The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPONENTS OF OXALIDACEAE FRUIT EXTRACTS

(Aktiviti Antioksida Dan Komponen Bioaktif Esktrak Buah Oxalidaceae)

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Abstract

The study was conducted to evaluate the Antioxidant Activity (AA), Total Flavonoid Content (TFC), and Total Phenolic Content (TPC) of Oxalidaceae fruit extracts. Two types of *Averrhoa carambola* L. and two types of bilimbi fruits were used in this study. Bilimbi fruits selected were Averrhoa *bilimbi* L. and *Averrhoa bilimbi* cv. while *Averrhoa carambola* L. selected were *Averrhoa carambola* L. (honey type) and another type of *Averrhoa carambola* L. which is known as tart type. The maturity stage for both *Averrhoa carambola* L. fruits (honey and tart type) were selected at commercial maturity stages which were stage 3 and stage 4. Antioxidant estimation of oxalidaceae fruit extracts were evaluated by using Total Flavonoid Content (TFC) and Total Phenolic Content (TPC), while antioxidant activity were evaluated using scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), β-carotene Bleaching (BCB) and Ferric Reducing Antioxidant Power (FRAP) assays. Phytochemical screening of alkaloids, flavonoids, terpenoids, steroids, tannins and saponins were also performed on all samples. *Averrhoa carambola* L. indicated positive results for all phytochemical screening conducted and similar results were observed from bilimbi fruits, except for alkaloids. *Averrhoa carambola* L. (tart type) of stage 4 possessed higher TPC and TFC followed by *Averrhoa carambola* L. (honey type) of stage 4. *Averrhoa carambola* L. (tart type) of stage 4 also exhibited high scavenging effect (74 %) as determined by DPPH assay with the value of EC_{50} 72.36 mg/ml while *Averrhoa carambola* L. (honey type) of stage 3 showed significantly lower percentage of scavenging effect. The results also demonstrated that FRAP values of *Averrhoa carambola* L. (honey type) of stage 4 and *Averrhoa carambola* L. (tart type) of stage 4 were higher than other samples (5.1023 mmol TE/g and 5.0759 mmol TE/g) and similar trends were observed for β-carotene Bleaching (BCB) assay, where *Averrhoa carambola* L. (honey type) of stage 4 had the highest value. There were strong positive correlations between antioxidant activity assays and TPC or TFC, hence indicating that the four types of Oxalidaceae fruits used in this study have the potential as natural antioxidant.

Keywords: antioxidant activity (AA), *Averrhoa carambola* L., *Averrhoa bilimbi* L., Total Flavonoid Content (TFC), Total Phenolic Content (TPC)

Abstrak

Kajian ini dijalankan untuk mengkaji aktiviti antioksida (AA), Jumlah Kandugan Flavonoid (TFC) dan Jumlah Kandungan Fenolik(TPC) pada ekstrak buah Oxalidaceae. Dua jenis *Averrhoa carambola* L. dan dua jenis belimbing buluh telah digunakan dalam kajian ini. Jenis belimbing buluh yang terpilih adalah *Averrhoa bilimbi* L. dan *Averrhoa bilimbi* cv. Manakala, *Averrhoa carambola* L. yang terpilih adalah *Averrhoa carambola* L. (jenis madu) dan *Averrhoa carambola* L. yang dikenali sebagai jenis masam. Tahap kematangan untuk kedua-dua jenis buah *Averrhoa carambola* L. (jenis madu dan masam) adalah mengikut tahap kematangan komersial iaitu tahap 3 dan tahap 4. Anggaran antioksida pada ekstrak buah Oxalidaceae diuji menggunakan TFC dan TPC, manakala aktiviti antioksida pula di tentukan dengan menggunakan esei radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH), pelunturan β-karotena dan ujian kuasa penurunan ferik. Penyaringan fitokimia alkaloid, flavonoid, terpenoid, steroid, tanin dan saponin juga dijalankan ke atas semua sampel. *Averrhoa carambola* L. menunjukkan keputusan positif bagi semua pemeriksaan fitokimia yang dijalankan dan keputusan yang sama diperhatikan daripada buah belimbing buluh kecuali alkaloid. *Averrhoa carambola* L. (jenis masam) dari tahap 4 memiliki jumlah kandungan fenolik dan flovonoid tertinggi diikuti oleh *Averrhoa carambola* L. (jenis madu) dari tahap 4. *Averrhoa carambola* L. (jenis madu) dari tahap 4 turut menunjukkan pencarian radikal tertinggi (74%) melalui ujian DPPH dengan jumlah EC_{50} 72.36 mg/ml sementara *Averrhoa carambola* L. (jenis madu) dari tahap 3 menujukkan jumlah peratus yang jauh lebih rendah. Keputusan turut menunjukkan bahawa jumlah FRAP bagi *Averrhoa carambola* L. (jenis madu) dari tahap 4 dan *Averrhoa carambola* L. (jenis tat) dari tahap 4 adalah lebih tinggi

daripada sampel yang lain (5.1023 mmol TE/g and 5.0759 mmol TE/g) dan tren yang sama turut ditunjukkan oleh ujikaji pelunturan β-karotena , di mana *Averrhoa carambola* L. (jenis madu) dari tahap 4 memiliki jumlah tertinggi. Terdapat korelasi positif yang kuat antara ujian aktiviti antioksida dan TPC atau TFC, dengan itu menunjukkan bahawa keempat-empat jenis buah Oxalidaceae yang digunakan dalam kajian ini mempunyai potensi sebagai antioksida semula jadi.

