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Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*)

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ABSTRACT

The total phenolic content and antioxidant activities of methanol, hexane and ethyl acetate extracts of the shoots of *Anacardium occidentale* were measured. Total phenolic content was assessed by the Folin–Ciocalteau assay whereas antioxidant activities were assessed by measuring the ability of the extracts to scavenge the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals, superoxide anion radicals and nitric oxide radicals as well as their ability to reduce ferric ions. Results indicated that the methanol extract of *A. occidentale* was the most potent reducing agent and radical-scavengers compared to the other two extracts. The ethyl acetate extract exhibited some antioxidant activities whereas the hexane extract is the least reactive. The order of the antioxidant potency of the plant extract is methanol > ethyl acetate > hexane. The methanol extract contained more than 7 fold of total phenolic content compared to the hexane and ethyl acetate extracts indicating the likely possibility that the observed antioxidant activities were partly contributed by the phenolics. The results suggest that the shoots of *A. occidentale* are a source of natural antioxidants.

1. Introduction

Plants have been used for years as a source of traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources for phytochemicals, many of which contain potent antioxidant activities. The Malaysian population particularly the Malays is well-known for consuming traditional vegetables and herbs, raw or cooked as accompaniments with their main meal. These vegetables are consumed mainly for their aroma and taste as well as an appetite inducer. Many of these vegetables are claimed to possess medicinal properties although there are no scientific evidence to support this claim. One of the commonly consumed vegetables is the shoot of *Anacardium occidentale*. *A. occidentale* has been used to treat various ailments including malaria and yellow fever (Akinpelu, 2001) as well as diarrhea (Goncalves et al., 2005).

A. occidentale or cashew plant is a member of the family Anacardiaceae and is a tropical tree indigenous to Brazil. However, it is now widely grown in other tropical places particularly in India. The biological activities of this plant is widely reported and it has been shown to possess anti-viral (Goncalves et al., 2005), anti-fungal (Schmourlo, Mendonça-Filho, Alviano, & Costa, 2005), anti-bacterial (Akinpelu, 2001) and anti-inflammatory activities (Mota, Thomas, & Barbosa Filho, 1985). Another study reported the ability of extracts of *A. occidentale* to provide protection against streptozotocin-induced diabetes in rats (Kamtchouing et al., 1998). The shoot of this plant extract was also able to inhibit copper-induced LDL oxidation (Roach, Salleh, Runnie, Mohamed, & Abeywardena, 2003). More recently, the leaves of *A. occidentale* was reported to provide vasorelaxation effect when studied using isolated rat aorta (Runnie, Salleh, Mohamed, Head, & Abeywardena, 2004).

The antioxidant activities and phenolic content of this plant have been reported but mainly in the nuts and stem barks (Kornsteiner, Wagner, & Elmadfa, 2006; Kubo, Masuoka, Ha, & Tsujimoto, 2006; Trevisan et al., 2006) and not much information is available on the shoots which are also commonly consumed. Two recent studies have reported the antioxidant activities of the leaves of this plant (Abas, Lajis, Israf, Khozirah, & Umi Kalsom, 2006; Runnie et al., 2004). In view of the limited data on the antioxidant activities and phenolic content of the shoots of *A. occidentale*, it was the aim of this study to attempt to provide further information on the phenolic content and antioxidant activities of this plant using several antioxidant assays. The assays consisted of estimating the ferric reducing capacity of the plant extracts and determining the scavenging effects of the plant extracts on the free radical $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, nitric oxide and superoxide anion. At the same time, the phenolic content of the plant was also estimated. As phenolic compounds are abundant in plants (Naczk & Shahidi, 2006), they may potentially be present in high concentrations in the

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shoots of *A. occidentale* and may act as potent antioxidants. Such information is valuable as plant extracts containing high antioxidant activities can prove beneficial for maintenance of optimal health. Furthermore, this may assist in providing evidence for the claimed medicinal properties of this plant.

2. Materials and methods

2.1. Chemicals

All reagents and chemicals used in the experiments were of analytical grade and obtained mostly from Sigma. Solvents used for extraction of plants were purchased from Fisher Scientific. The polyphenolic standards, gallic acid, quercetin and rutin were obtained from Sigma. Water used was of Millipore quality.

2.2. Preparation and extraction of plant extracts

The shoots of *A. occidentale* were purchased from the local wet market and were processed on the day of purchased. The samples were washed, air dried and this was followed by complete drying in an oven at 40 °C. The dried samples were ground to powder form and stored at −20 °C until further analysis.

The powdered shoots were extracted with methanol, ethyl acetate and hexane, solvents of varying polarity, at room temperature for 24 h with a mass to volume ratio of 1:20 (g/mL). The extracts were evaporated to dryness on the rotary evaporator at 37 °C and the residues were subsequently re-dissolved in 10% DMSO. Extracts were kept at −20 °C until the bioassay analyses.

