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Phytochemical study and antioxidant activity of the heartwoods of *artocarpus scortechinii* king

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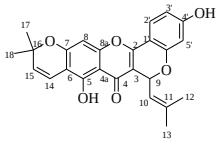
GRAPHICAL ABSTRACT



Cudraflavone A

ABSTRACT

Artocarpus scortechinii belongs to Moraceae family or mulberry family. There are around 50 species of Artocarpus present in South East Asia and Pacific Island which 20 species can found in Malaysia. Phytochemical study and antioxidant activity of the heartwoods of A. scortechinii have been carried out. The extraction process was conducted using cold extraction method with three different polarity of solvent which were n-hexane, dichloromethane and methanol. Fractionation of the methanol extract was performed using vacuum liquid chromatography while purification of fractions was carried out using column chromatography. Two pure compounds had been successfully isolated from the methanol extract. The structure of pure compounds was determined by proton nuclear magnetic resonance (^{1}H NMR), ^{1}H-^{1}H COSY and FTIR spectroscopies. The compounds were identified as cudraflavone A and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(3,3-dimethylallyl)flavone. Evaluation on the antioxidant activity of the crude extracts and pure compounds was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Both pure compounds were observed as inactive antioxidant agent.

Keywords: Artocarpus scortechnii, cudraflavone A, Antioxidant activity

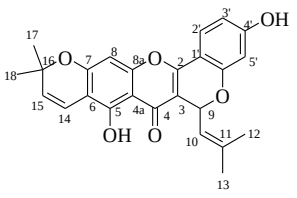
1. INTRODUCTION

Moraceae is a large primarily tropical family with several economically and ecologically important species such as breadfruit (*Artocarpus altitis*), paper mulberry (*Broussonetia papyrifera*) and figs (*Ficus*) [1]. This Moraceae family has around 50 genera and 1500 species all around the world that are mostly present in tropical forest. In Malaysia, this family has about 10 genera and 140 species which can be found mostly in lowland [2]

Artocarpus is a genus belonging to the mulberry family, Moraceae. Genus *Artocarpus* comprises about 50 species found in a Southeast Asia, the pacific northern Australia and the West Indies. Some of the species can suits with the cool climatic conditions growing in the tropical mountains as high as 1500 m. Many of the species are important food plants whose fruit and seeds are considered as food, such as breadfruit [2]. Some species have useful bark from which tannin is extracted.

Artocarpus scortechinii is known as "terap hitam" in Malaysia. It can be found throughout Malaysia in lowland forest and Sumatera, Indonesia. *Artocarpus* are rich in flavonoids and other phenolic compounds, for example chalcone, flavone, xanthone and stilbene that have biological activities such as antifungal and anticancer [1].

Artocarpus species are usually used in medication. The leaves of *A. heterophyllus* or jackfruit tree are useful for curing boils, fever, and skin diseases [4]. The ash of jackfruit leaves can be used to treat ulcers by burnt with corn and coconut shells. Besides, the roots part of jackfruit is used to treat diarrhea and fever while the woods are used as sedative for convulsions.



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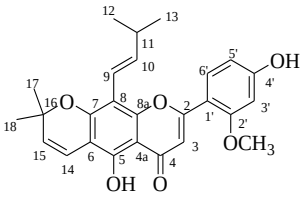

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2. EXPERIMENTAL

General experimental procedure

Thin layer chromatography (TLC) analysis was performed using 0.20 mm pre-coated silica gel aluminium plate (Merck 60 F254) with *n*-hexane and ethyl acetate (EtOAc) as the solvent system. The spots on the TLC plate was visualized by short UV light (254 nm) and then sprayed with vanillin sulphuric acid spraying agent. The reagent was prepared by mixing vanillin (0.5 g), MeOH (80 mL), acetic acid (10 mL) and concentrated sulphuric acid (5 mL).

Vacuum liquid chromatography (VLC) analysis was carried out for fractionation using Merck silica gel (230-240 mesh) with *n*-hexane and EtOAc as mobile phase. Gravity column chromatography (CC) was performed for purification process. Merck silica gel (70-230 mesh) will be used as the stationary phase with hexane and EtOAc as mobile phase.