Kata kunci: antioksida aktiviti (AA), *Averrhoa carambola* L., *Averrhoa bilimbi* L., Jumlah Kandungan Flavonoid, Jumlah Kandungan Fenolik

Introduction

Averrhoa bilimbi L. has been widely used in traditional medicine as a cure for cold, itches, boils, cough, diabetes, whooping cough, hypertension, syphilis and rheumatism [1]. There are two types of bilimbi; *Averrhoa bilimbi* L. that is green in colour while another type is known as *Averrhoa bilimbi* cv. that is yellow in colour as reported by Saidin [2]. According to Carolino et al. [3], *Averrhoa carambola* L. or known as star fruit is thermogenic, febrifuge, antipyretic, tonic, antiscorbutic, sweet and sour. There are two types of *Averrhoa carambola* L., where type one (tart type) is smaller in size, sour taste, richly flavoured and contain more oxalic acid content. The second type is larger in size, mild-flavoured with less oxalic acid and known as honey type. *Averrhoa carambola* L. are used to treat vomiting, headache and restlessness, and also as traditional medicine in Malaysia and China. Oxalidaceae family show high antioxidants activity and antioxidants play an important role especially in preventing the formation of free radicals. There are two types of antioxidants which are natural antioxidant and synthetic antioxidant. Synthetic antioxidant is man made antioxidant through chemical process while natural antioxidant is produce by human body or plant and normally regarded as safe. In fruits and vegetables, the examples of antioxidants are ascorbic acid, hydroxycarboxylic acids, flavonoids and caratenoids [1]. Besides showing antibacterial, anticarcinogenic, antiinflammatory, antioxidants that are isolated from plants also exhibit antiviral, antiallergic, estrogenic and immune-stimulating effects [1]. Shahidi et al. [4] claims that antioxidants have a few characteristics which are capable of reducing, delaying or preventing auto-oxidation where the antioxidants bind together with free radicals to decrease their destructive power, hence, reduce the harmful effect of oxidants.

Materials and Methods

Chemicals

Concentrated hydrochloric acid, 95% methanol, ferric chloride, ammonium chloroform, concentrated sulphuric acid, folin-ciocalteu reagent, sodium carbonate, Mayer‘s reagent, 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), 2, 2-diphenyl-2-picrylhydrazyl radical scavenging (DPPH), ethanol, trolox, β-carotene, linoleic acid, Tween 20, chloroform, acetic acid, ascorbic acid (AA), Butylated hydroxyltoluene (BHT), and Butylated hydroxylanisole (BHA).

Fruits (*Averrhoa bilimbi* and *Averrhoa carambola* L.)

Both types of *Averrhoa carambola* L. were taken from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, while both types of bilimbi were collected from Kampung Alai, Melaka.

Preparation for extraction process

The fruits were washed under running tape water, for about one minute before the fruits were sliced and dried using cabinet oven. About 100g of the fruits were ground and 20g of the ground sample was extracted with boiling water (600 ml) for 10 minutes before being blended and filrated with filter paper, Whatman No.4. The water was removed by using Rotary Evaporator at 70°C and the extracts were stored in amber bottles at 4^{o}C prior to further analysis [5].

Phytochemical screening : Test for flavonoids

The presence of flavonoids was detected by the colour changes from orange to magenta red colouration [6]. Two milligram of crude extract was mixed with 2 ml of distilled water and left for 10 minutes. After that, metallic magnesium and concentrated hydrochloric acid (about five drops) were added.

Test for steroids

According to Ciriaco et al. [7], the colour changed from violet to blue or green in samples indicated the presence of steroids. Two millilitre of sulphuric acid was added to 0.5g of crude extract. Then, 2 ml of acetic anhydride was added to the mixture and the changes were observed.

Test for saponins
Approximately 5 ml of distilled water was added to the 0.5g crude extract in a beaker before filtered into the test tube and the filtrate was shake vigorously for a stable persistent froth. The stable persistent froth was regarded as positive for the presence of saponins [7].

Test for tannins
According to Karumi et al. [8], a green precipitate or blue-black precipitate proved the presence of gallic tannin or catechol tannis. Around 10 mg of extract was added with distilled water until all the extract was immersed in the distilled water. One millilitre of extract was mixed with ten millilitre of distilled water before being filtered. Three drops of ferric chloride were added to the filtrate and the changes were observed for the presence of tannins.

Test for alkaloids
Ten millilitre of ammonium chloroform and five milligram of crude extract were mixed in the test tube, stirred and filtered into capped test tube. Ten drops of concentrated sulphuric acid was added and shake well. Acidic phase at the top layer and organic phase at the bottom layer were formed. Mayer's reagent was used to test the titrated acidic phase and a white precipitate indicates the presence of alkaloids [7].

Total phenolic content (TPC)
Total phenolic content was determined according the method of Singleton and Rossi [9]. Approximately 100 µl were mixed with 0.5 ml of Folin-Ciocalteu reagent and allowed to stand at room temperature for 5 minutes, and 1.5 ml of sodium carbonate solution (7.5%) was added to the mixture. After two hours, absorbance was read at 765 nm. Results were expressed as gallic acid equivalents.