2.3. Total phenolic analysis

The Folin–Ciocalteau method was used for total phenolic analysis (Singleton & Rossi, 1965). Ten milliliter of 1:10 Folin–Ciocalteau reagent was added to 200 μL of sample or standard. The mixture was mixed and incubated for 5 min before the addition of 7 mL of 0.115 mg/mL Na_2CO_3. The resulting solution was incubated a further 2 h before absorbance readings were taken at 765 nm. Gallic acid was used for the calibration curve and a concentration range of 50–200 mg/L were prepared and analysed as above. Results were expressed as mg gallic acid equivalents (GAE)/g dried plant material. All experiments were done in triplicate.

2.4. Ferric reducing activity (FRAP assay)

The ferric reducing activity of the plant extracts was estimated based on the Ferric reducing ability of plasma (FRAP) assay developed by Benzie and Strain (1996). The solutions for this assay consisted of 300 mmol/L acetate buffer, 10 mmol/L TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mmol/L of HCl and 20 mmol/L $FeCl_3 \cdot 6H_2O$. Reagent for this assay was prepared fresh by mixing 25 mL acetate buffer with 2.5 mL TPTZ solution and 2.5 mL $FeCl_3 \cdot 6H_2O$.

The assay was performed as followed: freshly prepared FRAP reagent was incubated at 37 °C for 5 min after which a blank reading was taken at 593 nm. Thereafter, 30 μL of extract or standard and 90 μL water was added to 900 μL of the FRAP reagent. Absorbance readings were recorded immediately upon addition of the FRAP reagent and again at 4 min after the start of the reaction. The change in absorbance in the 4 min time reaction was calculated and this was followed over a 5 h period. The absorbance was related to the absorbance changes of a Fe (II) standard solution tested in parallel. All results were based on three experiments and results were expressed as mmol ferric reducing activity of the extracts per g of dried weight. Quercetin and rutin were used as positive controls.

2.5. $ABTS^{\cdot+}$ radical scavenging activity

Determination of the $ABTS^{\cdot+}$ (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging effect of extracts of *A. occidentale* was performed according to the method of Re et al. (1998) with some modifications. Initially, the $ABTS^{\cdot+}$ radical was generated by reacting 7 mM $ABTS^{\cdot+}$ solution in water with 2.45 mM potassium persulfate in the dark for 12–16 h. Absorbance of the reactant was later adjusted to 0.700 ± 0.02 at ambient temperature at a wavelength of 734 nm. Radical scavenging reaction was started by addition of appropriately diluted extracts to the $ABTS^{\cdot+}$ solution so that when 10 μL of the extract was added to 1 mL of $ABTS^{\cdot+}$ solution, it would give a 20–80% inhibition of the absorbance. The absorbance of the mixture was recorded at 734 nm 1 min after addition of the sample and again at 20 min after the initial mixing. A Trolox calibration curve was constructed by measuring the reduction in absorbance of the $ABTS^{\cdot+}$ solution in the presence of different concentrations of Trolox (0–2000 μM). The $ABTS^{\cdot+}$ radical scavenging activity of the extracts was measured by comparing the ratios of the gradients of the concentration plot of the extracts with that of Trolox over a linear concentration range. Results were expressed as mmol Trolox equivalent (TE) antioxidant capacity per g dried plant sample. All determinations were performed in triplicate. Quercetin and rutin were used as positive controls.

2.6. $DPPH^{\cdot}$ radical scavenging activity

Radical scavenging activities by antioxidants in the plant extracts were evaluated using $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) radicals (Cos et al., 2002). Two hundred microliter of the plant extract was added to 1200 μL of a 0.04 mg/mL $DPPH^{\cdot}$ solution in methanol. A series of concentrations ranging from 25 to 400 μg dried extracts/mL were tested. The mixtures were shaken vigorously and incubated in the dark for 20 min after which the reduction of $DPPH^{\cdot}$ absorption was measured at 517 nm.

A Trolox calibration curve was constructed by measuring the reduction in absorbance of the DPPH solution in the presence of different concentrations of Trolox (0–400 μM). The DPPH radical scavenging activity of the extracts was measured using the Trolox standard curve and results were expressed as mmol Trolox equivalent (TE) antioxidant capacity per g dried plant sample. All determinations were performed in triplicate. Quercetin and rutin were used as positive controls.

Scavenging activity of the plant extracts was also estimated based on the percentage of the $DPPH^{\cdot}$ reduction by calculating the IC_{50} values (concentration in μg/mL that caused 50% inhibition of $DPPH^{\cdot}$ radicals) using a non-linear regression analysis.

