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Secondary Metabolites and Antioxidant Activity of Methanol Extract of the Root of Gitaan (*Willughbeia coreacea* Wall)

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Abstract: *Gitaan (Willughbeia coreacea), an endemic Indonesian plant belonging to the Apocynaceae family, presents potential as a source of antioxidants. This family contains various bioactive compounds, including triterpenoids, phenolics, alkaloids, and cardenolides. However, comprehensive information regarding the secondary metabolites and biological activities of W. coreacea still needs to be improved. This study aims to identify the secondary metabolites and assess the antioxidant activity of methanol extracts and fractions derived from the root of Gitaan. The root powder was subjected to maceration, followed by phytochemical analysis to identify secondary metabolites and evaluation of antioxidant activity using qualitative and quantitative DPPH (1,1-diphenyl-2-picrylhydrazyl) assays, with ascorbic acid serving as the positive control.The result indicated that the methanol extract and its fraction contained alkaloids, polyphenols, and terpenoids, whereas the ethyl acetate and n-hexane fractions contained alkaloids and polyphenols. Qualitative DPPH analysis revealed that all samples, except the n-hexane fraction, exhibited antioxidant properties, as evidenced by yellow stains on the thin-layer chromatography (TLC) plate after applying 50 ppm DPPH. Quantitative analysis demonstrated that the methanol extract, as well as the methanol, ethyl acetate, and n-hexane fractions, exhibited antioxidant activity with IC50 values of 44.1181 ppm, 45.4060 ppm, 57.2484 ppm, and 983.9576 ppm, respectively. Ascorbic acid showed a significantly lower IC50 value of 0.0445 ppm. Consequently, the methanol extract demonstrated superior antioxidant activity than the other fractions.*

Keywords: Antioxidant, Apocynaceae, Gitaan, Phytochemical, Willughbeia.

INTRODUCTION

Gitaan (*Willughbeia coreacea Wall*), belonging to the Apocynaceae family, is an endemic plant of Indonesia. This edible fruit plant is well-known among Southeast Asian inhabitants, particularly on the island of Borneo, due to its uniquely tasty fruit. Traditionally, various parts of this plant are utilized for medicinal purposes, such as treating typhus by consuming a decoction made from the root (Arung et al., 2009). Additionally, the wood of Gitaan can be carved for ornaments, and its latex is used to produce glue.

Members of the Apocynaceae family are recognized for their antioxidant properties. For example, the ethanol fraction of the Pulai stem bark (Alstonia scholaris) exhibited antioxidant activity with an IC50 value of 211.54 µg/mL (Zuraida et al., 2017). Similarly, methanol extracts and fractions of Catharanthus roseus flowers demonstrated antioxidant activities with IC50 values ranging from 50.069 ppm to 503.037 ppm (Indri et al., 2016). Furthermore, the n-hexane and ethyl acetate fractions, along with the ethanol extract of Kopsia arborea, showed weak antioxidant activity with IC50 values of 3524.05 ppm, 316.09 ppm, and 154.89 ppm, respectively (Purwanto et al., 2017).

According to the literature, secondary metabolites such as triterpenoids, iridoids, alkaloids, and cardenolides have been reported in plants from the Apocynaceae family (Chan et al., 2016). The predominant isolated compounds are indole alkaloids, which exhibit various biological activities (Liu et al., 2013; Kruakaew et al., 2017; Zhu et al., 2019; Zhang et al., 2020; Shi et al., 2022).

Despite its traditional medicinal use, phytochemical information and data on the biological activities of Gitaan (Willughbeia coreacea) grown in Melawi Regency, West Kalimantan, still need to be identified. Therefore, this study aims to elucidate the chemical profile of the secondary metabolites and evaluate the antioxidant activity of Gitaan root.

RESEARCH METHODS

Materials and Tools

The root of *W.coreacea*, ammonia (Merck), acetic acid (Merck), sulfuric acid (Merck), iron (III) chloride 5%, hydrochloric acid (Merck), magnesium powder, DPPH (1,1-diphenyl-2-picrylhydrazyl), ethyl acetate (Merck), chloroform (Merck), methanol (Merck), n-hexane (Merck), sodium hydroxide (Merck) 1%, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, and G60 F254 aluminium silica gel TLC plates (20 x 20 cm) were utilized in this study.

General laboratory glassware commonly used in organic chemistry laboratories, an analytical balance (BEL Engineering M124Ai), a rotary evaporator (HS 2005V-N), and a UV-Vis spectrophotometer (Shimadzu UV-Vis 2600) were also employed.

Procedures

Sample Preparation

The root of W. coreacea (2.8 kg) was cleaned and air-dried. Following drying, the sample was powdered using a wood crusher, resulting in 1.7 kg of powdered root.