Total flavonoid content (TFC)
The flavonoid content in extracts was determined according to Zhishen et al. [10], using a method based on the formation of a complex flavonoid–aluminium, having the maximum absorbanceat 510 nm. Quercetin was used as a standard. One millilitre of diluted samples/standards was separately mixed with 4ml of distilled water, 0.3ml of $NaNO_2$ (10%) and was incubated at dark place for 5 minutes. Then, 0.3ml of aluminium chloride ($AlCl_3$) and 2ml of sodium hydroxide was added and the solution was made up to 10 ml with distilled water. Reaction mixture was measured at 510 nm with UV–Vis spectrophotometer.

Ferric reducing antioxidant power (FRAP) assay
The ferric reducing antioxidant power assay was determined according to method of Oyaizu et al. [11]. Two hundred millilitre of buffer (3.2 ml of acetic acid mixed with 196.8 ml of distilled water and 0.62g of sodium acetate) (pH 3.6) was added with 20 ml of TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) (0.063g of TPTZ in mixture of 78.8µl of hydrochloric acid) and with 20 ml of $FeCl_3$ in the ratio of 10:1:1. The mixture of buffer, TPTZ and also ferum chloride is called FRAP reagent. 0.3 ml of samples/standards were added with 8.7ml of FRAP reagent and were make up until 9 ml. After that, the mixtures were incubated for 1 hour and the absorbance was measured by using UV-Vis spectrophotometer at 593 nm against the blank. Increasing in reducing power will show increasing in absorbance. Trolox was used as a standard for comparison.

2, 2-diphenyl-2-picrylhydrazyl radical scavenging (DPPH) assay
The scavenging activity was estimated according to the method of Tang et al. [12]. Six hundred microlitre of samples were added to 4.5ml of 0.1 mM DPPH in 95% of ethanol. The mixtures were shaken vigorously using vortex and left to stand for 20 minutes at room temperature in a dark room. Absorbance was read using spectrophotometer at 517 nm. The scavenging effects on the DPPH radical were calculated using the following equation (1):

$$\text{Scavenging effect (\%) (SE)} = \left[1 - \frac{\text{Absorbance of sample at 517nm}}{\text{Absorbance of control at 517nm}}\right] \text{x } 100 \quad (1)$$

Triplicate measurements were carried out and their scavenging effects were calculated based on the percentage of DPPH scavenged.

β-carotene-linoleate bleaching (BCB) assay

β-Carotene-linoleate bleaching assay was conducted according to method of Velioglu et al. [13]. β-carotene solution (2 mg in 10 ml chloroform), 60 µL of linoleic acid and 600µL Tween 20 were transferred into a round bottom flask and the mixture were evaporated at 40ºC for 10 minutes to remove the chloroform. 300 ml of distilled water was added to the mixture, and then shaken vigorously to form emulsion. 5 ml emulsion was then transferred to test tube containing 200µL samples/standards separately and immediately placed in a water bath at 50ºC. The absorbance was read at 20 minutes intervals for 2 hours at 470 nm. Degradation rates (DR) were calculated using the following equation (2)[14]:

$$\left(\ln \frac{a}{b}\right) \times \left(\frac{1}{t}\right) = DR_{\text{sample}} \text{ or } DR_{\text{standard}} \quad (2)$$

where In is natural log, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 20, 40, 60, 80, 100 or 120 minutes and t is the time.

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula equation (3):

$$AA = \left[\frac{DR_{\text{control}} - DR_{\text{sample or standard}}}{DR\text{control}}\right] \times 100 \quad (3)$$

Statistical analysis

All data were expressed as mean ± standard deviation. Data were analysed using one-way ANOVA using SAS 15.0. Duncan's multiple-range test was used to measure the difference between means. Pearson's correlation test was used to assess correlations between means. A significant difference was considered at the level of $p < 0.05$.

Results and Discussion

Phytochemical Screening

From the result, it was observed that *Averrhoa bilimbi* L. and *Averrhoa bilimbi* cv. showed negative result for alkaloids and positive result for other phytochemical while all extracts from *Averrhoa carambola* L. indicated positive results for the presence of phytochemicals. (Table 1). For saponin test, all samples showed positive result. Blue-black precipitate proved the presence of gallic, tannin or catechol, which can be seen at all of the extracts. The presence of flavonoids will be detected by the colour changes to magenta red or orange colouration according to Mojab et al. [6]. Light colour of red magenta from the screening of *Averrhoa bilimbi* L. , *Averrhoa bilimbi* cv., stage 3 *Averrhoa carambola* L. (honey type) and stage 4 *Averrhoa carambola* L. (tart type) indicate the presence of flavonoids. The stage 4 *Averrhoa carambola* L. (honey type) and stage 4 *Averrhoa carambola* L. (tart type) illustrated the high presence of flavonoids. Reddish brown precipitate explained the presence of terpenoids for all extracts.

According to Ciriaco et al. [7], if the filtrate turns red or pink in colour, the presence of steroids will be observed as shown by the positive results of all extracts. All results shown complies to the preliminary phytochemical screening on the bilimbi fruits extracts (bilimbi) reported by Wong et al. [15] who revealed the presence of flavonoids, saponins and terpenoids but no alkaloids. A study conducted by Vasconcelos et al. [16] and Shinu Thomas et al. [17], demonstrated the presence of tannins, alkaloids, flavonoids, saponins, and also steroids in the *Averrhoa carambola* L. Krishnaiah et al. [18] postulated that phytochemical is a natural bioactive compound found in plant that can be divided into two groups which are primary compounds (for examples; amino acid, sugars, protein and chlorophyll) and secondary compounds (for examples; alkaloids, terpenoids, phenolic). These two groups are divided based on the compounds functions in plant metabolism.

