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RESEARCH ARTICLE

Phytochemicals and Antioxidant Activity of *Anacardium occidentale* L.

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ABSTRACT

Anacardium occidentale, locally known as "*pokok gajus*" is known for its medicinal purposes. The objectives of this study are to isolate compounds from the stem bark extract of *A. occidentale* and to study the antioxidant properties of the extracts. Cold maceration method was performed for the extraction of *A. occidentale* stem bark. Purification of the crude extracts using column chromatography successfully isolated two compounds. The isolated compounds were elucidated using spectroscopic methods including Fourier Transform Infrared and Nuclear Magnetic Resonance spectroscopy. These compounds were identified as stearic acid and β-sitosterol. The antioxidant potential of the crude extracts was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results revealed that methanol extract was most effective in the capability to scavenge free radicals as it exhibited the highest radical scavenging activity towards DPPH with an IC_{50} value of 87.67 µg/mL. Total phenolic content and total flavonoid content of the extracts were measured. Both total phenolic and flavonoid contents showed a good correlation with the antioxidant activity indicated that both compounds were the major contributors to the antioxidant capacity of the extracts. Based on the findings, *A. occidentale* can be considered as an effective source of natural antioxidants.

Keywords: *Anacardium occidentale*, isolation, spectroscopy, antioxidant

1. INTRODUCTION

The enormous variety of Malaysian flora with a diverse range of chemicals is one of the major aspects that essentially make natural products as excellent sources in every screening method. Natural products are among the most prominent sources of leading substances in drug development and have been regarded as an important source of medicinal agents and bioactive compounds since the dawn of historical times. The presence of over 200,000 natural metabolites containing a variety of unique bioactive properties emphasizes the importance of natural resources in the discovery of new drugs (Boy et al., 2018). *Anacardium occidentale* is one of the medicinal plant species found in the tropical regions of Malaysia. *A. occidentale,* known as *pokok gajus*. It belongs to the plant family of Anacardiaceae, popular for its medicinal purposes and being traditionally used for the treatment of many diseases (Runjala and Kella, 2017). Phytochemical studies on this species revealed the presence of various secondary metabolites, which are of potential medicinal uses. Studies have found that some of these compounds extracted from *A. occidentale* exhibit biological activities such as antioxidant, anti-

inflammatory, antibacterial, antimicrobial, antitumor, anti-mutagenic, and anti-allergic (Olatunji, 2015; Uni et al., 2018; Yuliana et al., 2014; Aracelli et al., 2016). The main focus was on the extraction, isolation, and characterization of compounds from the stem barks of *A. occidentale.* The objectives of this study are to isolate phytochemical constituents in *A. occidentale* that grow in Malaysia and evaluate its antioxidant properties.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The stem barks of *A. occidentale* were collected from Kelantan in September 2019. The stem barks were dried and chopped into small pieces before grinding it into fine powdered sample. The extraction of stem barks of *A. occidentale* was carried out using cold maceration technique. The grinded stem barks of *A. occidentale* (900 g) were sequentially extracted using *n*-hexane, chloroform, and methanol (2.5 L each) at room temperature for 72 hours. The crude extracts were then filtered out and the filtrate was then concentrated using a rotary evaporator, yielding *n*-hexane crude extract (0.92 g, 0.10%), chloroform extract (1.29 g, 0.14%), and methanol extract (3.30 g, 0.37%).

2.2. Isolation of chemical constituents

Purification of the crude extract was carried out by gravity column chromatography (CC) using Merck silica gel 60 (70-230 mesh). The components were eluted initially with *n*-hexane and the polarity of the eluent was gradually increased by the addition of a higher percentage of diethyl ether. Eluents were then collected and analyzed using TLC. Fractions with similar TLC profiles were pooled together and combined. The combined fractions with a single spot on the TLC were labelled as AO61 and AO93. Fraction A061 was obtained from the purification of n-hexane extract using CC had afforded white solid of stearic acid **(1)** (0.01 g, 1.09%); m.p. 65-68°C; IR (ATR) νmax cm-1 : 3500-2500 (O-H), 2915 (sp3 C-H), 1701 (C=O); 1H NMR (CDCl3, 300 MHz) : δ 2.35 (2H, t, J = 7.5 Hz, H-2), 1.64 (2H, m, H-3), 1.29 (14 × 2H, m, H-4 - H-17), 0.88 (3H, t, J = 6.6 Hz, H-18). Fraction AO92 was yielded from the purification of n-hexane extract using CC had yielded white solid of β-sitosterol **(2)** (0.02 g, 2.17%); m.p. 130-132°C; IR (ATR) νmax cm-1 : 3413 (OH), 2934 (sp3 C-H), 1642 (C=C alkene), 1052 (C-O); 1H NMR (CDCl3, 300 MHz) : δ 3.54 (1H, m, H-3), 5.37 (1H, m, H-6), 0.70 (3H, s, H-18), 1.03 (3H, s, H-19), 0.94 (3H, d, J = 6.0 Hz, H-21), 0.85 (3H, d, J = 6.9 Hz, H-26).