2.7. Superoxide anion radical ($O_2^{\cdot-}$) scavenging activity

The superoxide radical scavenging activity was measured based on the method by Siddhuraju and Becker (2007). The reaction mixture contained 0.1 M phosphate buffer, pH 7.4, 150 μM nitroblue tetrazolium (NBT), 60 μM phenazine methosulphate, 468 μM NADH and different concentrations of the plant extracts, added in that sequence. The mixture was incubated in the dark for 10 min at 25 °C and the absorbance was later read at 560 nm. Quercetin and rutin were used as the positive control and the results were expressed as percentage inhibition of the superoxide radical. The superoxide anion radical scavenging activity of the extracts was also measured using the Trolox standard curve and results were expressed as mmole Trolox equivalent (TE) antioxidant capacity per g dried plant sample. All determinations were performed in triplicate.

2.8. Nitric oxide scavenging activity

The nitric oxide scavenging activity was conducted based on the method by Rai, Wahile, Mukherjee, Saha, and Mukherjee (2006). 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered-saline was mixed with 0.5 mL of different concentrations of the plant extracts and incubated in the dark at room temperature for 150 min. The control was run as above but the sample was replaced with the same amount of water. After the incubation period, 1 mL of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was added to 0.5 mL of the reaction mixture. After 5 min incubation, 1 mL of 0.1% naphthyl ethylene diamine dihydrochloride was added, mixed and incubated for 30 min at 25 °C. The absorbance of the chromophore formed was read at 540 nm. Quercetin and rutin were used as positive control and results were expressed as percentage inhibition of nitric oxide. The nitric oxide scavenging activity of the extracts was also measured using the Trolox standard curve and results were expressed as mmole Trolox equivalent (TE) antioxidant capacity per g dried plant sample. All determinations were performed in triplicates.

3. Results and discussion

3.1. Total phenolic analysis

The Folin–Ciocalteau assay is a fast and simple method to rapidly determine the content of phenolics in samples. Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities (Rice-Evans, Miller, & Paganga, 1996). This information has led to the determination of the total phenolic content of the sample understudy. Furthermore, several studies have reported a significant correlation between antioxidant activity present in some tropical vegetables with their total phenolic content (Kaur & Kapoor, 2002; Maisuthisakul, Suttajit, & Pongsawatmanit, 2007), suggesting that plants containing high phenolics can be a good source of antioxidants. In addition, various studies have shown that phenolic antioxidants such as quercetin have potential application as therapeutic drugs against free radical reactions (Yao et al., 2007).

In this work, solvents of varying polarity were used to extract and separate the medium and high polarity antioxidants from the non-polar antioxidants in the shoots of *A. occidentale*. In this case, hexane extracted the least polar antioxidants whereas methanol extracted the most polar antioxidants. Extraction of *A. occidentale* with methanol showed the highest yield at 120 mg/g dried weight and this was followed by hexane and ethyl acetate at 80 and 40 mg/g dried weight, respectively (Table 1).

The total phenolic content of the three extracts of *A. occidentale* are presented in Table 1. The methanol extract of *A. occidentale* exhibited the highest total phenolic content at 307.33 ± 0.11 mg GAE/g, approximately 8 fold more than the ethyl acetate extract and 11 fold more than the hexane extract. Several parameters can influence phenolic yield during extraction process and this include extraction temperature, solvent type and solvent concentration (Li, Smith, & Hossain, 2006). A preliminary study conducted in our lab demonstrated that the 24 h incubation at room temperature yielded the highest total phenolic content and antioxidant activities. A comparison of the total phenolic content of our samples with that of several tropical plants demonstrated a much higher total phenolic content in *A. occidentale* than the tropical plants (Maisuthisakul et al., 2007; Runnie et al., 2004; Wong, Leong, & Koh, 2006). Furthermore, the methanol extract of our plant had a higher total phenolic content than the methanol extract of oregano (Skerget et al., 2005), a herb commonly consumed in the western countries and known to possess high antioxidant activities (Su et al., 2007). This suggests the potential of *A. occidentale* to be utilized as a source of nutritional phenolics.

A study has reported the presence of several phenolic acids in the leaves of *A. occidentale*, mainly gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, cinnamic acid, *p*-coumaric acid and ferulic acid (Kogel & Zech, 1985). Analysis of the individual phenolics present in the shoots of *A. occidentale* would be beneficial in providing information on the types of phenolics present in the sample.

Table 1
Yield and total phenolic content of methanol, ethyl acetate and hexane extracts of *A. occidentale*

	Methanol extract	Ethyl acetate extract	Hexane extract
Yield (mg/g dried weight)	120	40	80
Total phenolic content (mg GAE/g[a])	307.33 ± 0.11	38.95 ± 0.25	28.55 ± 0.21

[a] Total phenolic content is expressed as mg gallic acid equivalents (GAE) in 1 g of dry weight material ± std dev.