The structure of pure compounds were elucidated by using ^{1}H Nuclear Magnetic Resonance (^{1}H NMR), Infrared Spectroscopy (IR) and ^{1}H-^{1}H COSY. The ^{1}H NMR spectra were recorded using Bruker Avance spectrometer (400 MHz). And chemical shifts recorded in δ ppm in deuterated acetone (CD_3COCD_3) as a solvent. The IR spectra was recorded using Perkin-Elmer series 1600.

Plant materials

The heartwoods of *A. scortechinii* were collected from forest of Bukit Fraser, Pahang, Malaysia in December 2013, dried at room temperature and ground into small pieces. Then the extract were filtered and concentrated by using a rotary evaporator. The extraction was repeated twice by adding fresh *n*-hexane. The same extraction procedure repeated by using CH_2Cl_2 and MeOH. The extracts were concentrated to give sticky dark gummy of *A. scortechinii*.

Extraction and isolation

The MeOH crude extract of *A. scortechnii* (3 g) was fractionated using Vacuum Liquid Chromatography (VLC) with column size 4.0 cm × 7.0 cm and packed with Merck silica gel 230-400 mesh (75.0 g). The solvent system used was a combination of *n*-hexane:EtOAc, and EtOAc:acetone with increasing polarity by 10% to obtain 21 fractions. Fractions with similar colour and TLC profile pattern were combined to give three major fractions ASCHM 1, ASCHM 2 and ASCHM 3.

Purification of ASCHM 1 with *n*-hexane:EtOAc in order of increasing polarity by 5% by using CC yielded cudraflavone A (9 mg, 3.27%) as a yellow solid with R_f value = 0.81 (*n*-hexane:EtOAc = 4:1); m.p. 270-272^{0}C (lit. [5] 265-272^{0}C) ; IR (ATR) ν_{max} cm^{-1} : 3403 (OH), 2976 (sp^3 CH), 1622 (C=O), 1580 and 1458 (C=C), 1233 (C-O) **(Figure 1)**; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.47 (6H, s, H-12 and H-13), 1.69 (3H, s, H-17), 1.96 (3H, s, H-18), 5.48 (1H, d, *J* = 9.2 Hz, H-9), 5.77 (1H, d, *J* = 10.0 Hz, H-15), 6.20 (1H, d, *J* = 9.2 Hz, H-10), 6.45 (1H, d, *J* = 2.0 Hz, H-5'), 6.65 (1H, dd, *J* = 8.6 and 2.0 Hz, H-3'), 6.68 (1H, d, *J* = 10.0 Hz, H-14), 7.72 (1H, d, *J* = 8.6 Hz, H-2') and 13.29 (1H, s, 5-OH). (**Figure 2**).

Purification of ASCHM 1 (0.27 g, 9.16%) was conducted using CC and eluted with *n*-hexane:EtOAc in order of increasing polarity to yield 4′,5-Dihydroxy-6,7-(2,2-dimethylpyrano)-2′-methoxy-8-(3,3-dimethylallyl)flavone **(2)**, (24.5 mg, 8.91%) as yellow solid with a yellow spot on TLC plate; R_f value = 0.71 (hexane:EtOAc = 3:2) [31]; m.p. 259-261ºC (lit. [6] 258-260ºC) ; IR (ATR) ν_{max} cm^{-1} : 3393 (OH), 2939 (sp^3 CH), 1621 (C=O), 1580 and 1462 (C=C), 1207 (C-O) **(Figure 4)**; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.10 (6H, d, *J* = 6.8 Hz, H-12 and H-13), 1.70 (3H, s, H-18), 1.97 (3H, s, H-17), 2.45 (1H, m, H-11), 4.01 (3H, s, 2'-OCH_3), 5.49 (1H, d, *J* =9.6 Hz, H-15), 6.23 (1H, d, *J* = 9.6 Hz, H-14), 6.46 (1H, d, *J* = 2.4 Hz, H-3'), 6.61 (1H, d, *J* = 16.4 Hz, H-9), 6.65 (1H, dd, *J* = 8.8 and 2.4 Hz, H-5'), 6.71 (1H, dd, *J* = 16.4 and 7.2 Hz, H-10), 6.79 (1H, s, H-3), 7.75 (1H, d, *J* = 8.8 Hz, H-6') and 13.69 (1H, s, 5-OH) (**Figure 5**).