Table 1: Phytochemical screening of Oxalidaceae fruit extracts

Samples	**Alkaloids**	**Saponin**	**Tannin**	**Flavonoids**	**Terpenoids**	**Steroids**
Averrhoa bilimbi L.	-	+	+	+	+	+
Averrhoa bilimbi cv.	-	+	+	+	+	+
Stage 3 Averrhoa carambola L. (Honey type)	+	+	+	+	+	+
Stage 4 Averrhoa carambola L. (Honey type)	+	+	+	+	+	+
Stage 3 Averrhoa carambola L. (Tart type)	+	+	+	+	+	+
Stage 4 Averrhoa carambola L. (Tart type)	+	+	+	+	+	+

+ = presence; - = absence

Total Phenolic Content (TPC)

Ikram *et al.,* [19] stated that different phenolic compounds have different responses in the Folin-Ciocalteau method. Reducing agents for example ascorbic acid may interfere with the results, thus, the Folin-Ciocalteau reagent method may overestimate TPC. Depending on their chemical structure, the molecular antioxidant response of phenolic compounds in methyl linoleate varies remarkably. A proper characterisation of individual phenolic compounds has to be done in order to predict the antioxidant content of an extract, not only depends on the basis of its phenolic content. Total phenolic assay is simple, precise and sensitive [20] as it measures the reducing capacity of sample. However, it may not suitable for measuring the antioxidant activity of every sample [21]. Table 2 showed the TPC for *Averrhoa bilimbi* and *Averrhoa carambola* L. Extract of *Averrhoa bilimbi* L. showed lowest phenolic content than other extracts. TPC for previous study on *Averrhoa bilimbi* done by Ikram *et al.,* [19] reported that the TPC of *Averrhoa bilimbi L* for methanol extract was 12.61mg GAE/g of sample. However, finding exhibited higher value of TPC. Narain *et al.,* [22] reported 67.13mg GAE/g of TPC presence in *Averrhoa carambola* L. extract which is slightly lower than the result obtained. Extrinsic factors (such as agronomic, environmental, handling and storage), different plants, procedures and standards used to express TPC will absolutely give different level of TPCs [23].

Table 2: Total Phenolic Content (TPC) of Oxalidaceae Fruit Extracts

Samples	**TPC (mg GAE/g)**
Averrhoa bilimbi L.	41.00±2.75E
Averrhoa bilimbi cv.	53.01±4.36D
Averrhoa carambola L. Stage 3 (honey type)	72.42±2.98C
Averrhoa carambola L. Stage 4. (honey type)	87.65±2.57B.
Averrhoa carambola L. Stage 3 (tart type)	79.38±1.53A
Averrhoa carambola L. Stage 4 (tart type)	89.50±0.76A.

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are characterised by a benzo-pyrone structure which is ubiquitous in fruits and vegetables. According to Chang et al. [24], this method determines flavones and flavonols. The $AlCl_3$ forms acid stable complexes with either keto group (C-4) or hydroxyl group (C-3 or C-5) of flavones and flavonols. In this study, water extract of stage 4 *Averrhoa carambola* L. (honey type) and stage 4 *Averrhoa carambola* L. (tart type) samples showed the highest amount of flavonoid content as compared to other samples with the values of 41.63 mg quercetin equivalent/gram and 48.61 mg quercetin equivalen/gram. These values are slightly higher as compared to TFC of *Averrhoa carambola* L. reported by Narain et al. [22]. Higher antioxidant activity observed may be due to synergistic activity between phenolic compounds found in the samples.

Iker et al. [25] reported that flavonoid accumulates after slight heat treatment in plant in many different species. Actually, flavonoid content appeared to be closely connected with colour. Herb honey with strong red or green colours such as raspberry and black chokeberry have high flavonoid content while those with pale yellow or pale green colours such as camomoli and mint contained a small amount of flavonoid content [26]. As *Averrhoa bilimbi* has pale green colour so it has lower amount of TFC while stage four of *Averrhoa carambola* L. has yellow colour, so it is expected to posses higher amount of TFC. According to Harris [27], maturity and weather condition will affect the physicochemical characteristics of bilimbi.

Table 3: Total Flavonoid Content (TFC) of Oxalidaceae Fruit Extracts

Samples	**TFC (mg QE/g)**
Averrhoa bilimbi L	23.32±3.50DE
Averrhoa bilimbi cv.	19.62±1.44E
Averrhoa carambola L. Stage 3 (honey type)	26.60±0.82D
Averrhoa carambola L. Stage 4 (honey type)	41.63±0.25B
Averrhoa carambola L. Stage 3 (tart type)	34.26±1.73C
Averrhoa carambola L. Stage 4 (tart type)	48.61±0.25A

Antioxidant activity: **Ferric reducing antioxidant power (FRAP)**

For the measurements of the reductive ability, ferric oxide (Fe^{3+}) to ferrous oxide (Fe^{2+}) transformation in the presence of Oxalidaceae fruit extracts samples were investigated using FRAP assay developed by Oyaizu [11]. The reducing power ability of Fe^{3+} was carried out by varying the concentration of extracts. The result of this analysis as shown in Figure 1 showed that reducing activity increases as the amount of extracts increased.