3.2. Antioxidant activity

Various antioxidants are present in plant samples, therefore measuring each one of the antioxidants can be tedious and time-consuming. Hence, several methods have been developed to measure antioxidant activities as a whole and these methods are more useful and can be applied to the *A. occidentale* samples. Furthermore, these methods are reliable and easy to perform and can be used for determining hydrophilic and lipophilic antioxidant capabilities of plant samples (Arnao, Cano, & Acosta, 2001; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006). Protective effects of nutritional antioxidants in health come from their ability to scavenge free radicals by acting as a hydrogen/electron donor or direct reaction with them; chelate transition metal ions (thus preventing the formation of free radicals via the Fenton reactions); inhibit radical-producing enzymes such as cyclooxygenase and lipoxygenase or increase the expression of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. In this study, the ability of antioxidants in the shoots of *A. occidentale* to scavenge free radicals and reduce ferric ions was investigated.

3.3. Ferric reducing activity

The ferric reducing assay gives fast and reliable results in the *A. occidentale* samples. In this assay, samples are used in a redox-linked reaction whereby the antioxidants present in the sample act as the reductants while the reagent, containing excess ferric ions act as the oxidants. Reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples. The reaction rate of the samples and positive control was followed over a 5 h period and demonstrated that all the samples had reached a plateau by the 3rd hour of incubation (Fig. 1). Both methanol extract and quercetin (0.1 mg/mL) showed a rapid reaction in the first 4 min and this was subsequently followed by a much slower increase. Fig. 2 shows linear dose-response for the controls and the extracts. The reducing capability of the methanol extracts increases with increasing concentration of the sample. The hexane and ethyl acetate extracts were non-reactive, hence did not demonstrate any dose-response effect.

The relative antioxidant activity in the 0–4 min time reaction of the samples was compared with a standard curve of $FeSO_47H_2O$. The ferric reducing activity of the methanol, ethyl acetate and hex-

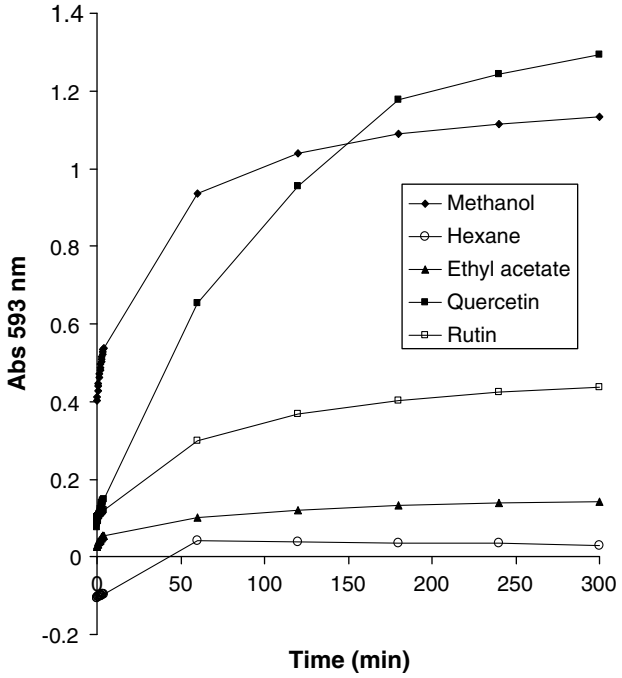

Fig. 1. Ferric reducing activity of methanol, hexane and ethyl acetate extract of *A. occidentale*. The reaction time (min) of the methanol, hexane and ethyl acetate extract of *A. occidentale* in the ferric reducing activity assay was followed initially every 15 s for 4 min and subsequently every hour for 5 h. The graph was plotted against absorbance at 593 nm. The results are average of 3 readings ± std dev.