DPPH radical scavenging assay

Radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as the reagent and prepared by dissolved 3.943 mg of DPPH in 100 mL methanol to obtain 100 mM solution. The sample (100 µL) was added into the microplate. 100 µL DPPH solution was then added into the sample and DPPH is replaced with methanol as a blank solution. Then, the solution were kept in the dark at room temperature around 30 minutes to allow it completely react. The absorbance will be read at 517 nm and the percentage of DPPH scavenging is measured by formula below

$$\% \text{ DPPH scavenging} = \frac{\text{A blank DPPH} - \text{A sample}}{\text{A blank DPPH}} \times 100$$

All test will be carried out in replicates. The SC_{50} value will be obtained by plotting the DPPH scavenging percentage of each sample against the concentration **.**The SC_{50} value was calculated as the concentration of each sample required to scavenge the DPPH radical by 50%.

3. RESULTS AND DISCUSSION

Compound **(1)** was isolated as a pale yellow solid (9 mg, 3.27 %) with m.p., 270-272^{0}C (lit. [30] 265-272^{0}C) from the MeOH crude extract of *A. scortechinii*. It showed a yellow spot after sprayed with vanillin sulphuric acid spraying agent. The IR spectrum of compound **(49) (Figure 1)** exhibited absorption band for hydroxyl group at 3403 cm^{-1}, *sp*^{3} C-H stretching at 2976 cm^{-1}, a carbonyl group at 1662 cm^{-1} conjugated with an aromatic ring and also chelated with a hydroxyl group, aromatic C=C at 1580 cm^{-1} and 1458 cm^{-1} and C-O stretching at 1233 cm^{-1}.

The ^{1}H NMR spectrum (**Figure 2**) showed a singlet signal at downfield region (δ 13.29) that was assigned for the chelated hydroxyl group (5-OH). The characteristic of dimethylpyrano group was observed at δ 1.69 (3H, H-17), and 1.96 (3H, H-18) which represented by two singlets together with two doublets (*J* = 10.0 Hz) at δ 5.77 and at δ 6.68 for the olefinic protons, H-15 and H-14. 3,3-Dimethylallyl group was observed at δ 5.48 (1H, d, *J* = 9.2 Hz, H-9), and δ 6.21 (1H, d, *J* = 9.2 Hz, H-10) with two methyl groups as a singlet that integrated for six proton at δ 1.47 (H-12 and H-13) attached at C-11. ABX splitting pattern for 1',4',6'-trisubstituted benzene ring of ring B was also observed as doublet resonated at δ 7.72 (1H, d, *J* = 8.6 Hz, H-2'), a doublet at δ 6.45 (1H, d, *J* = 2.0 Hz, H-5') and doublet of doublets at δ 6.65 (1H, *J* = 8.6 and 2.0 Hz, H-3').

Table 1: ^{1}H NMR Data of compound (50) and 4',5-Dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(3,3-dimethylallyl)flavone

