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ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM THE LEAVES AND STEM BARKS OF *Artocarpus scortechinii* KING

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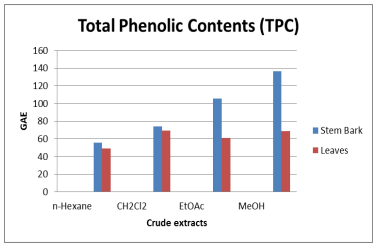
Graphical abstract



Abstract

The antioxidant activities of extracts (*n*-hexane, dichloromethane, ethyl acetate and methanol) from the leaves and stem barks of *Artocarpus scortechinii* were evaluated using various biochemical assays. The quantification of the Total Antioxidant Capacity was measured using ferric reducing antioxidant potential (FRAP) and 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) assays. While, the qualitative of The Total Phenolic Content (TPC) was determined *via* standard gallic acid calibration graph which was expressed as mg gallic acid equivalent (GAE)/g of dry weight (dw) using Folin Ciocalteau's reagent. Among all the extracts tested, the methanolic extract of the stem barks showed the highest phenolic content with TPC value of 136.84 mg GAE/g dry weight (dw). FRAP results were expressed as mM equivalent to $FeSO_4.7H_2O$ by calculating from the standard $FeSO_4.7H_2O$ calibration graph. The ethyl acetate extract of the stem barks showed the most significant reducing potential in the range between 0.27-2.47 mM FRAP. $ABTS^{+\bullet}$ radical scavenging capacity showed that the ethyl acetate extract of the stem barks had the highest scavenging capacity at concentration 1.0 mM with percentage of 90.9%.

Keywords: *Artocarpus, Artocarpus scortechinii,* antioxidant activities

Abstrak

Aktiviti antioksidan ke atas ekstrak (*n*-heksana, diklorometana, etil asetat dan metanol) daripada daun dan kulit batang *Artocarpus scortechinii* telah dianalisa melalui pelbagai ujian biokimia. Kuantifikasi jumlah kapasiti antioksidan telah diukur menggunakan ujian potensi penurunan ferik antioksidan (FRAP) dan 2,2'-azino-bis(3-etil-benzothiazolin-6-asid sulfonik) (ABTS). Manakala, kualitatif jumlah kandungan fenolik telah ditentukan melalui graf kalibrasi asid galik piawai yang dinyatakan sebagai mg asid galik kesetaraan (GAE)/g berat kering (dw) menggunakan reagen Folin Ciocalteau. Di antara semua ekstrak yang diuji, ekstrak metanol kulit batang menunjukkan kandungan fenolik tertinggi dengan nilai TPC 136.84 mg GAE/g berat kering (dw). Keputusan FRAP dinyatakan sebagai kesetaraan mM terhadap persamaan $FeSO_4.7H_2O$ dengan mengira dari graf kalibrasi $FeSO_4.7H_2O$ piawai. Ekstrak etil asetat kulit batang menunjukkan potensi penurunan paling signifikan pada julat 0.27-2.47 mM FRAP. Keupayaan memerangkap radikal $ABTS^{+\bullet}$ menunjukkan ekstrak etil asetat kulit batang adalah yang tertinggi pada kepekatan 1.0 mM dengan peratusan 90.9%.

Kata kunci: *Artocarpus*, *Artocarpus scortechinii,* aktiviti antioksidan

1.0 INTRODUCTION

Artocarpus plant belongs to Moraceae family. It consists of about 50 species distributed over tropical forest of the world [1-2]. *Artocarpus* are known for their edible fruits such as *Artocarpus heterophyllus* (jackfruit), *Artocarpus altilis* (breadfruit) and *Artocarpus integer* (cempedak). More interestingly, they had been reported widely as traditional folk medicine for the treatment of malaria fever, liver cirrhosis, hypertension and diabetes [3-7]. Almost whole parts of these species had its own benefit on human for medicinal purposes. The root can be used to cure asthma and fever, the seed can be used to relieve diarrhea, the wood acts as sedative, the leaves acts as antisyphilitic in human while the leaves ash can be used to relieve ulcer and wounds [8].

Artocarpus had been reported containing a lot of isoprenylated phenolic compounds, including flavone, isoflavone, chalcone, xanthone and stilbene type of compounds [9-11]. Many bioassays studies had been conducted on *Artocarpus* species including antioxidant, antiproliferative, anticancer, antimicrobial and tyrosinase inhibitory activities [12-17].

A. scortechinii King is a rare species locally known as *terap hitam*. It can be found scattered throughout Malaysia in lowland forest and Sumatra, Indonesia. This species had been identified to have very close similarity with *A. elasticus* (terap nasi) [4-5].