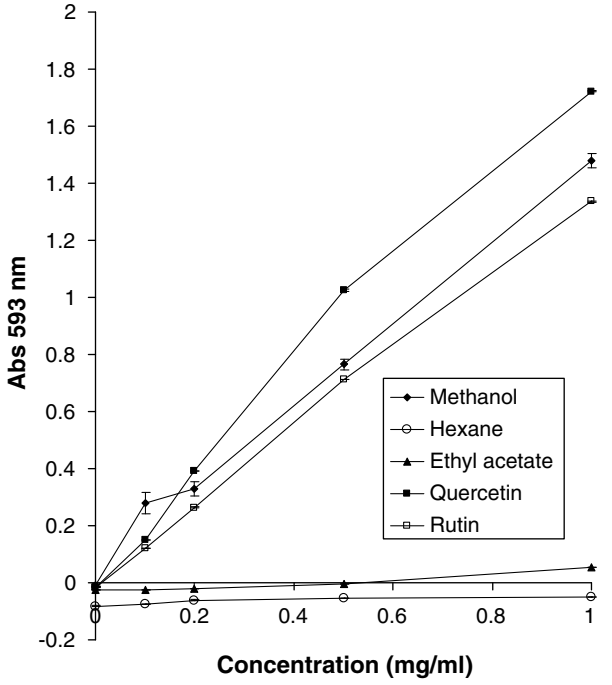

Fig. 2. Dose-response line of the methanol, ethyl acetate and hexane extract of *A. occidentale* in the ferric reducing activity assay. The ferric reducing activity assay was conducted over the concentration range of 0.1–1.0 mg/ml and the reducing capabilities of the methanol, ethyl acetate and hexane extracts of *A. occidentale* were measured at an absorbance of 593 nm. Results are average of 3 readings ± std dev.

ane extracts of *A. occidentale* is shown in Table 2. Only the methanol extract of *A. occidentale* was able to reduce ferric ions efficiently and its activity was comparable to rutin. The hexane extract did not show any reducing capacity whereas the ethyl acetate extract had very low reducing activity. The ferric reducing capacity of the methanol extract of *A. occidentale* was higher when compared with several tropical plants (Runnie et al., 2004), supporting the antioxidative potential of this plant.

The reductive ability of the samples assessed in this study suggests that the extracts were able to donate electron, hence they should be able to donate electrons to free radicals in actual biological or food systems, making the radicals stable and unreactive. Pure gallic acid and ferulic acid which were detected in the leaves of *A. occidentale* (Kogel & Zech, 1985) were able to reduce ferric ions when assessed by FRAP assay (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005), indicating that the results obtained may have partly been contributed by these phenolics. Other than phenolics, a recent study by Trevisan et al. (2006) reported the presence of anacardic acids, derivatives of salicylic acid, in the nuts of *A. occidentale* which exhibited high antioxidant activity when measured using the hypoxanthine/xanthine oxidase system. There is a possibility that anacardic acids may also be present in the shoots of *A. occidentale* which may have contributed to the observed antioxidant activity.

3.4. Radical scavenging abilities of A. occidentale

One of the most important mechanisms of action of antioxidants is by scavenging of reactive oxygen and nitrogen radicals and phenolics have been shown to exert their effect via this mechanism (Firuzi, Lacanna, Petrucci, Marrosu, & Saso, 2005; Soobrattee et al., 2005). In this study, synthetic $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals were utilized to assess radical scavenging abilities of *A. occidentale* extracts. These methods are widely used for the determination of the radical scavenging abilities of plants. Although these radicals may not be biologically relevant, they provide an indication of hydrogen/electron-donating capacity of plants and are hence a useful measure of *in vitro* antioxidant activity.

3.5. $ABTS^{\cdot+}$ radical scavenging activity

The methanol extract of *A. occidentale* exhibited the highest radical scavenging activities when reacted with the $ABTS^{\cdot+}$ radicals (Table 2). In contrast, the hexane and ethyl acetate extracts only showed low activities, approximately 4–7 folds lower than the methanol extract. The methanol extract was more potent than rutin in scavenging $ABTS^{\cdot+}$ radicals but was less active than quercetin.

Fig. 3 demonstrated a steady increase in the percentage inhibition of the $ABTS^{\cdot+}$ radicals by the methanol extract of *A. occidentale* and maximum inhibition was achieved above 1000 μg/mL of the dry extract. The hexane extract is non-reactive, showing a percentage inhibition of less than 10% at the highest concentration studied (2 mg dried extract/mL). In contrast, the ethyl acetate extract of *A. occidentale* did not show a leveling off at the highest concentration, however its radical scavenging effects were much less than the methanol extract.

3.6. $DPPH^{\cdot}$ radical scavenging activity

The radical scavenging activities of the extracts of *A. occidentale* were estimated by comparing the percentage inhibition of formation of $DPPH^{\cdot}$ radicals by the extracts and those of Trolox. Fig. 4 depicts a steady increase in the percentage inhibition of the absorbance of the DPPH radicals by the extracts up to a concentration of 300 μg dried extract/mL, after which there was a leveling off with much slower increase in inhibition. This pattern of DPPH inhibition is commonly observed with plant extracts (Kumaran & Karunakaran, 2007). An exception was observed in the hexane extract

Table 2
Antioxidant activities of *A. occidentale*[a]

Antioxidant activity	Methanol extract	Ethyl acetate extract	Hexane extract	Rutin	Quercetin
Ferric reducing activity (mmol/g dried weight)	3.591 ± 0.004	0.0904 ± 0.0001	ND	3.356 ± 0.002	13.248 ± 0.002
$ABTS^{\cdot+}$(mmol TE/g dried weight)	2.28 ± 0.01	0.581 ± 0.001	0.32 ± 0.03	1.113 ± 0.004	4.80 ± 0.03
$DPPH^{\cdot}$ (mmol TE/g dried weight)	2.52 ± 0.05	0.97 ± 0.02	0.41 ± 0.03	4.13 ± 0.01	4.82 ± 0.01
Superoxide anion (mmol TE/g dried weight)	0.83 ± 0.01	0.701 ± 0.001	0.615 ± 0.005	0.52 ± 0.01	0.871 ± 0.004
Nitric oxide (mmol TE/g dried weight)	0.52 ± 0.02	0.42 ± 0.02	0.18 ± 0.02	0.91 ± 0.02	1.11 ± 0.01

ND: Ferric reducing activity was not detected.
[a] Results expressed are the average of triplicates ± std dev.

