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Measurement of Antioxidant Activity and Structural Elucidation of Chemical Constituents from *Aglaia oligophylla* Miq.

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Abstract. *Aglaia oligophylla* Miq. is a shrub of approximately 25 meters tall under the *Meliaceae* family which possess distinct pharmacological properties including insecticides, anti-inflammatory and anticancer. The purpose of this study is to evaluate the antioxidant capacity of the trunks and stem of *Aglaia oligophylla* plant extracts and to isolate their chemical constituents. The antioxidant activity was evaluated as the reducing antioxidant capacity includes of cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) assays. In CUPRAC assay, ethyl acetate extract of the trunks part exhibited the strongest reducing capacity with the value of 1543 mg Trolox equivalent (TE)/g and for the stem part, methanol extract showed the strongest reducing antioxidant capacity with the value of 1059 mg TE/g. In FRAP assay, the methanol extracts of both trunks and stem of the plant showed the strongest reducing power with the values of 1269 mg TE/g and 1084 mg TE/g respectively. Repeated column chromatographic separation on chloroform extract of the trunks part afforded one triterpenes which was suggested to be stigmasterol (**1**) whilst the separation on methanol extract of the trunks obtained a triterpene, *β*-sitosterol (**2**). In the stem of *A. oligophylla*, the column chromatographic separation on petroleum ether extract afforded a new triterpene, namely oligophyllic acid (**3**) while the separation on chloroform and ethyl acetate extracts gave compound **2**. All the chemical constituents were elucidated by comparison with literature review reported previously. Based on the value of CUPRAC and FRAP antioxidant assays of the plant extracts, *A. oligophylla*, shows great potential for the possibility of discovery and development of health promoting supplement from the extracts and chemical constituents.

Keywords: *Aglaia oligophylla*, stigmasterol, *β*-sitosterol, FRAP, CUPRAC, antioxidant, reducing power.

1. Introduction

The contribution of free-radical-induced damage in oxidative stress to the pathogenesis and pathophysiology of chronic health diseases had been confirmed by previous studies [1]-[3]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) causes damage of cellular macromolecules including DNA lesion which will leads to degenerative diseases. Antioxidants are the substances that capable of delaying or quenching the oxidation through scavenging or reducing of free radicals, and are considered important nutraceuticals on account of many health benefits [4]. The employment of medicinal plant and natural product as the alternative sources of antioxidant in prevention of degenerative diseases is receiving great attention worldwide due to their distinctive pharmacological properties with minimal adverse effects as compared to synthetic antioxidants, which showed potential toxicity and carcinogenicity [5].

Aglaia oligophylla is a tree with a Latin name, *oligophylla* which means a few leaves. It distributes widely in Brunei, India, Indonesia, Malaysia, Philippines, Singapore and Thailand. The objective of this study was focused on the antioxidant properties and phytochemistry of *A. oligophylla* due to the interesting

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biological activities of the extracts and phytochemicals of the plant species reported by previous research. Various types of natural products were obtained from *A. oligophylla* including bisamides, flavaglines and triterpenoids which were found to exhibit insecticidal properties [6], [7]. The antioxidant activities of *A. oligophylla* in this study were conducted using electron transfer reaction assays which were CUPRAC and FRAP assays. Electron transfer based assays measure the free radical scavenging capacity of the sample which involved redox reaction with oxidant as the indicator of reaction endpoint and as the probe of monitoring reaction [8].

2. Materials and Methods

2.1. Plant Material

The trunks and stem of *A. oligophylla* were collected from Hutan Simpan Ayer Hitam, Puchong, Malaysia and voucher specimen was deposited at the Herbarium of Faculty of Forestry, Universiti Putra Malaysia. The plant samples were air-dried and cut into small pieces prior to extraction.

2.2. Extraction and Isolation

The finely ground air-dried trunks (1.5 kg) and stem (1.6 kg) of the plant were extracted using cold maceration method. The plant samples were extracted successively with hexane/petroleum ether, chloroform, ethyl acetate and methanol at room temperature for 72 hours and were repeated twice with the same solvent system. The filtrates were concentrated under reduced pressure to give hexane (1.5 g), chloroform (4.7 g), ethyl acetate (3.5 g) and methanol (6.7 g) extracts of the trunks part of plant as well as petroleum ether (67.1 g), chloroform (30.0 g), ethyl acetate (2.0 g) and methanol (11.2 g) extracts of the stem part of plant. The extracts were fractionated using gravity column chromatography and the fractions collected were monitored and pooled using thin layer chromatography. The pure compounds were obtained from purification by recrystallization and various chromatographic techniques.