Position	Compound (50)	4',5-dihydroxy-6,7-(2,2-dimethyl pyrano)-2'-methoxy-8-(3,3-dimethylallyl) flavone [29]
	1H NMR δ (ppm)	1H NMR δ (ppm)
2		
3	6.79 (1H, s)	6.76 (1H, s)
4		
4a		
5		
6		
7		
8		
8a		
9	6.61 (1H, d, J = 16.4 Hz)	6.56 (1H, d, J = 16.0 Hz)
10	6.71 (1H, dd, J = 16.4 and 7.2 Hz)	6.73 (1H, dd, J = 16.0 and 7.2 Hz
11	2.45 (1H, m)	2.45 (1H, m)
12/13	1.10 (6H, d, J = 6.8 Hz)	1.09 (6H, d, J = 6.8 Hz)
14	6.23 (1H, d, J = 9.6 Hz)	6.22 (1H, d, J = 9.2 Hz)
15	5.49 (1H, d, J =9.6 Hz)	5.48 (1H, d, J =9.2 Hz)
16		
17	1.97 (3H, s)	1.96 (3H, s)
18	1.70 (3H, s)	1.69 (3H, s)
1'		
2'		
3'	6.46 (1H, d, J = 2.4 Hz)	6.44 (1H, d, J = 2.0 Hz)
4'		
5'	6.65 (1H, dd, J = 8.8 and 2.4 Hz)	6.65 (1H, dd, J = 8.4 and 2.0 Hz)
6'	7.75 (1H, d, J = 8.8 Hz)	7.73 (1H, d, J = 8.4 Hz)
2'-OCH3	4.01 (3H, s)	4.00 (3H, s)
4'-OH		
5-OH	13.69 (1H, s)	13.6713.67 (1H, s)

The ^{1}H-^{1}H COSY spectrum (**Figure 3**) supported the correlations between neighbouring protons in this compound. Cross peak was observed between H-9 and H-10, H-14 and H-15. There were also cross peak between H-2' and H-3', H-3' and H-5'. Based on the spectral data obtained and comparison with literature data [5], compound **(1)** was identified as cudraflavone A.

Compound **(2)** was isolated as a pale yellow solid (24.3 mg, 8.84%) with m.p. 259-261ºC, (lit. 258-260 ºC [31]). It showed a yellow spot after sprayed with vanillin sulphuric acid spraying agent. The IR spectrum **(Figure 4)** of compound **(2)** exhibited an absorption band at 3393 cm^{-1} that was indicated to OH group. Absorption frequency corresponding to *sp*^{3} CH was observed at 2939 cm^{-1}. In addition, a medium intensity peak was observed at 1621 cm^{-1} for C=O. Conjugation effect between the C=O and aromatic ring lowered the C=O absorption frequency. Absorption band for C=C aromatic and C-O were observed at 1580 cm^{-1}, 1462 cm^{-1} and 1207 cm^{-1} respectively.

The ^{1}H NMR spectrum (**Figure 5**) showed characteristic signal of chelated hydroxyl group (5-OH) which appeared as a singlet at downfield region δ 13.69. The characteristic signals of 2,2-dimethylpyrano group was also observed which represented by two singlets, each integrated for three proton at δ 1.70 and δ 1.97 (H-17 and H-18), together with two doublets (*J* = 9.6 Hz) at δ 5.49 and (*J* = 9.6 Hz) at δ 6.23 for the olefinic proton, H-14 and H-15. 3,3-dimethylallyl group was observed at δ 6.61 (1H, d, *J* = 16.4 Hz, H-9), δ 6.71 (1H, dd, *J* = 16.4 and 7.2 Hz, H-10), and δ 2.45 (1H, m, H-11) with two methyl groups as doublet that integrated for six proton at δ 1.10 (6H, d, *J* = 6.8 Hz, H-12 and H-13) attached at C-11. The spectrum also revealed a methoxyl group which appeared as a singlet at δ 4.01 (3H, s, 2'-OCH_3). ABX splitting pattern for 1',2',4'-trisubstituted benzene ring of ring B was also observed as doublet of doublets resonated at δ 6.65 (1H, dd, *J* = 8.8 and 2.4 Hz, H-5'), a doublet at δ 7.75 (1H, d, *J* = 8.8 Hz, H-6') and another doublet at δ 6.46 (1H, d, *J* = 2.4 Hz, H-3'). The ^{1}H NMR data of compound **(2)** were compared with literature data [6] of the same compound isolated previously from *A. anisophyllus* which shown in **Table 1**.

The ^{1}H-^{1}H COSY spectrum **(Figure 6)** supported the correlations between neighbouring protons in this compound. Cross peaks were observed between H-9 and H-10, and between H-11 and H-12/13. There were also cross peak between H-14 and H-15, H-5' and H-6', H-5' and H-3'. Based on its spectral data and comparison with reported data, compound **(2)** was elucidated as 4′,5-dihydroxy-6,7-(2,2-dimethylpyrano)-2′-methoxy-8-(3,3-dimethylallyl)flavone.