By reducing ferric ion to give more active ions, the common metal ions such as Fe^{3+} can be reduced by the antioxidant that provokes the antioxidant to behave as prooxidant [14]. The reaction results in a change of colour from brownish to dark green corresponding to the reduction of Fe^{3+}complex to Fe^{2+}complex. The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects [28].

The FRAP values obtained from this finding are slightly lower than values reported by Yan et al. [29] and Wong et al. [15], where bilimbi and *Averrhoa carambola* L. extracts had FRAP values of 1.76 ± 0.87 mmol TE/g and 4.86 ±

0.57 mmol TE/g respectively. The difference may be due to the types and the quantity of phytochemical and the food matrix presence in the sample [30]. Environmental factors such as climatic growth condition, ripening stage, temperature, duration of storage and thermal treatment may have influenced the antioxidant activity [31,32].

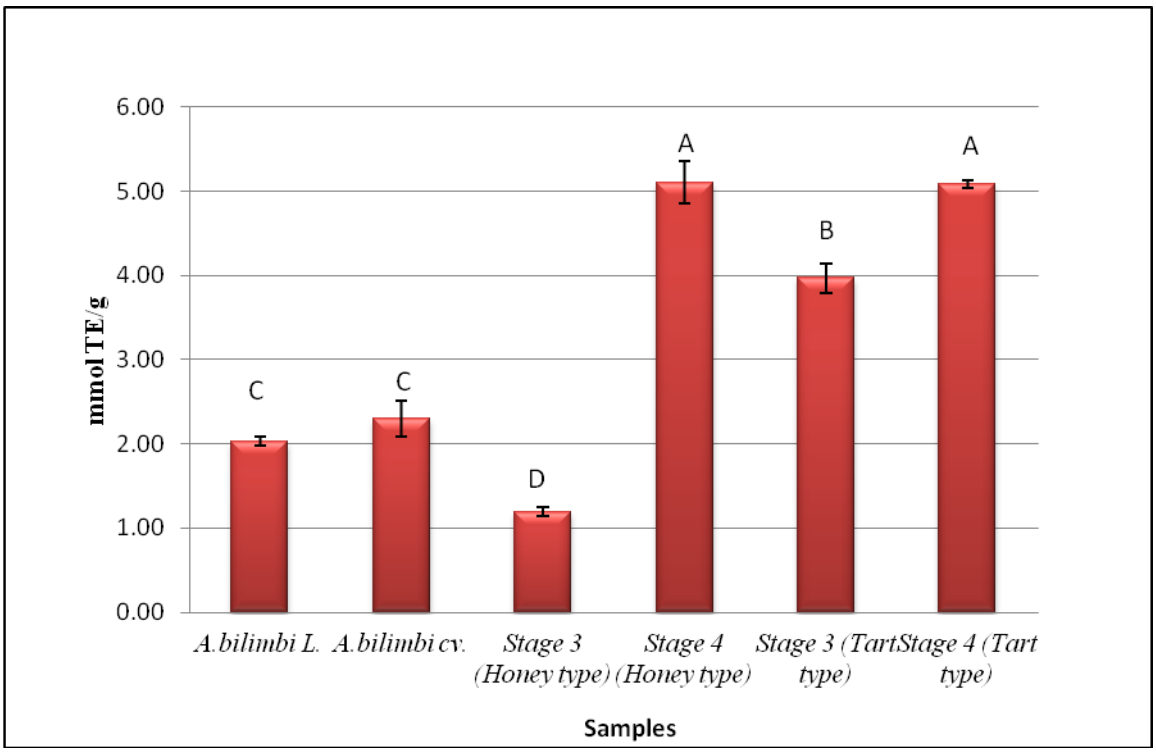

Figure 1: The values of Ferric reducing antioxidant power (FRAP) of Oxalidaceae fruit extracts

2, 2-diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH)

DPPH assay is one of the most widely used methods for screening antioxidant activity of sample extract. The assay is based on the measurements of the antioxidant ability to scavenge the stable radical DPPH. DPPH is a stable nitrogen centred free radical which produces violet colour in methanol solution [33]. DPPH radicals react with suitable reducing agents, during which electrons become paired off and the solution losses colour stoichiometrically depending on the number of electrons taken up [34]. In this assay, the solution progressively reduced to yellow coloured product diphenylpicryl hydrazine with the addition of the extracts in a concentration dependent manner. The absorbance was measured at 517 nm.

The DPPH free radical scavenging activity of *Averrhoa bilimbi* and *Averrhoa carambola* extracts are shown in Table 4. *Averrhoa bilimbi* cv. and stage 3 Averrhoa carambola L. (honey type) showed the lowest DPPH free radical scavenging activity. Water extract of *Averrhoa bilimbi* showed significantly higher in radical scavenging activity than methanol extract according to Ikram et al. [19]. They also stated that DPPH free radical scavenging activity for water extraction of sample was 38.09 ± 1.87% for raw *Averrhoa bilimbi* respectively. Narain et al. [22] reported that 66.37 ± 2.44% value of DPPH assay for *Averrhoa carambola* L. extracts. Higher phenolic content especially in stage 4 *Averrhoa carambola* L. (honey type) could be the main reason for its higher antioxidant activity towards DPPH radicals.