2.3. *In Vitro* Antioxidant Assays

The reducing antioxidant capacity of the extracts were investigated using CUPRAC and FRAP assays. The extract sample was prepared in concentration of 1000 ppm, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the standard in both CUPRAC and FRAP assays and the results were expressed as mg TE/g of extract. The absorbance of the sample mixtures was read via spectrophotometer (BMG Labtech, Germany). All the assays were done in duplicate and were repeated thrice at room temperature. The result was expressed as mean for duplicate measurements.

The CUPRAC assay was performed according to the adapted method described previously [9]. Working CUPRAC reagent was prepared by mixing 10 mM of copper(II) chloride, 7.5 of mM neocuproine and 1 M of ammonium acetate buffer of pH 7.0. Extract, water or Trolox (50 µL) was mixed with 1.0 mL of CUPRAC reagent and allowed to stand in dark for 30 minutes before the absorbance measured at wavelength of 450 nm.

The FRAP assay was conducted based on method described previously [10]. The FRAP reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM of 2,4,6-tris(2-pyridyl)-s-triazine in 40 mM hydrochloric acid and 20 mM iron(III) chloride hexahydrate. Extract, water or Trolox (50 µL) was mixed with 1.0 mL of FRAP reagent and was allowed to stand in dark for 30 minutes before the absorbance measured at wavelength of 595 nm.

3. Results

3.1. Antioxidant Activity

The reducing capacities of the extracts were verified via CUPRAC and FRAP assays (Fig. 1). In CUPRAC assay, all extracts showed significant reducing antioxidant capacity with values of more than 600 mg TE/g. Ethyl acetate extract of trunk parts of the plant and methanol extract of stem part of the plant exhibited the strongest reducing powers with values of 1543 mg TE/g and 1059 mg TE/g respectively. The strength of antioxidant activity of the trunks part extracts in CUPRAC assay was in the following order: ethyl

acetate > hexane > methanol > chloroform extracts whereas the strength of antioxidant activity of the stem extracts were ranked as: methanol > ethyl acetate > petroleum ether > chloroform extracts.

In FRAP assay, hexane, chloroform and ethyl acetate extracts of the trunks of plant gave significant reducing antioxidant capacity with values of more than 400 mg TE/g whilst petroleum ether, chloroform and ethyl acetate extracts of the stem of plant exhibited moderate reducing power with values of not more than 200 mg TE/g. Meanwhile, the methanol extracts of trunks and stem of the plant showed the strongest reducing antioxidant capacity in FRAP assay with values of 1269 mg TE/g and 1084 mg TE/g respectively. The FRAP reducing power of the trunks of plant extracts was ranked in the following order: methanol > ethyl acetate > hexane > chloroform extracts while the FRAP reducing power of the stem of plant extracts was in the order of methanol > petroleum ether > chloroform > ethyl acetate extracts.

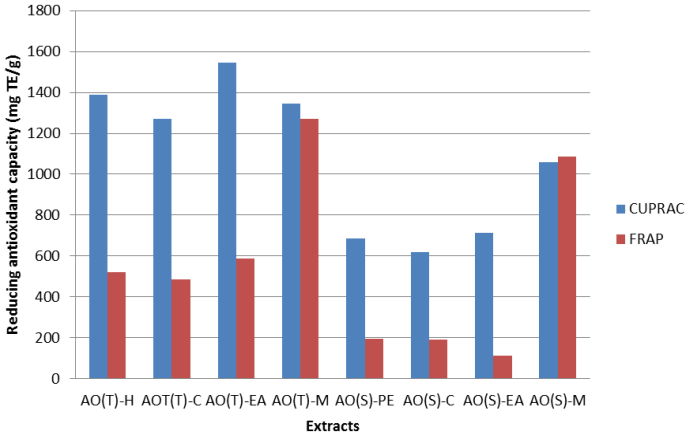

Fig. 1: Cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) of hexane (H), petroluem ether (PE), chloroform (C), ethyl acetate (EA) and methanol (M) extracts of trunks (T) and stem (S) of *Aglaia oligophylla* (AO).