Extraction and Fractionation

The powdered root of W. coreacea (1.7 kg) was macerated in methanol for three consecutive 24-hour periods at room temperature. The crude methanol extract was subsequently reduced using a vacuum rotary evaporator. The methanol extract was then partitioned sequentially using n-hexane and ethyl acetate. The obtained methanol, n-hexane, and ethyl acetate fractions were concentrated using the vacuum rotary evaporator to obtain their respective masses.

Phytochemical Assays (Harborne, 1998)

The crude methanol extract and fractions were tested for flavonoids, polyphenols, alkaloids, terpenoids, and steroids. Positive results were indicated by the formation of characteristic colours or precipitates for each test.

Antioxidant Activity (Molyneux, 2004)

Antioxidant activity was assessed both qualitatively and quantitatively. The qualitative test was performed using thin-layer chromatography (TLC). The TLC plate was eluted with a mixture of n-hexane and ethyl acetate (6:4). After elution, the plate was observed under UV light at 366 nm and 254 nm. Subsequently, the TLC plate was sprayed with a 50 ppm DPPH solution. Compounds with antioxidant activity appeared as yellow spots against a deep violet background.

The quantitative antioxidant activity was evaluated using the DPPH assay. Each extract and fraction was dissolved in the respective solvents to concentrations of 5, 10, 15,

20, and 25 ppm, except for the n-hexane fraction, which was prepared at 25, 50, 100, 200, and 400 ppm. For each concentration, 2 mL of the sample was mixed with 2 mL of a 50 ppm DPPH solution. The mixture was vortexed and incubated for 30 minutes at room temperature, protected from light. The absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The same procedure was followed for the positive control (ascorbic acid) at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 ppm. Each concentration of all tested samples was evaluated in five replicates. The percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{DPPH abs.} - \text{sample abs.}}{\text{DPPH abs.}} \times 100\ \%$$

Linear regression was applied in order to obtain IC_{50} value of each sample.

RESULTS AND DISCUSSION

Extraction and Fractionation

The methanol extract was partitioned with *n*-hexane and ethyl acetate. The results of the extraction and fractionation processes are presented in Table 1. It shows that semi-polar compounds are predominantly contained in the root of the gitaan.

Table 1 Weight and Yield of Extract and Fractions

Samples	Weight (gram)	Yield (%)
Methanol extract	45.836	2.694
n-hexane fraction	0.474	1.034
Ethyl acetate fraction	25.718	56.108
Methanol fraction	1.823	3.977
Solid residue	17.500	38.179

Phytochemical Test

The results of phytochemical test (Table 2) showed alkaloids, flavonoids, polyphenols, and steroids/terpenoids.

Table 2 Results of Phytochemical Screening of Extract and Fractions of Gitaan

	MeOH Extract	Fractions		
		MeOH	EtOAc	*n*-hexane
Flavonoids	+	+	+	-
Polyphenols	+	+	+	+
Alkaloids				
Wagner	+	+	+	+
Meyer	+	+	+	-
Dragendorf	+	+	+	-
Terpenoids	+	+	-	-
Steroids	-	-	-	-

Note: (+) = detected (-) = undetected

The major secondary metabolites in the methanol extract and fractions were flavonoids, polyphenols, alkaloids, and terpenoids, whereas the *n*-hexane fraction contained only polyphenols and alkaloids.

Antioxidant activity by DPPH

To identify which stains showed antioxidant activity in extract and fractions, qualitative DPPH was applied by TLCs as can be seen in Figure 1. The TLC plates revealed yellow stains with a deep violet background after being sprayed by 50 ppm DPPH indicating all samples except for *n*-hexane fraction were active for antioxidant. Changing a deep violet of DPPH to yellow color occurs due to radical of DPPH reacted to antioxidant molecules in tested samples by releasing its radical hydrogens to form a stable 2,2-diphenyl-1-picrylhdrazine as the electrons pairs off (Molyneux, 2004).

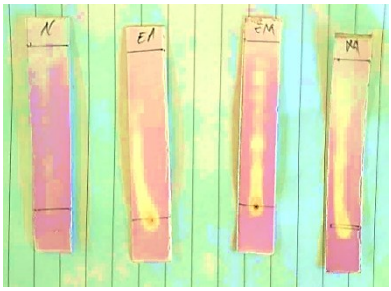

Figure 1 TLCs of methanol extract and fractions after DPPH sprayed. N: *n*-hexane fraction, EA: ethyl acetate fraction, EM: methanol fraction, M: methanol extract

The quantitative DPPH assay was applied after conducting the qualitative one. Figures 2-5 showed correlations between the tested samples' concentration and inhibition percentages. Thus, IC50 values were obtained for methanol extract, methanol, ethyl acetate, and *n*-hexane fractions by 44.1181 ppm, 45.4060 ppm, 57.2484 ppm, and 983.9576 ppm, respectively.