The higher the antioxidant activity of the extracts, the more the DPPH bleaching will occur. Thus, the greater the bleaching action of DPPH, the higher the percentage of scavenging effect [19]. Percentage scavenging of Oxalidaceae fruits extracts in this study were in descending order of ascorbic acid (AA) > BHA/BHT > Stage 4 *Averrhoa carambola* L. (honey type) > Stage 4 *Averrhoa carambola* L. (tart type)> Stage 3 *Averrhoa carambola* L. (tart type) > *Averrhoa bilimbi* L. > *Averrhoa bilimbi* cv. > Stage 3 *Averrhoa carambola* L. (honey type).

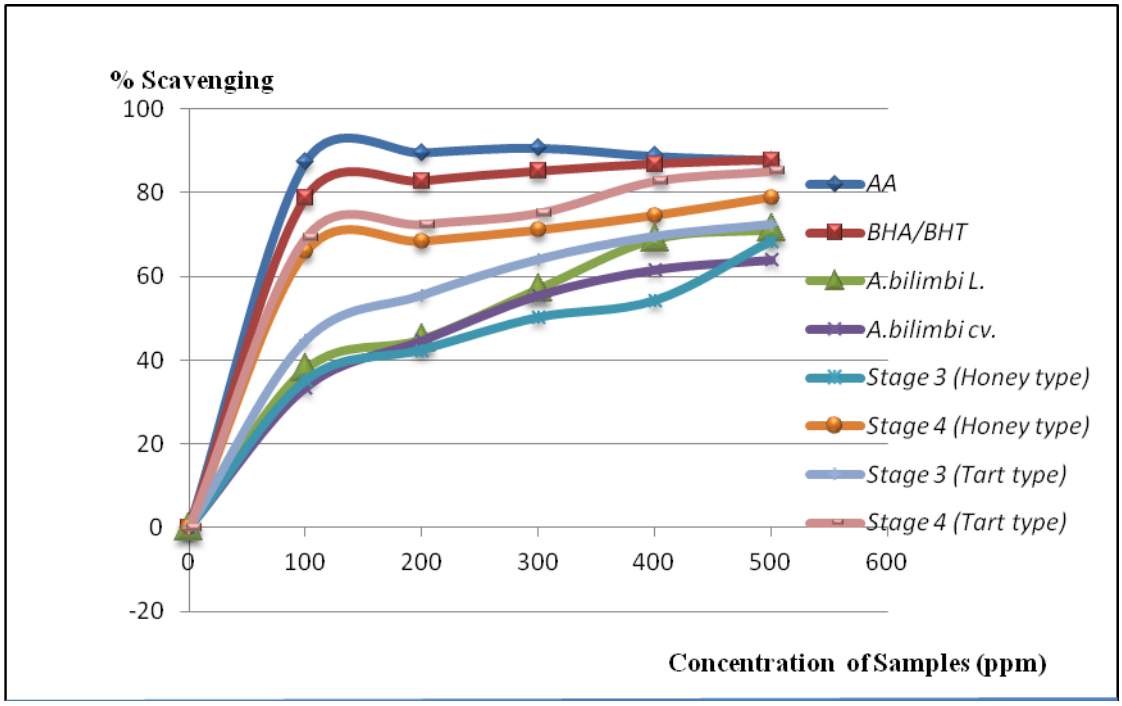

Figure 2: Percentage (%) scavenging of Oxalidaceae fruit extracts

β-carotene-linoleate bleaching assay (BCB)

Antioxidant activity of extract is strongly dependent on the solvent, due to different antioxidant potential of compound with different polarity [35]. In the β-carotene bleaching assay, oxidation of linoleic acid release linoleic acid peroxide as free radicals during incubation at 50ºC that will oxidize beta-carotene resulting in discolouration, hence decreasing the absorbance value [36]. A linear relationship was found between the ability of the sample extract to inhibit oxidation and antioxidant activity. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. There was a correlation between degradation rate and the bleaching of β-carotene, where the extract with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. However, in this study, extracts showed lower antioxidant activity than the standard BHA/BHT combination. The antioxidant activity of standard BHA/BHT was 92.43 ± 0.01%. The antioxidant activity of samples were in decreasing order of BHA/BHT > *Averrhoa carambola L.* (Stage 4- honey type) > *Averrhoa carambola* (Stage 4- tart type) >*Averrhoa bilimbi* cv. > *Averrhoa bilimbi* L.> *Averrhoa carambola* (Stage 3-honey type) > *Averrhoa carambola* (Stage 3- tart type). Ikram et al. [19] reported that antioxidant activity of methanolic extract of *Averrhoa bilimbi* is about 91.89%. However, our findings showed lower activity and this may be due to different extraction method used or due to the difference in species of *Averrhoa bilimbi* used.

Previous study on *Averrhoa carambola* L. as reported by Mishra et al. [37] showed a moderate antioxidant activity. In this fruit, as both types of *Averrhoa carambola* L. from stage 3 illustrated the lower antioxidant activity, which means have lowest β-carotene degradation rate among all the extracts while both types of *Averrhoa carambola* L. from stage 4 exhibited the highest β-carotene degradation rate. Ikram et al. [19], proved that antioxidant activity of methanolic extract of *Averrhoa carambola* L. is higher, which about 95.89%. Othman et al. [38] stated that water extracts had higher antioxidant activity than methanolic extracts in cocoa beans study. Antioxidant activity of *Averrhoa bilimbi* and *Averrhoa carambola* extracts also depends on the type and polarity of the extracting solvent, the isolation procedures and purity of active compounds as well as the assay techniques and substrate used [39].