4. Phytochemistry

The plant extracts were subjected to fractionation to obtain pure compounds. The chloroform and methanol extracts of the trunk part afforded stigmasterol (**1**) (300.0 mg) and *β*-sitosterol (**2**) (1.0 mg) respectively. The petroleum ether stem extract afforded a new compound named oligophyllic acid (**3**) (41.8 mg) whilst the chloroform and ethyl acetate extracts of the stem part isolated compound **2** (2.1 mg) (Fig. 2). The spectral data of the isolated chemical constituents was as following:

Stigmasterol (**1**). White solid, m.p. 136-137 ℃ (m.p. 135-136 ℃) [11]; EIMS *m/z* (rel. intensity): 412 $[M]^+$ (27); IR (cm^{-1}, UATR) ν_{max}: 3422, 2935, 1724, 1455, 756; ^1H and ^{13}C NMR spectral data were in good agreement with previously reported data [11].

β-Sitosterol (**2**). Colorless crystal, m.p. 134-135 ℃ (m.p. 136-138 ℃) [12]; EIMS *m/z* (rel. intensity): 414 $[M]^+$ (84); IR (cm^{-1}, UATR) ν_{max}: 3442, 2938, 2868, 1646, 1462, 1378, 1050; ^1H and ^{13}C NMR spectral data were in good agreement with previously reported data [13].

Oligophyllic acid (**3**). White solid, m.p. 274-276 ℃; HR-EIMS *m/z* (rel. intensity): 505.3521 $[M+H]^+$ (100); IR (cm^{-1}, UATR) ν_{max}: 3413, 2949, 1706, 1459, 1382, 1168; 762; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) refer to Table 1.

The molecular formula of compound **3** was assigned as $C_{32}H_{56}O_4$ in accordance with the high resolution electron ionization mass spectrometry (HR-EIMS) which gave an $[M+H]^+$ adduct ion peak at *m/z* 505.3521 ($C_{32}H_{57}O_4$ is calculated for 505.7925). The detailed analysis of the spectral data of compound **3** indicated the compound is closely related to foveolin B (**4**), a dammarane triterpene isolated from the bark of *Aglaia foveolata* Pannell [14]. Compound **3** is different from foveolin B with the point of attachment of the carboxylic acid group at C-1 and the substitution of hydroxyl group with propyl group at C-4. The compound was identified as 20,24-epoxy-25-hydroxy-4-propyl-2-secodammarane-2-oic acid and given trivial name ‘oligophyllic acid’. The connectivity of protons and carbons of compound **3** can be seen by analyzing the two-bond and three-bond correlations in the HMBC spectrum (Fig. 3).

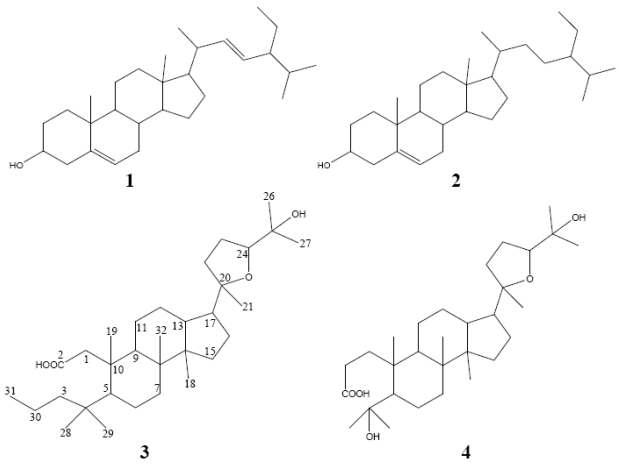

Fig. 2: Structures of stigmasterol (**1**), *β*-sitosterol (**2**), oligophyllic acid (**3**) and foveolin B (**4**).

Table 1: ^{1}H NMR, ^{13}C NMR and HMBC spectral data of oligophyllic acid (**3**)