Antioxidant activity (DPPH assay)

DPPH assay was carried out on all the crude extracts of *A. komendo* and pure compounds isolated from *A. scortechinii*. The colour changes from purple to yellow indicates the antioxidant activity of the samples. The SC_{50} of the crude extracts which are labelled as AKLD, AKLM, AKLH, ASHM and two pure compounds (**1**) and (**2**) are shown in **Table 2.** They were determined by plotting the DPPH scavenging percentage of each sample against the concentration **Figure 7**. Based on the results, AKLD crude showed the highest scavenging activity with SC_{50} value of 60.03 ppm followed by AKLM crude and AKLH crude with SC_{50} values of 65.97 and 160.25 ppm respectively.

Table 2: Antioxidant Results of the Crude Extracts and Pure Compounds

Samples	**Scavenging Capacity (%)**						**SC_{50} (ppm)**
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	1000 ppm	
AKLD	85.04	93.91	107.91	107.91	124.36	102.67	60.03
AKLM	95.19	93.27	95.62	97.54	97.22	106.52	65.97
AKLH	40.06	53.95	81.84	92.84	103.52	99.68	160.25
ASHM	61.47	65.02	69.02	72.67	78.07	80.03	334.3
(49)	ND	ND	ND	ND	ND	ND	ND
(50)	10.72	12.01	12.01	14.26	14.75	14.95	ND