Table 4: The antioxidant activity of Oxalidaceae fruit using BCB assay

Samples	**BCB (%)**
BHA/BHT	97.43 ± 0.01%A
Averrhoa bilimbi L	67.65 ± 2.12%D
Averrhoa bilimbi cv.	87.65 ± 3.12%C
Averrhoa carambola L. (Stage 3- Honey type)	57.65 ± 4.42%E
Averrhoa carambola L. (Stage 4- Honey type)	90.34 ± 1.65%B
Averrhoa carambola L. (Stage 3- Tart type)	47.35 ± 1.97%E
Averrhoa carambola L. (Stage 4- Tart type)	94.28 ± 1.42%A

Values are expressed as mean ± standard deviation. Means within column (A-E) marked with different letters were not significantly different at the level of $p>0.05$

Correlation between antioxidant activity (AA) with TPC and TFC

The Pearson correlation coefficient between antioxidant assays with TPC and TFC of Oxalidaceae fruit extracts showed positive correlations except for BCB assays and is shown in Table 5. The correlation ($r= 0.432$) for β-carotene indicate that it has moderate positive relationship with TPC. DPPH and FRAP assay showed strong positive relationship with TPC. From this correlation, it can be suggested that, although the plant may contain other antioxidants such as ascorbate, proteins and carotenoids, these antioxidants do not contribute significantly to the antioxidant activity. Hence, it can be postulated that the phenolic compounds may be a major contributor to the antioxidant activity of the Oxalidaceae fruit.

Previous study done by Soong and Barlow [40], suggested that there was a strong correlation between antioxidant assay and TPC. However there are findings indicates that antioxidant assay and TPC do not correlate to each other [41]. There are several reasons that caused variation in the correlations between assays such as due to high content of reducing agents such as ascorbic acid, minerals and carotenoids in fruit [42], and also high protein content or genetic and agronomic and environmental influences such as varieties (cultivar/sub-species) and agronomic and environmental factors (climate, soils and light exposure) [43].

Table 5: Correlation matrix showing relationship between antioxidant assays (DPPH, FRAP and BCB assays)

	TPC	**TFC**
DPPH	0.8368	0.9576
FRAP	0.9344	0.8884
BCB	0.432	0.173

Conclusion

Antioxidant content of fruits is most probably due to the presence of high phenolic compounds in the Oxalidaceae fruits. Most fruits gave positive results in all phytochemical screening conducted. TPC and TFC were found to be highest in stage 4 of *Averrhoa carambola* L. (tart type). Antioxidant activity from the 3 assays also indicated stage 4 of *Averrhoa carambola* L. (tart type) possessed highest antioxidant activity in all the antioxidant assays conducted. Pearson correlation between the assays indicated that the antioxidant might be contributed by the phenolic compounds presence in Oxalidaceae fruits.

Acknowledgement

The authors would like to thank the Faculty of Applied Science, Universiti Teknologi MARA for the facilities and Research Cluster fund 600-RMI/DANA 5/3/CG (18/2012) for the fund.

References

1. Abas, F., Lajis, N.H., Israf, D.A., Khozirah, S. & Kalsom, Y.U. (2006). Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chemistry*: 566–573.
2. Saidin, I., (2000). *Sayuran tradisional ulam dan penyedap rasa*, Vol.1, *University kebangsaan Malaysia Publication*, 42.
3. Carolino, R.O.G., Beleboni, R.O., Pizzo, A.F., Flavio, D.V., Norbert, G.C., Miguel, M.N., Wagner , D.S. & Joaquim, C.N. (2005). Convulsant activity and neurochemical alterations induced by a fraction obtained from fruit *Averrhoa carambola* L. (Oxalidaceae: Geraniales). *Neurochemistry International*, *46*: 523–531.
4. Shahidi, F. & Wanasundra, P.K.J.P.D. (1992). Phenolic antioxidants, *Critical Reviews In Food Science and Nutrition*, *32*: 2017-2021.
5. Duh, P.D. & Yen, G.C. (1997). Antioxidative activity of three herbal water extracts. *Journal of Food Chemistry*, *60*: 639-645.
6. Mojab, F., Kamalinejad, M., Ghaderi, N. & Vahidipour, H.R. (2003). Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*, 2: 77-82.
7. Ciriaco, P.O.P. (1978). Phytochemical,Microbiological and Pharmacological Screening of Medicinal Plants: A supplement of the ACTA MANILANA. Philipines. *GMS Publication Corporatio:* 233-256.
8. Karumi, Y., Onyeyili, P. A. & Oyuybuaja, V.O. (2004). Identification of active principles of *M. Balsamia* (Balsam Apple) leaf extract. *Journal Medical Sciences*, 4: 179-182.
9. Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Methods in Enzymology*, *29*: 152–178.
10. Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64:* 555-559.
11. Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidants activities of products of browning reaction prepared from glucosamine, *Japanese Journal of Nutrition*, *44*: 307-315.
12. Tang, S. Z., Kerry, J.P., Sheehan, D. & Buckley, D.J. (2002). Antioxidative mechanism of tea catechins in chicken meat systems. *Journal Food Chemistry, 76:* 45-51.
13. Velioglu, Y.S., Mazza, G., Gao, L. & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry*, *46*: 4113–4117.
14. Thaipong, K., Boonprakob, U., Crosby, K., Zevallos, L.C. & Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19: 669-675.
15. Wong, K.C., & Wong, S.N. (1995). Volatile constituents of *Averrhoa bilimbi* L. fruit.*Journal of Essential Oil Research* ,7: 691-693.
16. Vasconcelosa, C.M.L. & Conde-Garciaa, E.A. (2006). Electrophysiological effects of the aqueous extract of *Averrhoa carambola L*. leaves on the guinea pig hearts. *Phytomedicine*, *13*: 501–508.
17. Shinu, T., Patil, D.A., Patil, A.G. & Chandra, N. (2008). Pharmacognostic Evaluation and physicochemic analysis of *Averrhoa Carambola* L. fruit. *Journal of Herbal Medicine & Toxicology, 2- 51-54.*
18. Krishnaiah, D., Devi, T., Bono, A. & Sarbathly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research, 3(2):* 067-072.
19. Ikram, E.H.K., Khoo, H.E., Jalil, A.M.M., Ismail, A., Idris, S., Azlan, A., Nazri, H.S.M., Dito, N.A.M. &