H/C	δ_H (multiplicity, *J* Hz)	δ_C	HMBC	δ_H* (multiplicity, *J* Hz)	δ_C*
1	2.72 (*d*, 13.7) 2.32 (d, 13.7)	46.1	C_2, C_5, C_{10}	-	34.9
2	-	178.0	-	2.18 2.44	27.8
3	-	23.9	-	-	179.7
4	-	48.1	-	-	76.8
5	-	51.7	-	-	53.3
6	-	23.9	-	-	22.7
7	-	30.6	-	-	34.9
8	-	41.4	-	-	40.2
9	-	44.4	-	-	42.5
10	-	44.2	-	-	41.5
11	-	21.5	-	-	21.4
12	-	28.0	-	-	27.2
13	-	45.0	-	-	43.2
14	-	51.5	-	-	50.4
15	-	35.7	-	-	31.3
16	-	26.6	-	-	26.0
17	-	50.8	-	-	51.9
18	1.00 (*s*)	15.7	C_8, C_{13}, C_{14}, C_{15}, C_{16}	0.84 (*s*)	16.1
19	0.97 (*s*)	20.2	C_1, C_6, C_9, C_{10}	0.99 (*s*)	20.7
20	-	87.7	-	-	86.6
21	1.10 (*s*)	23.3	C_{16}, C_{17}, C_{22}	1.12 (*s*)	22.0
22	-	36.8	-	-	37.5
23	-	27.4	-	-	26.0
24	3.72 (*t*, 6.9)	84.7	-	3.73 (*dd*, 7.0, 7.0)	84.5
25	-	72.8	-	-	71.6
26	1.10 (*s*)	25.3	C_{24}, C_{25}	1.19 (*s*)	27.8
27	1.13 (*s*)	22.9	C_{24}, C_{25}, C_{26}	1.09	24.5
28	1.26 (*s*)	30.6	-	1.29	34.6
29	1.13 (*s*)	26.1	C_4, C_5, C_{30}	1.24 (*s*)	27.4
30	1.43 (*m*)	32.2	-	0.96 (*s*)	15.5
31	1.20 (*m*)	32.2	C_3, C_4	-	-
32	0.85 (*s*)	16.6	C_8, C_9, C_{14}	-	-

*Roux *et al.* [11]

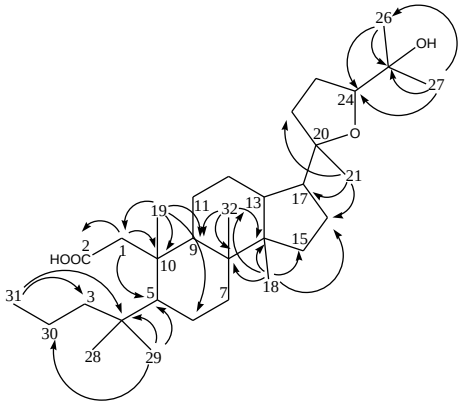

Fig. 3: Selected HMBC correlations of oligophyllic acid (**3**).

5. Discussion

In this study, we applied two *in vitro* methods namely CUPRAC and FRAP to investigate the antioxidant properties of *Aglaia oligophylla*. CUPRAC and FRAP are the electron transfer-based assays that evaluated the antioxidant capacity of the substance in term of their ability to transfer single electron and reduce the chromogenic redox reagents. The assays measured the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the amount of the antioxidants in the sample. In CUPRAC assay, copper(II)-neocuproine (Cu(II)-Nc) was used as the chromogenic oxidant which will change color from blue to yellow when reduced. The chemical reaction involved in the assay was as shown below [15]:

$$Cu(Nc)_2^{2+} + \text{antioxidant} \rightarrow Cu(Nc)_2^{+} + \text{oxidized antioxidant} + H^{+}$$

On the other hand, FRAP utilized ferric-tripyridyltriazine (Fe(III)-TPTZ) complex as the chromogenic oxidant. The brown color Fe(III)-TPTZ will be reduced to ferrous-tripyridyltriazine (Fe(II)-TPTZ) complex which is blue color in the redox reaction. The chemical reaction involved in FRAP was as following [16]:

$$[\text{Fe(III)-TPTZ}]^{3+} + \text{antioxidant} \rightarrow [\text{Fe(II)-TPTZ}]^{2+} + \text{oxidized antioxidant} + H^{+}$$

The results from the antioxidant assays indicated that all the extracts possessed stronger antioxidant potency towards CUPRAC compare to FRAP. Copper which has a lower redox potential than iron, improves the redox cycling and hence the copper reduction is more sensitive than iron reduction. The low redox potential of copper also improves the selectivity of its reactions towards common interferences such as sugars and citric acid as compare to iron. Therefore, CUPRAC served as a more sensitive indicator than FRAP. CUPRAC is a better antioxidant assay than FRAP as it could detect all classes of antioxidants with trace of interference from reactive radical and the kinetics reaction of copper are faster than iron [17].