ND – not determined

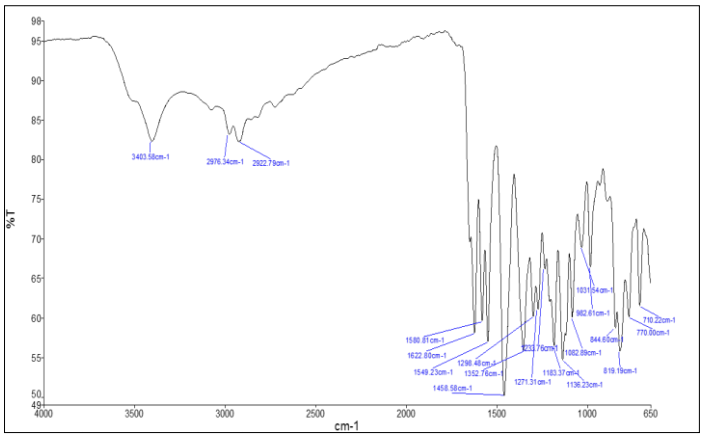

Figure 1: Infrared spectrum of cudraflavone A **(1)**

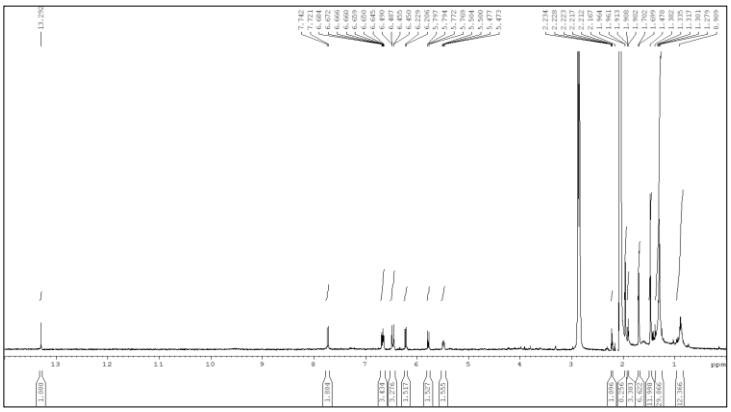

Figure 2: ^{1}H NMR spectrum of cudraflavone A **(1)**

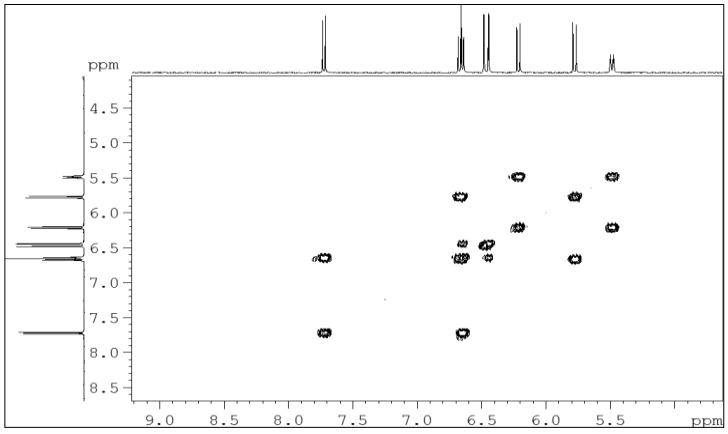

Figure 3: ^{1}H-^{1}H COSY of cudraflavone A **(1)**

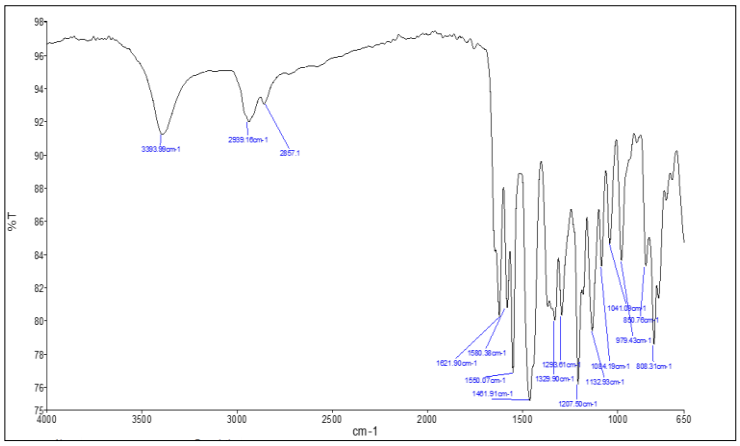

Figure 4: Infrared spectrum of 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(3,3-dimethylallyl)flavone **(2)**

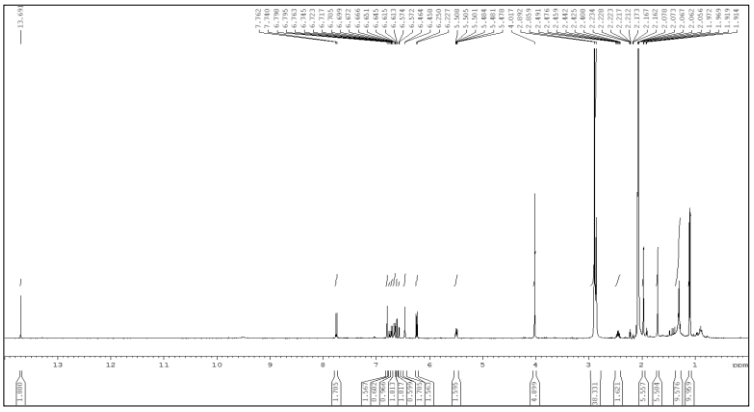

Figure 5: ^{1}H NMR spectrum of 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(3,3-dimethylallyl)flavone **(2)**

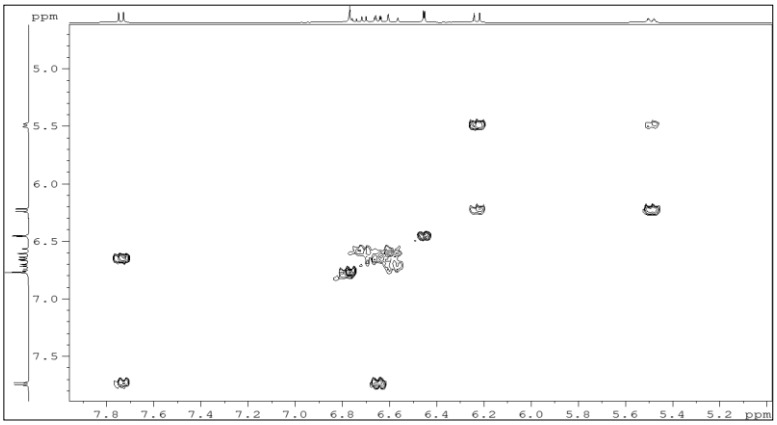

Figure 6: ^{1}H-^{1}H COSY of 4'5,-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-(3,3-dimethyl)flavone **(2)**

