemicals. All other chemicals were prepared with analytic grade.

2.3. Procedures

2.3.1. Phytochemical assay

The phytochemical assay was carried out according to the methods described by Tapwal *et al*. (2016) with slight modifications. In particular, phenolic and tannin were evaluated by ferric chloride test, flavonoid was evaluated by addition of magnesium and hydrochloric acid, steroid was evaluated by a mixture of acetate anhydrate and sulfuric acid, terpenoid was evaluated by addition of sulfuric acid, and alkaloid was evaluated



A B C

Fig. 1. Kemenyan resin samples collected from Pakpak Bharat (A), North Tapanuli (B), and Humbang Hasundutan (C) with class quality premium grade (I) and lowest grade (II).

by using Mayer's and Wagner's reagents.

2.3.2. Phenolic content

Evaluation of the total phenolic content (TPC) was performed following a modification of a previously described method (Tambe and Bhambar, 2014). The kemenyan resin solution was used in a final concentration of 40 mg/mL. Gallic acid was used as a standard, and the corresponding concentrations were prepared in the range of 0.8 to 4 mg/mL at the final reaction. Each kemenyan resin, standard solution, or methanol as a blank was added to a solution of 0.25 mL of FC reagent in 1.4 mL aquadest. After 8 min incubation, 0,75 mL of sodium carbonate (7.5%) was added to the mixture, and its absorbance at 715 nm was recorded after 2 h incubation. TPC was calculated by plotting the absorbance value in the standard calibration curve of gallic acid. All the tests were conducted in triplicate. The content of total phenol was expressed as mg of gallic acid equivalents (GAE) per g of kemenyan resin.

2.3.3. Flavonoid content

Evaluation of the total flavonoid content (TFC) was carried out according to a modification of a previously described colorimetric method (Tambe and Bhambar, 2014). The kemenyan resin solution was used in the final concentration of 20 mg/mL. Quarcetin was used as a standard, and the corresponding concentrations were prepared in the range of 1 to 20 mg/mL at the final reaction. Each kemenyan resin, standard solution, or methanol as a blank was added to a 0.15 mL solution of $NaNO_3$ (5%) in 2.6 mL aquadest and incubated for 8 min. Then, 0,15 mL of $FeCl_3$ (10%) was added, and the resulting mixture was further incubated for 6 min. A solution of NaOH (1 N) was added to obtain a reaction volume of 5 mL, and the absorbance of this mixture at 510 nm was recorded after 30 h incubation. TFC was calculated by plotting the absorbance value in the standard calibration curve of quarcetin. All the tests were conducted in triplicate. The content of total flavonoid was expressed as mg of quarcetin equivalents (QE) per g of kemenyan resin.

2.3.4. Antioxidant assay

The antioxidant activity of kemenyan resin for DPPH free radical was determined according to a previous method (Kuspradini *et al*., 2016) with slight modifications. In a series of test tubes, kemenyan resin, cinnamic acid, ethyl cinnamate, and vanillin solutions were diluted with methanol affording concentrations in the range of 10 to 100 mg/L, and 0.25 mL of a DPPH (1 M) solution was added to obtain a final reaction volume of 2.5 mL. After 30 min incubation at room temperature, the absorbance was monitored at 517 nm. The methanolic solution without sample and gallic acid was selected as blank and positive controls, respectively. All the tests were conducted in triplicate. The inhibitory effect of DPPH was calculated using to the following formula:

Scavenging activity (%)
= [(absorbance of the control - absorbance of the sample) / absorbance of the control] × 100%

The antioxidant activity was expressed as IC_{50}, which refers to the amount of sample necessary to reduce the initial DPPH concentration by half.