Artocarpus species have been proven rich with flavonoids based on the previous. However, no scientific report had been published on the antioxidant property of *A. scortechinii*. Due to limited bioactivity study on this species, several antioxidant assays were carried out to evaluate the antioxidant properties of crude extracts from the leaves and stem barks of *A. scortechinii*.

2.0 EXPERIMENTAL

2.1 Plant Material

The leaves and stem barks of *A. scortechinii* were collected from forest at Bukit Fraser located in Pahang, Malaysia in December 2013 with voucher specimen SK2327/14. The plant samples were authenticated by Dr Shamsul Khamis, botanist from Forest Department, Universiti Putra Malaysia (UPM) and all samples were deposited at the Herbarium of University Putra Malaysia, Serdang, Selangor.

2.2 Extraction of the Leaves and Stem Barks of *A. scortechinii*

The powdered leaves of *A. scortechinii* (4 kg) were sequentially extracted using *n*-hexane, dichloromethane, ethyl acetate and MeOH at room temperature for three days each. The solvents were removed by filtration. The extraction was repeated twice by adding new solvent. All filtrates were concentrated using rotary evaporator to yield the dark green of *n*-hexane (36.67 g, 0.92%), CH_2Cl_2 (43.75 g, 1.09%), EtOAc (45.27 g, 1.13%) and MeOH (127.14 g, 3.18%) crude extracts.

The dried stem barks of *A. scortechinnii* (2.0 kg) were also extracted using *n*-hexane, CH_2Cl_2, EtOAc and MeOH at room temperature for three days each. The solvent was removed using rotary evaporator to give dark gummy of *n*-hexane (4.88 g, 0.20%), CH_2Cl_2 (19.38 g, 0.78%), EtOAc (5.52 g, 0.22%) and MeOH (39.57 g, 1.58%) crude extracts.

All crude extracts from the leaves and stem barks were subjected to antioxidant test using ferric reducing antioxidant potential (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays.

2.3 Total Phenol Content (TPC)

Determination of the total phenolic content on the crude extracts was carried out using method by Kassim *et al*. [18] with minor modification. Crude extracts (40 µL) were mixed with Folin-Ciocalteau's reagent (20 µL) and were let to stand at room temperature for 5 minutes. Sodium carbonate (80 µL) and distilled water (60 µL) were then added to previous mixture in 96-well microtiter plate. The 96-well plate was incubated for 90 minutes in the dark before the absorbance was recorded at 760 nm. Gallic acid with various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mM) were used to construct the calibration graph. The TPC value was expressed as mg gallic acid equivalent (GAE)/g of dry weight of the extracts.

2.4 Antioxidant Assay

The total antioxidant capacity was measured using ferric reducing antioxidant potential (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods. Both methods were measured using microplate reader EPOCH (BioTek).

2.5 Ferric Reducing Antioxidant Potential (FRAP) Assay

Experiment was carried out according to Channarong *et al*. [19] with minor modification. FRAP reagent was freshly prepared, consist of stock solution with ratio 10:1:1 of acetate buffer (300 mM), TPTZ (10 mM) in HCl (40 mM) and $FeCl_3.6H_2O$ (20 mM) solutions. Sample (5 µL), methanol (15 µL) and FRAP reagent (150 µL) were added to the 96-well microtiter plate. The absorbance was recorded after 10 minutes of incubation at 37°C at 573 nm. $FeSO_4.7H_2O$ solution (0.1 mM - 1.0 mM) was used to build up calibration curve of standard antioxidant.

2.6 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Assay

The ABTS assay was conducted as described by Zou *et al*. [20] with minor modification. ABTS and potassium persulfate were dissolved with distilled water to obtain concentration 7 mM and 4.9 mM respectively. Equal amount of these two solutions were mixed and let stand for 12 to 16 hours at room temperature before use. The ABTS radical was added with distilled water to absorbance of 0.7 at 734 nm. Sample (10 µL) was added to 96-well plates together with ABTS solutions (190 µL). The absorbance was recorded after 30 minutes incubation in dark at room temperature. The percentage of antioxidant activity was calculated using the following formula:

$$\text{SC conc.} = \frac{\text{Abs (ABTS)} - \text{Abs (ABTS+Sample)}}{\text{Abs (ABTS)}} \times 100$$

2.7 Statistical Analysis

Data of percentage inhibition was analyse using SPSS 16.0 software. The Independent t-test was used to analyse the data and data was considered to be significant if the probability $p<0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Total Phenolic Content (TPC)

The phenolic contents of eight crude extracts were measured using the Folin Ciocalteau's reagent. The absorbance was recorded by microplate reader EPOCH (Bio Tek) ELISA at 760 nm. TPC results were expressed as mg gallic acid equivalents/g of dry weight (mg GAE/g dw). The results obtained are shown in the Table 1.