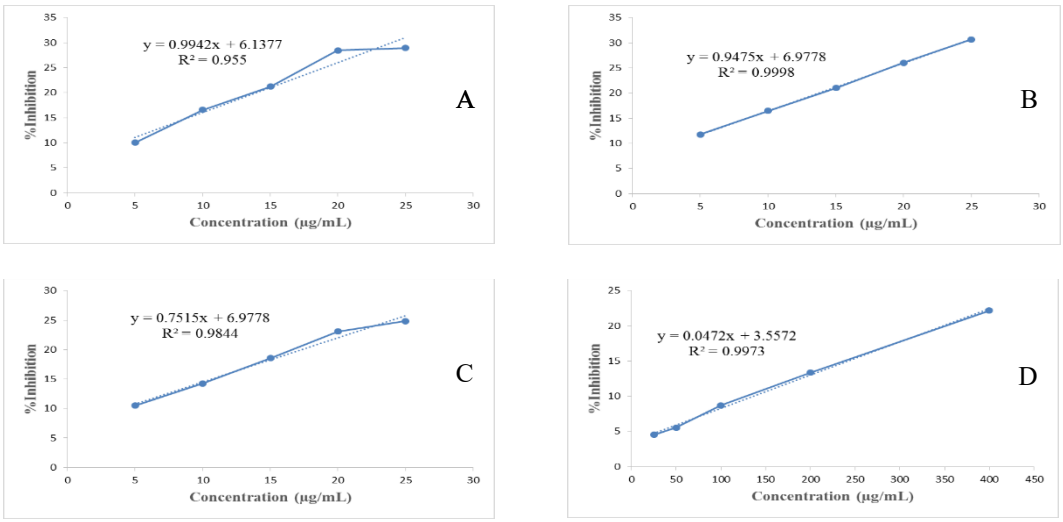

Figure 2 Correlations between concentration and % inhibition of methanol extract (A), methanol fraction (B), ethyl acetate fraction (C), and *n*-hexane fractions (D)

Antioxidant activity can be categorized as very strong (IC50 < 50 ppm), strong (IC50: 50 ppm-100 ppm), moderate (IC50: 100 ppm -150 ppm), weak (IC50: 150 ppm-200 ppm, and very weak (IC50 > 200 ppm) (Molyneux, 2004) and based on IC50 values, both methanol extract and methanol fraction exhibited extreme antioxidant activity, in contrast to *n*-hexane fraction which was weak. These results suggested that flavonoids, polyphenols, alkaloids, and terpenoids, identified by the phytochemical test, were synergistic responses to antioxidant activity. Furthermore, ethyl acetate and *n*-hexane fractions without terpenoids showed weaker antioxidant activity than the formerly

tested samples. This study revealed that the presence of terpenoids strengthened antioxidant activity. Regarding mechanism, Jafar et al. (2020) have stated that polyphenols and alkaloids can donate their radical hydrogens to neutralize and stop chain reactions, whereas, for terpenoids, the mechanism is by capturing reactive species such as superoxide. In comparison between IC50value of ascorbic acid with 0.0537 ppm (Fig 3) and IC50 values of tested samples, it can be seen clearly that antioxidant activity of extract and its fractions of the root of gitaan are weaker than the ascorbic acid.

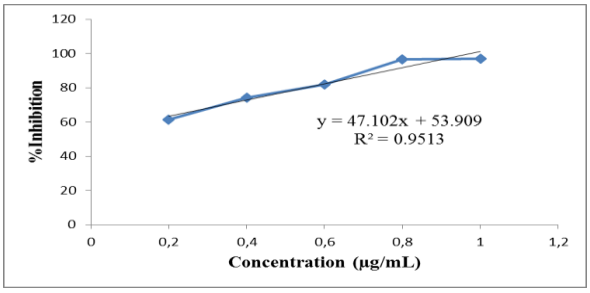

Figure 3 Correlations between concentration and % inhibition of ascorbic acid

CONCLUSIONS

This study revealed that the root of Gitaan (Willughbeia coreacea) contains flavonoids, polyphenolics, alkaloids, and terpenoids. Both the methanol extract and its fractions exhibited antioxidant properties. The methanol extract demonstrated higher antioxidant activity than its fractions; however, its activity was lower than that of ascorbic acid. Consequently, further purification of the methanol extract using various chromatographic techniques, including reversed-phase high-performance liquid chromatography (RP-HPLC), is necessary to isolate pure compounds and elucidate their structures responsible for the observed antioxidant activities.

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