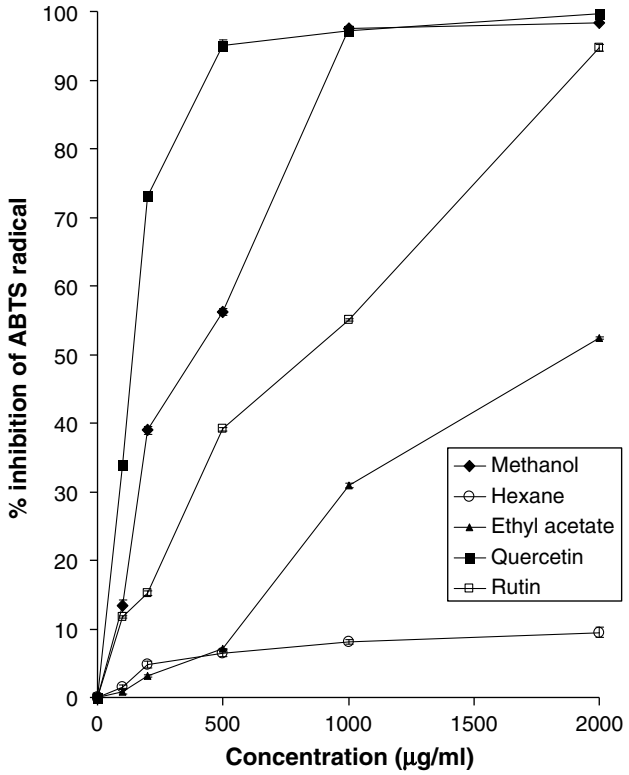

Fig. 3. $ABTS^{\cdot+}$ radical scavenging capacity of methanol, ethyl acetate and hexane extracts of *A. occidentale*. The radical scavenging ability of varying concentrations (0–2000 μg/ml) of methanol, ethyl acetate and hexane extracts of *A. occidentale* was analysed by measuring their inhibitory effects on the absorbance of the $ABTS^{\cdot+}$ radicals. Absorbance of the reaction was measured at 734 nm. The analyses were performed in triplicates and results were expressed as% inhibition of the absorbance of $ABTS^{\cdot}$ radicals ± std dev.

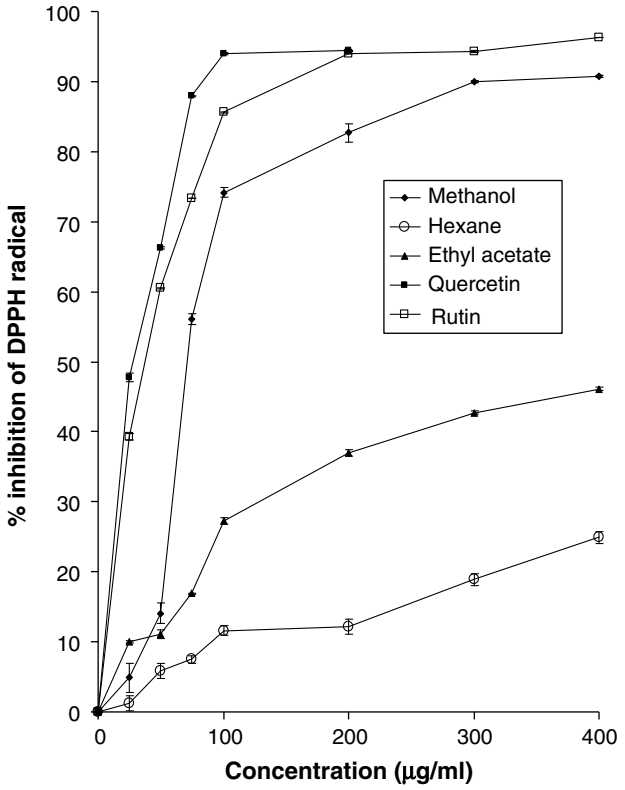

Fig. 4. $DPPH^{\cdot}$ radical scavenging capacity of methanol, ethyl acetate and hexane extracts of *A. occidentale*. The radical scavenging ability of varying concentrations (0–400 μg/ml) of methanol, ethyl acetate and hexane extracts of *A. occidentale* was analysed by measuring their inhibitory effects on the absorbance of the $DPPH^{\cdot}$ radicals. Absorbance of the reaction was measured at 517 nm. The reaction was performed in triplicates and results were expressed as% inhibition of the absorbance of the $DPPH^{\cdot}$ radicals ± std dev.