Table 1 Total Phenolic Contents of *A. scortechinii* Crude Extracts

Crude extract	GAE	±SD
Stem Barks		
AScSH	55.56	2.66
AScSD	73.93	9.16
AScSE	105.56	10.57
AScSM	136.84	1.19
Leaves		
AScLH	48.93	0.34
AScLD	69.16	1.66
AScLE	60.83	2.05
AScLM	69.05	1.00

ASc= *A. scortechinii*, S = Stem barks, L = Leaves, = *n*-hexane, D= Dichloromethane, E = Ethyl acetate, M = Methanol

Among all crude extracts tested, MeOH extract of the stem barks (AScSM) showed the highest phenolic content with TPC value of 136.84 mg GAE/g dw and followed by EtOAc and CH_2Cl_2 extracts of the stem barks with TPC value of 105.56 and 73.93 mg GAE/g dw respectively. The lowest phenolic content is *n*-hexane extract of the leaves (AScLH) with TPC value of 48.93 mg GAE/g dw. Figure 1 shows the total phenolic contents in *A. scortechinii* crude extracts. Crude extracts of the stem barks contained higher value of phenolic content compared to the crude extracts of the leaves.

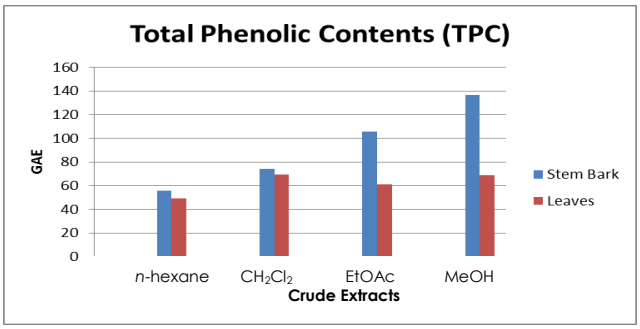


Figure 1 Total Phenolic Contents (mg GAE/g dw) in the Crude Extracts of the Leaves and Stem Barks of *A. scortechinii*

The result showed that the phenolic contents depend on the polarity of the extract. The most polar extract gave the highest phenolic contents. The phenolic content followed the order: methanol > ethyl acetate > dichloromethane > *n*-hexane extracts [21].

3.2 Ferric Reducing Antioxidant Potential (FRAP) Assay

FRAP assay is one of the reducing antioxidant methods. FRAP values were expressed as mM FRAP equivalent to $FeSO_4.7H_2O$. The ability to produce Fe(II) from Fe(III) is defined as "antioxidant power" [6]. The result of FRAP assay of eight crudes extracts were presented in Table 2.

The ethyl acetate crude extract of the stem barks (AScSE) showed the most significant reducing potential in the range between 0.27-2.47 mM FRAP equivalent compared to other extracts. The higher the FRAP values, the higher the antioxidant capacities. It shows that ethyl acetate extract of stem barks (AScSE) have highest reducing ferric ions which act as reducing agent [22].

The FRAP values are also depend on to the presence of the groups such as flavonoids, rosmarinic acids, coumarins or monoterpenes in the plant extract [23].

Table 2 Reducing ability of the Crude Extracts from *A. scortechinii*

Samples	mM FRAP Equivalent to $FeSO_4.7H_2O$				
	0.2 mM	0.4 mM	0.6 mM	0.8 mM	1.0 mM
Stem Barks					
AScSH				***	***
	0	0	0	0.08 ± 0.04	0.07 ± 0.04
AScSD	***	***	***	***	***
	0.11 ± 0.04	0.15 ± 0.06	0.17 ± 0.06	0.34 ± 0.13	0.52 ± 0.05
AScSE	*	*	**	**	**
	0.27 ± 0.10	0.48 ± 0.24	1.19 ± 0.29	1.73 ± 0.28	2.47 ± 0.95
AScSM	*	***	*	**	***
	0.44 ± 0.07	0.63 ± 0.14	0.98 ± 0.10	1.08 ± 0.09	1.11 ± 0.06
Leaves					
AScLH	***	***	***	***	***
	0.06 ± 0.02	0.14 ± 0.03	0.22 ± 0.05	0.32 ± 0.06	0.36 ± 0.07
AScLD	***	***	***	***	***
	0.09 ± 1.13	0.25 ± 0.04	0.31 ± 0.06	0.49 ± 0.05	0.51 ± 0.04
AScLE	***	***	***	***	***
	0.10 ± 0.05	0.20 ± 0.04	0.30 ± 0.03	0.57 ± 0.11	0.77 ± 0.02
AScLM	***	***	***	***	***
	0.13 ± 0.03	0.35 ± 0.04	0.41 ± 0.03	0.79 ± 0.10	0.86 ± 0.08
Standards					
Trolox	0.43 ± 0.08	0.87 ± 0.11	1.23 ± 0.20	1.69 ± 0.38	2.43 ± 0.40
BHT	0.33 ± 0.09	0.64 ± 0.11	1.24 ± 0.07	1.62 ± 0.13	1.89 ± 0.02
AA	0.07 ± 0.08	0.24 ± 0.13	0.33 ± 0.08	0.59 ± 0.11	0.60 ± 0.06