2.3.5. Toxicity assay

The brine shrimp lethality test (BSLT) method was used to investigate the toxicity activity, according to the method described by Mirzaei *et al.* (2013). The kemenyan resin was added in gradient concentrations to a vial containing 4.5 mL of the brine shrimp solution. Ten brine larvae developed for 48 h were then added to the vial. The control solution was prepared with methanol, Tween80, and seawater. The vial was incubated in the darkness at room temperature for 24 h. Feeding and air were not allowed during the experiment. After 24 h, the number of dead shrimps was counted, and the toxicity of the sample was determined. The percentage of mortality was calculated and analyzed using the probit value method. The toxicity activity was expressed as LC_{50}, which indicates the amount of sample necessary to reduce ten brine shrimp larvae by half.

3. RESULTS and DISCUSSION

3.1. Solubility of kemenyan resin

A preliminary study was conducted to assess the solubility of kemenyan resin in three different solvents, namely methanol, ethanol, and ethyl acetate. Methanol provided the best result (data not shown), which was expected considering that kemenyan resin is mainly constituted by polar compounds (Kiswandono *et al*., 2016). Therefore, methanol was selected as the solvent for this study.

3.2. Phytochemical assay

The qualitative result of the phytochemical assay showed that all the samples contained phenolic, flavonoid, tannin, and terpenoid compounds (Table 1). Total phenolic and flavonoid contents were investigated by using the Folin-Ciocalteu method and AlCl3 as reactant. The results are presented in Table 1.

The measurement of the TPC and TFC values showed that all kemenyan samples tested contained similar amounts of phenolic and flavonoid compounds (44.75-66.41 mg GAE/g and 143.23-160.89 mg QE/g, respectively). Tannins and terpenoids were also present in all the samples, whereas steroids and alkaloids were absent. Phenolic and polyphenol compounds, which originate from secondary metabolites, have one or more hydroxyl groups located in the benzene ring, and their scavenging properties for free radicals are well known (Evans *et al*., 1997; Halliwell and Gutteridge, 2006). Free radicals are key intermediates in diseases such

Table 1. Phytochemical assay of kemenyan resin from some locations in North Sumatera Province

No	Origin	Class	Phenolic		Flavonoid		Tannin	Terpenoid	Steroid	Alkaloid
			A	B*	A	B**				
1	Pakpak Bharat	I	+	52.42 ± 0.48	+	160.23 ± 12.39	+	+	-	-
		II	+	56.37 ± 0.27	+	153.96 ± 11.42	+	+	-	-
2	North Tapanuli	I	+	44.75 ± 6.42	+	143.23 ± 18.46	+	+	-	-
		II	+	66.41 ± 10.92	+	156.11 ± 4.60	+	+	-	-
3	Humbang Hasundutan	I	+	47.72 ± 9.82	+	159.41 ± 14.12	+	+	-	-
		II	+	58.81 ± 2.29	+	160.89 ± 13.67	+	+	-	-

Note: A = qualitative test, B = quantitative test, * = mg GAE/g sample, ** = mg QE/g sample, + = detected, - = not detected

as diabetes mellitus, cancer, liver diseases, renal failure, and degenerative diseases (Silva *et al*., 2016).

3.3. Antioxidant and toxicity assay

The radical scavenging and antioxidant activity of kemenyan resin was evaluated by the DPPH assay due to its low cost, easiness, and widespread use (Apak *et al*., 2016, Kim *et al*., 2010; Lee *et al*., 2004, Park *et al*., 2004; Park *et al*., 2012; Hou *et al*., 2003; Kim *et al*., 2014). The antioxidant activities (IC_{50}) for all the samples were found to lie in the range 15.28-31.74 mg/L (Table 2). The IC_{50} value of first class kemenyan resin was higher (15.28-16.50 mg/L) than second class kemenyan resin (21.12-31.74 mg/L). This result indicates that kemenyan resin has high capacity of inhibition of free radical molecules of DPPH. Kemenyan resin with higher grade (first class) originated from all sites showed higher inhibition activities compared with those of lower grade quality (second class) (Table 2). Furthermore, the standard samples used as positive control, i.e., vanillin, cinnamic acid, and ethyl cinnamate (Pauletti *et al*., 2006), showed no inhibition activity of free radical molecules. Meanwhile, gallic acid showed stronger activity than butylated hydroxytoluene, which were also tested as positive controls. Gallic acid is commonly used as positive control in antioxidant assays (Sharma and Vig, 2013; Kim *et al*., 2014; Noreen *et al*., 2017), since its three -OH molecules render it very active compared with other constituents (Karamac *et al*., 2005). Pauletti *et al*. (2006) reported that *Styrax* Genus is composed of 30 phenolic compounds related to benzoic and cinnamic acids. The difference in the antioxidant activity has been reported to depend on the capacity of polyphenol compounds for stabilizing the DPPH free radical molecules (Karamac *et al*., 2005). When comparing kemenyan resin of first and second class, this difference is also related to the purity, quality, and the quantity of constituents. In this context, it is worth noting that first class kemenyan resin has a clearer appearance than second class kemenyan resin (Fig. 1).