which did not show a leveling off with increasing concentration, however, the radical scavenging activity of this extract was much lower than the methanol and ethyl acetate extract. This suggests that the antioxidants in the hexane extract are weak radical-scavengers and required extremely high concentration to have a significant effect. Overall, the methanol, ethyl acetate and hexane extracts of *A. occidentale* were able to inhibit the formation of $DPPH^{\cdot}$ radicals with a percentage inhibition of 90.7 ± 0.2, 46.1 ± 0.2 and 24.9 ± 0.8%, respectively at the highest concentration (Fig. 4). The methanol extract of *A. occidentale* has an IC_{50} value of 72 μg dried extract/mL which is inversely related to its antioxidant ability. When the radical scavenging activity of the samples was compared with Trolox, it showed the methanol extract to be almost 3 and 6 times more potent than the ethyl acetate and hexane extracts, respectively (Table 2). The $DPPH^{\cdot}$ radical scavenging capacity of the methanol extract of *A. occidentale* is almost similar to those of rutin and quercetin, suggesting its potency.

When the ratio between the concentration of the extracts of *A. occidentale* and quercetin giving the same percentage of inhibition of the $ABTS^{\cdot+}$ radicals were estimated, it was noted that the ratio was similar as for the DPPH assay. Subsequently, when the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical scavenging capacity of the plant was compared with Trolox, similar values were obtained suggesting that these two assays were comparable (Katalinic, Milos, Kulisic, & Jukic, 2006).

Although both $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ are radicals, their reactions with antioxidants such as phenolics have been shown to differ and reaction of phenolics and $ABTS^{\cdot+}$ are usually more rapid. Furthermore, the reaction kinetics between the two radicals also differ over a similar concentration range (Campos & Lissi, 1996), possibly due to the differences in which the radicals are generated.

A comparison between the $DPPH^{\cdot}$ radical scavenging ability of *A. occidentale* and some common western herbs (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003) indicated that the former was more potent whereby its IC_{50} values were approximately 10 times lower

than the western herbs. This suggests that *A. occidentale* can potentially exert its radical scavenging effects at a much lower concentration than the western herbs. Comparison between the IC_{50} values of our sample (72 μg dried extract/mL) and culinary spices such as basil and parsley, which had IC_{50} values of 0.49 and 12.0 mg/mL of the dried extract, respectively, further demonstrated the potency of *A. occidentale* (Hinneburg, Dorman, & Hiltunen, 2006).

3.7. Superoxide anion (0_2^-) scavenging activity

Superoxide anion is a free radical created from the normal process of energy generation in the human body. Superoxide anion is toxic to cells and tissues and can act as precursors to other reactive oxygen species (Korycka-Dahl & Richardson, 1978).

Fig. 5 shows the superoxide radical scavenging capacity of the extracts of *A. occidentale*, measured by the PMS–NADH superoxide generating system. The extracts demonstrated a dose-response inhibition of the superoxide anion radicals. The methanol and ethyl acetate extracts of *A. occidentale* exhibited superoxide anion radical scavenging activity at all the concentrations. In addition, the methanol extract has a similar superoxide scavenging capacity as quercetin at a concentration of 200 μg dried extract/mL, showing more than 60% inhibition of the superoxide radicals. The hexane extract was much less reactive, only showing a percentage inhibition of less than 40% at the highest concentration.

When the superoxide anion radical scavenging activities of the plant was expressed as Trolox equivalent, the methanol extract exhibited the highest potential, 3 fold more and 6 fold more than the ethyl acetate and hexane extracts, respectively (Table 2).