2.3. Antioxidant aactivity

Free radical scavenging activity of crude extracts of *A. occidentale* stem barks was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Johari and Khong (2019) with some modifications. The crude extracts were dissolved in methanol (MeOH) to obtain a series of stock solutions with different concentrations. The stock solution was serially diluted to a final concentration of 1000, 500, 250, 125, and 62.5 µg/mL. A solution of DPPH in methanol was prepared by dissolving DPPH (8 mg) in MeOH (100 mL). The DPPH methanolic solution (100 µL) was then added to aliquots of stock sample (50 µL). The mixture was shaken and allowed to stand at room temperature under dark conditions for 30 minutes. The absorbance was measured at 517 nm by using microplate reader and a commercial vitamin C was used as a positive control. All measurements were carried out in triplicate.

2.4. Total phenolic content (TPC)

Total phenolic contents of *A. occidentale* bark extracts were measured using the Folin-Ciocalteu method as described by Madjitoloum et al. (2018) with slight modifications. An aliquot (25 µL) of each crude extract and distilled water were mixed with 125 µL of diluted Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand in the dark at room temperature for 10 minutes to enable reagents to react completely with the phenolates. A 125 µL of 7.5% Na_2CO_3 was then added to the mixture to quench residual reagents followed by incubation for 30 minutes at room temperature. Absorbances were measured at 760 nm against the reagent blank using a spectrophotometer. All experiments were performed in triplicate. Total phenolic contents of the samples were determined from the calibration curve equation, $y = 0.0056x + 0.0353$ with $R^2 = 0.9964$ and were expressed in miligrams of gallic acid equivalents per gram of extract (mg GAE/g).

2.5. Total flavonoid content (TFC)

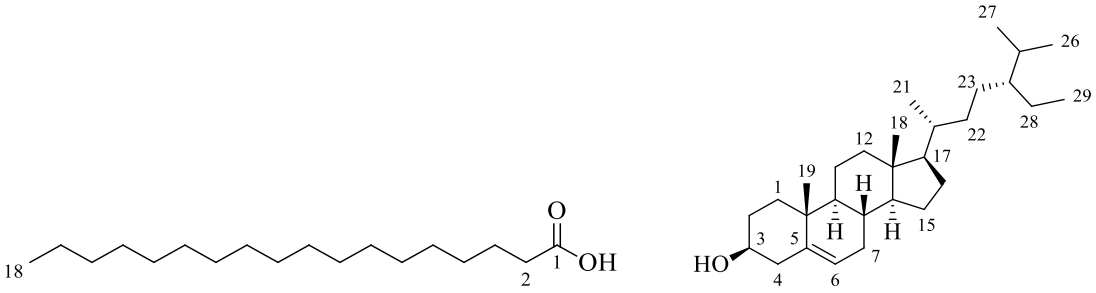
Total flavonoid contents of *A. occidentale* bark extracts were measured using aluminium chloride colorimetric method (Madjitoloum et al., 2018). A 120 µL of 5% $NaNO_2$ was added into a 25 µL aliquot of crude extracts and were incubated at room temperature for 5 minutes. A 15 µL of diluted aluminum chloride, 10% $AlCl_3$ was added into the mixture. After 6 minutes, 50 µL of 1M NaOH was added and absorbances were measured at 510 nm against the reagent blank. All experiments were performed in triplicate. Total flavonoid contents of the samples were determined from the calibration curve equation, $y = 0.0012x + 0.0224$ with $R^2 = 0.9863$ and were expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g).

3. RESULTS AND DISCUSSION

3.1. Characterization of isolated compounds

Compound **(1)** was characterized and the IR spectrum showed a broad absorption band at 3500-2500 cm^{-1} indicated the presence of O-H group of an acid. A strong absorption band at 2915 cm^{-1} represented the stretching vibration of sp^3 C-H group, while a strong stretching absorption at 1701 cm^{-1} was assigned to the C=O stretching of an acid. The ^1H NMR spectrum showed a triplet signal at δ 0.88 with a *J* value of 6.6 Hz indicated the presence of H-18 methyl proton. A peak was observed at δ 1.29 represented a long chain of methylene protons comprising of 14 overlapping methylene groups. A multiplet signal at δ 1.64 indicated two protons of H-3 which attached to methylene adjacent to carboxyl group. A triplet peak at δ 2.35 with *J* value of 7.5 Hz corresponded to the methylene protons adjacent to carboxyl group. Based on the above data, compound **(1)** was identified as stearic acid.