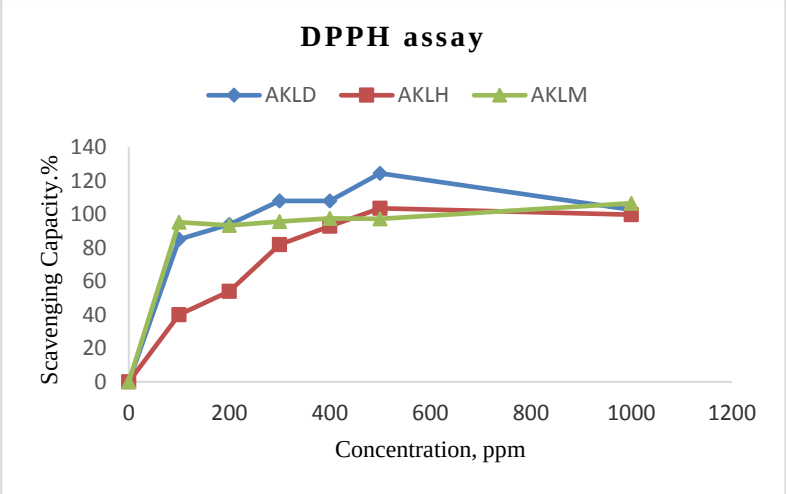

Figure 4.2: DPPH scavenging percentage of the Crude Extracts against the concentration

4. CONCLUSION

Cold extraction of the heartwood of *A. scortechinii* had successfully afforded dark gummy methanol crude extract (39.57 g, 1.58%). Two pure compound were successfully isolated from this extract which were identified as cudraflavone A **(1)** and 4′,5-dihydroxy-6,7-(2,2-dimethylpyrano)-2′-methoxy-8-(3,3-dimethylallyl)-flavone **(2).** The structure of pure compounds were elucidated by using ^{1}H Nuclear Magnetic Resonance (^{1}H NMR), ^{1}H-^{1}H COSY and Infrared (IR) Spectroscopies.

AKLD crude showed the highest scavenging activity with SC_{50} value of 60.03 ppm followed by AKLM crude and AKLH crude with SC_{50} values of 65.97 and 160.25 ppm respectively. Compounds **(1)** and **(2)** were observed as inactive antioxidant agents.

REFERENCES

[1] Corner, E. J. H (1988). *Wayside Trees of Malaya Vol 2*. (3rd ed.) Kuala Lumpur: The Malayan Nature Society Ng, F. S. P. and Phil, D. (1978). *Tree Flora of Malaya Vol 3*. Singapore: Longman Malaysia Sdn. Bhd. 119-134

[2] Jagtap, U. B., and Bapat, V. A. (2010). Artocarpus: A Review of its Traditional Uses, Phytochemistry and Pharmacology. *Journal of ethnopharmacology*,*129*(2), 142-166

[3] Zerega, N. J., Supardi, M. N., & Motley, T. J. (2010). Phylogeny and Recircumscription of Artocarpeae (Moraceae) with a Focus on Artocarpus.*Systematic Botany*, *35*(4), 766-782

[4] Likhitwitayawuid, K., Chaiwiriya, S., Sritularak, B., and Lipipun, V. (2006). Anthiherpetic Flavones from the Heartwood of Artocarpus gomezius Chemistry and Biodiversity 3:1138-1143.

[5] Lathiff, S. M., Jemaon, N., Abdullah, S. A., and Jamil, S. (2014). Flavonoid from *Artocarpus anisophyllus* and their Bioactivities. Natural Product Communicatios. 10(3): 393-396