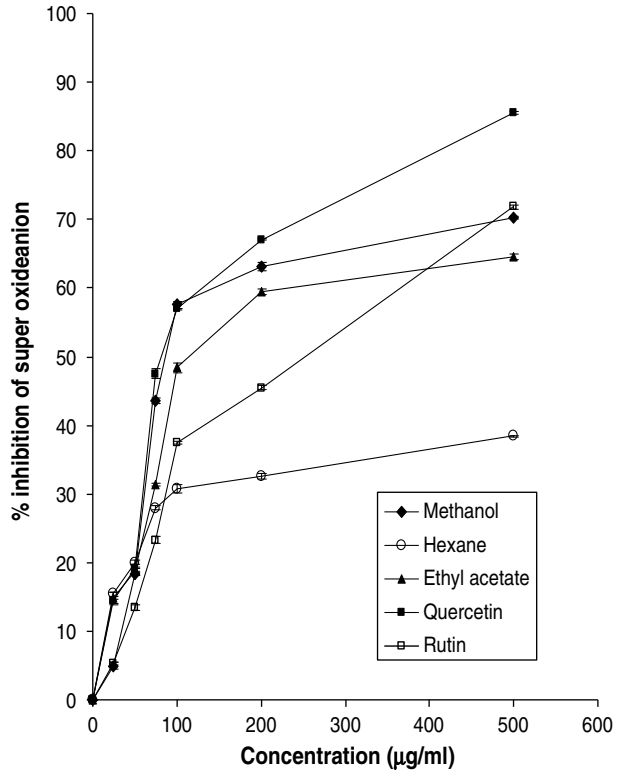

Fig. 5. Superoxide anion radical scavenging capacity of methanol, ethyl acetate and hexane extracts of *A. occidentale*. The radical scavenging ability of varying concentrations (0–500 μg/ml) of methanol, ethyl acetate and hexane extracts of *A. occidentale* was analysed by measuring their inhibitory effects on the absorbance of the superoxide anion reaction product. Absorbance of the reaction was measured at 560 nm. The reaction was performed in triplicates and results were expressed as% inhibition of the absorbance of superoxide anion reaction product ± std dev.

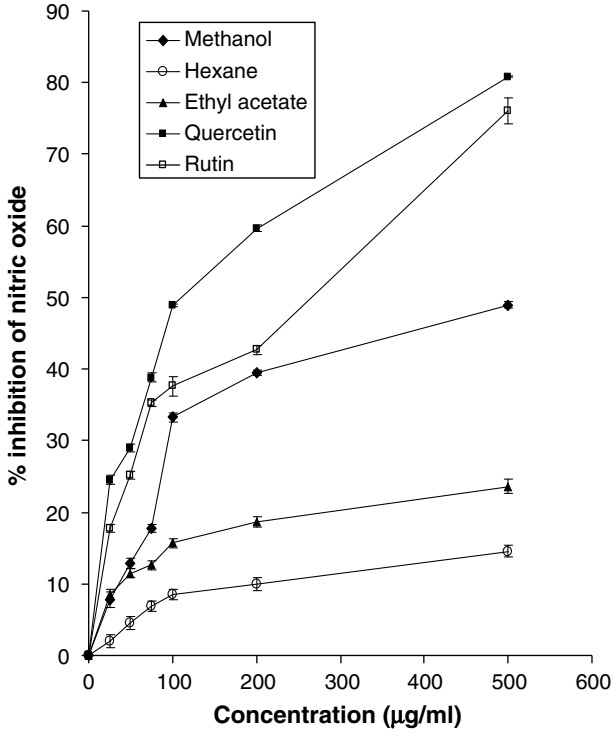

Fig. 6. Percentage inhibition of nitric oxide radicals in the presence of different concentration of the extracts of *A. occidentale*. The radical scavenging ability of varying concentrations (0–500 μg/ml) of methanol, ethyl acetate and hexane extracts of *A. occidentale* was analysed by measuring their inhibitory effects on the absorbance of the nitric oxide reaction product. Absorbance of the reaction was measured at 540 nm. The reaction was performed in triplicates and results were expressed as% inhibition of the absorbance of nitric oxide reaction product ± std dev.

3.8. Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

Overall, the methanol extract of *A. occidentale* showed the highest nitric oxide scavenging ability compared to the ethyl acetate and hexane extracts (Fig. 6). At the highest concentration (500 μg/mL of dry extract), the methanol extract inhibited almost 50% of nitric oxide compared to 24% and 15%, respectively for the ethyl acetate and hexane extracts. However, compared to the nitric oxide scavenging activity of quercetin and rutin, the methanol extract of *A. occidentale* could be considered a moderate nitric oxide scavenger. When the scavenging capacity was expressed as Trolox equivalent, it showed that the methanol extract was more potent than the other two extracts (Table 2).

4. Conclusion

This study showed that among the methanol, hexane and ethyl acetate extracts of *A. occidentale*, the methanol extract possess significant antioxidant activities and their potency is in the order of methanol > ethyl acetate > hexane. Overall, the methanol extract of *A. occidentale* is the most potent in scavenging the DPPH and ABTS radicals, superoxide anion and nitric oxide as well as in reducing ferric ions whereas the hexane extract contained the weakest antioxidants. In addition, the antioxidant activity of the methanol extract was comparable to rutin and quercetin, phenolic compounds which have been reported to contain potent antioxi-

dant activities. The presence of high levels of phenolic compounds in the methanol extract may have partly contributed to the observed antioxidant activities. This study provided evidence on the potential health benefits of *A. occidentale*. In vivo studies are beneficial to further understand the mechanism of action of this plant as an antioxidant.

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