Compound **(2)** was also characterized using IR and ^1H NMR. The IR spectrum showed a broad absorption band at 3413 cm^{-1} indicated the presence of hydroxyl group. A sharp peak appeared at 2934 cm^{-1} represented the stretching vibration of sp^3 C-H group. A peak indicated the presence of C=C alkene was observed at 1642 cm^{-1}, while a strong absorption band centered at 1052 cm^{-1} was assigned to the C-O stretching of a secondary alcohol. The ^1H NMR spectrum showed a multiplet signal at δ 3.54 represented the oxymethine proton of H-3, a proton attached to the hydroxy group of C-3. The presence of olefinic proton of H-6 was observed at δ 5.37. Two methyl singlets appear at δ 0.70 and 1.03 represent protons of H-18 and H-19, respectively. Another two methyl protons of H-21 and H-26 were observed as doublet signals centered at δ 0.94 and 0.85 with *J* value of 6.0 and 6.9 Hz, respectively. Based on the above data, compound **(2)** was identified as β-sitosterol.



(1) **(2)**

3.2. Antioxidant activity

The crude extracts of *A. occidental* were analyzed for antioxidant activity using DPPH free radical scavenging assay. Vitamin C was used as the positive control and the half-maximal inhibitory concentration (IC_{50}) of each extract was measured and expressed as µg/mL, which is defined as the concentration of substrate required to inhibit 50% of the radical (Ajileye et al., 2015). The result of DPPH radical scavenging assay of *A. occidentale* stem bark extract in is tabulated in Table 1. The methanolic extract was most effective in its ability to scavenge free radical as it exhibited the highest radical scavenging towards DPPH from 38.39% at 62.5 µg/mL to 92.91% at 1000 µg/mL. The results revealed that methanolic extract exhibited strong antioxidant activity in the DPPH assay with an IC_{50} value of 87.67 µg/mL. This finding is similar to previous study which showed high antioxidant capacity of stem barks and leaves extracts of *A. occidentale* (Olatunji, 2015; Uni et al., 2018).

Table 1. IC_{50} value of *A. occidentale* extracts

Sample	Percentage inhibition (I%) [Concentration in ppm]					IC_{50} (µg/mL)
	62.5	125	250	500	1000	
AOBH	3.32	9.42	16.86	23.32	36.05	1981
AOBC	4.30	7.80	15.43	20.45	45.29	1272
AOBM	38.39	60.00	87.89	92.20	92.91	87.67
Vitamin C	90.87	94.66	96.73	98.28	99.83	4.52

*****AOBH- *A. occidentale* hexane extract; AOBC- *A. occidentale* chloroform extract; AOBM- *A. occidentale* methanol extract

3.3. Total phenolic content and total flavonoid content

The total phenolic contents of *A. occidentale* stem bark extracts were measured using the Folin-Ciocalteau method with gallic acid as a reference standard. The value of total phenolic content of each extract was determined from the calibration curve of gallic acid ($y = 0.0056x + 0.0353$) with R^2 value of 0.9964. Based on the results tabulated in Table 2, the methanolic extract displayed the highest phenolic content which was 81.91 mg GAE/g followed by chloroform and hexane extracts. Meanwhile, the total flavonoid content of *A. occidentale* extracts were determined using aluminium chloride colorimetric method with quercetin as a reference standard. The values of total flavonoid contents of the extracts were measured from the quercetin calibration curve equation ($y = 0.0012x + 0.0224$) with R^2 value of 0.9863. The highest value of total flavonoid content was observed in methanolic stem bark extract with the value of 56.33 mg QE/g. Both phenolic and flavonoid content showed a positive correlation with antioxidant activity of *A. occidentale* extracts. The total phenolic and total flavonoid content were significantly correlated with antioxidant activity with the correlation coefficients of $R^2 = 0.8668$ and $R^2 = 0.9188$, respectively. These results indicated that flavonoids and phenolic compounds were the major factors contributing to the antioxidant capacity of *A. occidentale* bark extracts.

Table 2. Total phenolic content and total flavonoid content of *A. occidentale*

Sample	TPC (mg GAE/g)	TFC (mg QE/g)
AOBH	6.20	14.67
AOBC	6.73	18.83
AOBM	81.91	56.33

*****AOBH- *A. occidentale* hexane extract; AOBC- *A. occidentale* chloroform extract; AOBM- *A. occidentale* methanol extract

4. CONCLUSION

The study on the phytochemicals and biological activities of *A. occidentale* was successfully carried out. Purification of *n*-hexane crude extract had successfully yielded stearic acid **(1)** and β-sitosterol **(2)**. The methanolic extract of *A. occidentale* exhibited strong antioxidant activity in DPPH with IC_{50} value 87.67 μg/mL. The total phenolic and flavonoid contents showed good correlation with the antioxidant activity indicated that both compounds contributed to the antioxidant capacity of the extracts. In conclusion, the stem barks of *A. occidentale* can be considered as an effective source of natural antioxidants.

Declaration of Interest
There is no conflict of interest regarding this study.

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