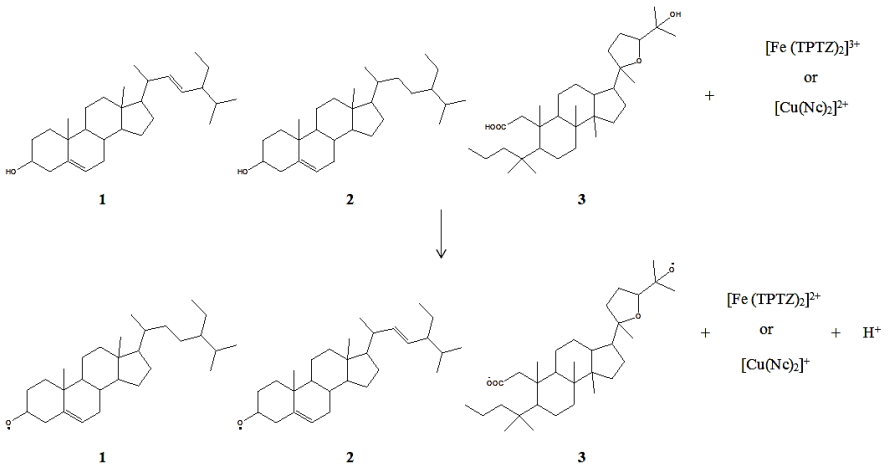

Fig. 4: The proposed mechanism of antioxidant activity of stigmasterol (**1**), *β*-sitosterol (**2**), oligophyllic acid (**3**) in CUPRAC and FRAP assays.

Fig. 1 also indicated that extracts from the trunks of *A. oligophylla* showed better antioxidant property than the extracts of stem bark part of the plant. This may be explained by the presence of stigmasterol (1) in the trunks part of the plant, which was absence in the stem bark of the plant, enhanced the antioxidant properties of the extracts of trunks part of the plant. Chromatographic separation of this active extracts afforded stigmasterol (1), *β*-sitosterol (2) and oligophyllic acid (3). The isolated compounds are triterpenes with tetracyclic skeleton, which may be responsible for the antioxidant properties of the plant extracts. The presence of electron-rich oxygen atoms in hydroxyl and carboxyl groups of the compounds enhanced the ability of the compounds to donate electron to the free radical, which thus increased the oxidative properties of the compounds [18]. The proposed mechanism of antioxidant activity of stigmasterol (1), *β*-sitosterol (2) and oligophyllic acid (3) in CUPRAC and FRAP assays were as shown in Fig. 4 [19].

The promising antioxidant activities of plant extracts from CUPRAC and FRAP assays indicated the potential of developing stigmasterol (**1**), *β*-sitosterol (**2**) and oligophyllic acid (**3**) as antioxidant agents. Numerous of previously studies showed that stigmasterol (**1**) and *β*-sitosterol (**2**) were neither genotoxic nor cytotoxic effect toward normal cell line [20], [21]. Oligophyllic acid (**3**) may show low cytotoxicity as foveolin B (**4**), which possessed similar structure with oligophyllic acid, demonstrated negligible cytotoxicity towards several cancerous cell lines [22]. Since a safety evaluation of the compounds is vital in regard to their probable beneficial use in human health, thus stigmasterol (**1**), *β*-sitosterol (**2**) and oligophyllic acid (**3**) may serve as the good candidates for pharmaceutical purposes with their minimal cytotoxicity.

6. Conclusions

Secondary metabolites from medicinal plants afford a vast array of bioactivities including anticancer, antioxidant and anti-inflammatory [23]. Natural antioxidants of plant origin such as terpene derivatives have been reported to possess antioxidative properties and several terpene rich plants extracts such as rosemary and sage were marketed as dietary supplement [24]. The reducing antioxidant capacities of the extracts evaluated by CUPRAC and FRAP assays indicated strong antioxidant activity of *Aglaia oligophylla*. The separation and purification of *A. oligophylla* active extracts afforded triterpenes (stigmasterol, *β*-sitosterol and oligophyllic acid) as the main phytochemicals. Strong antioxidant property of the extract highlights the possibility of the plant being developed as health promoting supplement particularly for the prevention of degenerative diseases. The chemical constituents afforded from active extract can also be a good candidate for pharmaceutical purposes. The *in vivo* study of *A. oligophylla* extracts as antioxidant agent and its toxicity should be further investigated.

7. Acknowledgements

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