A linear relationship between total phenolic content, total flavonoid content, and antioxidant activity of kemenyan resin was also observed. The total phenolic content showed higher relationship (r = 0.6) than the total flavonoid acid content (r = 0.3), which is indicative of phenolic compounds playing an important role in the antioxidant activity with the donation of one or more hydroxyl groups. Experimentally, this could be observed in the change of DPPH color from purple to yellow. Previous studies reported that the total phenolic content has a strong positive correlation with the antioxidant capacity (Sadeghi *et al*., 2015). Due to the redox property of phenolic compounds, they can

Table 2. The antioxidant and toxicity activity of kemenyan resin and standard compounds

No	Origin	Class	DPPH IC_{50} (mg/L)	LC_{50} (mg/L)
1	Pakpak Bharat	I	16.50 ± 4.98	nd, > 500
		II	27.88 ± 2.52	nd, > 500
2	North Tapanuli	I	16.02 ± 7.54	nd, > 500
		II	21.12 ± 4.28	nd, > 500
3	Humbang Hasundutan	I	15.28 ± 2.93	nd, > 500
		II	31.74 ± 4.61	nd, > 500
4	Gallic acid (positive control)		5.23 ± 0.25	
5	Butylated hydroxytoluene (positive control) *)		9.0- 30.0	
6	Vanillin		nd	
7	Cinnamic acid		nd	
8	Ethyl cinnamate		nd, (> 1000)	

Note: nd= not detected; *) Kim *et al*. (2010) and Lee *et al*. (2004).

act as potent reducing agents, hydrogen donors, and singlet oxygen quenchers, leading to the antioxidant activity (Khoddami *et al*., 2013; Yashin *et al*., 2017). Flavonoids have higher antioxidant activities (Yashin *et al*., 2017), but their antioxidant activity strongly depends on their chemical structure, which is particularly influenced by the number and position of hydroxyl groups attached to the two aromatic rings (Promden *et al*., 2014; Park *et al*., 2004). A BLST test (Table 2) showed that all samples have LC_{50} values above 500 mg/L, which is indicative of a low toxicity according to the value reported by McLaughlin and Rogers, 1998 (LC_{50} > 200 mg/L). Furthermore, increasing the concentration of kemenyan resin up to a level <500 mg/L still resulted in low mortality rate (<50%). This experiment determined that all samples exhibited LC_{50} values above 500 mg/L, which renders kemenyan resin originated from North Sumatera a potential candidate as antioxidant bioactive compound.

4. CONCLUSION

In this study, we investigated the bioactive properties of six kemenyan resin samples from North Tapanuli, Pakpak Bharat, and Humbang Hasundutan - North Sumatera Province. All samples contained phenolic and flavonoid compounds, which can serve as natural sources of free radical scavengers. The antioxidant activities of the samples were in the range 15.28- 31.74 mg/L, in which the phenolic compounds play an important role with the donation of one or more hydroxyl groups. Antioxidant compounds can protect against the negative effect of many physiological and pathological processes; however, the antioxidant activity of Kemenyan resin in Indonesia has been scarcely studied. According to our results, kemenyan resin has prospective value as a natural antioxidant resource.

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