ASc= *A. scortechinii*, S = Stem barks, L = Leaves, H = *n*-hexane, D= Dichloromethane, E = Ethyl acetate, M = Methanol. Value are mean ± STDEV for 3 replicates experiment; * $p < 0.05$, ** $p < 0.01$, *** $p<0.001$ compared with control

3.3 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Assay

Another method to test antioxidant is ABTS assay. ABTS acts as radical scavengers to their hydrogen and electron donating capacity. The strongest antioxidant activities in ABTS method achieved by compounds with many hydroxyl groups [20]. Table 3 summarizes the data obtained for ABTS assay on crude extracts of *A. scortechinii*.

Table 3 ABTS radical scavenging assay on crude extracts of *A. scortechinii*

Samples	Scavenging Percentage (%)				
	0.2 mM	0.4 mM	0.6 mM	0.8 mM	1.0 mM
Stem Barks					
AScSH	***	***	***	***	***
	14.92 ± 1.27	15.47 ± 0.54	22.72 ± 0.42	28.88 ± 1.09	26.28 ± 1.46
AScSD	*	*	***	***	***
	39.97 ± 8.53	50.65 ± 2.86	58.18 ± 0.39	60.78 ± 1.51	63.52 ± 1.12
AScSE	**	***	*	*	*
	68.65 ± 7.07	87.13 ± 0.92	90.62 ± 0.48	90.69 ± 0.27	90.89 ± 0.19
AScSM	***	***	*	**	*
	85.42 ± 1.27	90.14 ± 0.17	90.42 ± 0.19	90.49 ± 0.10	90.62 ± 0.10
Leaves					
AScLH	*	**	**	**	***
	26.01 ± 3.92	43.29 ± 2.09	49.13 ± 4.28	53.54 ± 3.98	61.58 ± 1.22
AScLD	*	*	***	***	**
	55.03 ± 1.13	57.88 ± 3.99	68.77 ± 1.01	69.69 ± 0.76	80.86 ± 3.07
AScLE	*	*	***	**	***
	34.26 ± 4.56	56.74 ± 2.02	67.56 ± 1.43	71.33 ± 2.38	77.09 ± 3.29
AScLM	***	*	***	***	***
	13.02 ± 1.69	33.97 ± 7.93	51.69 ± 1.16	55.25 ± 0.40	56.03 ± 1.39
Standards					
Trolox	25.20 ± 6.20	51.54 ± 10.0	89.98 ± 1.89	91.12 ± 0.27	91.55 ± 0.10
BHT	52.30 ± 2.26	76.88 ± 2.81	80.98 ± 3.67	90.09 ± 0.41	90.16 ± 0.15
AA	78.09 ± 3.09	90.92 ± 0.33	91.15 ± 0.25	91.15 ± 0.10	91.42 ± 0.18

ASc= *A. scortechinii*, S = Stem barks, L = Leaves, H = *n*-hexane, D= Dichloromethane, E = Ethyl acetate, M = Methanol. Value are mean ± STDEV for 3 replicates experiment; * $p < 0.05$, ** $p < 0.01$, *** $p<0.001$ compared with control

Comparison of the antioxidant activity between the crude extracts showed that the methanol extract of the stem barks inhibited higher with percentage more than 80% in all concentration. However, ethyl acetate extract of the stem barks (AScSE) gave the most significant value with scavenging percentage of 90.9% which is slightly higher than the methanol extract of the stem barks (90.6%) at concentration 1.0 mM.

It showed that, the ethyl acetate extract of stem barks (AScSE) has strong antioxidant activity compared to other extracts.

4.0 CONCLUSIONS

All crude extracts of the leaves and stem barks of *A. scortechinii* were evaluated for their total phenolic content (TPC) and antioxidant activities (FRAP and ABTS assays). Methanol extract from the stem barks (AScSM) showed the higher TPC value of 136.84 mg GAE/g dry weight (dw). The ethyl acetate extract of the stem barks (AScSE) showed the most significant reducing potential and highest inhibition percentages in both FRAP and ABTS assays. It can be concluded that the ethyl acetate extract from the stem barks (AScSE) has potential to be a source of natural antioxidant.

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