Mokhtar, R.A.M. (2009). Antioxidant activity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis, 22*: 388-393.
20. Stratil, P., Klejdus, B. & Kubán , V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables—Evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry, 54*: 607–616.
21. Huang, D., Ou, B. & Prior, R.L. (2005). The chemistry behind antioxidant activity assays. *Journal of Agricultural and Food Chemistry*, 53: 1841–1856.
22. Narain, N., Bora, P.S., Holschuch, H.J. & Vasconcelos, M.A.D.S. (2001). Physical and chemical composition of carambola fruit (*Averrhoa carambola* L.) at three stages of maturity. *Cienc.Technol.Aliment, 3:* 144-148.
23. Huda-Faujan, N., Noriham, A., Norrakiah, A.S. & Babji, A.S. (2007).Antioxidative activities of water extracts of some Malaysian herbs. *ASEAN Food Journal. 14(1*): 61-68.
24. Chang, C.C., Yang, M.H., Wen, H.M. & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10(3)*: 178-182.
25. Iker, H., Leonor, A., Frank, V.B. & Sergi, M.B. (2005). Opinion: How relevant are flavonoids as antioxidants in plants? *The Avi Publishing Company*, 970
26. Cao, G., Sofic, E. & Prior, R.L. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationship. *Free Radical Biology and Medicine, 22:* 749 -760.
27. Harris, R.S. (1996). Effects of agricultural practices on foods of plant origin**.** *The Avi Publishing Company*, 670.
28. Benzie, I.F.F. & Strain, J.J. (1996). The Ferric reducing ability of plasma (FRAP) as a measure of Antioxidant Power‘. *Analysis of Biochemistry, 23:* 70-76.
29. Yan, L.Y., Teng, L.T. & Jhi, T.J. (2006).Antioxidant Properties of Guava Fruit: Comparison with Some Local Fruits. *Sunway Academic Journal, 3:* 9-20.
30. Prior, R.L. & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *Horticultural Science, 35(4):* 588–592.
31. Gazzani, G., Papetti, A., Massolini, G. & Daglia, M. (1998). Anti and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry, 46*: 4118–4122.
32. Mathew, L., George, S. T., Babylatha, A. K. & Geetha, C. K. (1993) Flowering and fruit development in bilimbi (*Averrhoa bilimbi* L.). *Journal Food Science and Agriculture*, 41: 41-42.
33. Prior, R.L., Wu, X. & Schaich, K., (2005). Standardized methods for the determination of antioxidant activity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*: 4290–4302.
34. Subhasree, R.B., Laxmi, K.R., Lijina, S.R. & Rajasekaran, P. (2009). Evaluation of antioxidant potential in selected green leafy vegetables. *Journal Food Chemistry, 115*: 1213–1220.
35. Lee, W.Y., Emmy, H.K.I. & Amin, I. (2007). Antioxidant activity and phenolic content of selected commercially available cruciferous vegetables. *Malaysian Journal of Nutrition*, 13: 71-80.
36. Talcott, S.T., Howard, L.R. & Brenes, C.H. (2000). Antioxidant changes and sensory properties of carrot puree processed with or without periderm tissue. *Journal ofAgricultural and Food Chemistry, 48*: 1315–1321.
37. Mishra, N., Dubey, A., Singh, N. & Gupta, P. (2010). Antimicrobial, antioxidant and chemopreventive potential of vitamin c rich fruits. *International Journal of Applied Biology and Pharmaceutical Technology, 3:* 915-920.
38. Othman, A., Amin, I., Nawalyah, A.G. & Ilham, A. (2007). Antioxidant activity and phenolic content of cocoa beans. *Food Chemistry, 100:* 1523-1530.
39. Amin, I., Zamaliah, M.M. & Chin, W.F. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Journal Food Chemistry, 87*: 581–586.
40. Soong, Y.Y. & Barlow, P.J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Journal Food Chemistry, 88 (3):* 411–417.
41. Amin, I. & Lee, W.Y. (2005). Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *Journal of the Science of Food and Agriculture, 85*: 2314–2320.
42. Deepa, N., Kaur, C., Singh, B. & Kapoor, H.C. (2006). Antioxidant activity in some red sweet pepper cultivars. *Journal of Food Composition and Analysis, 19*: 572–578.
43. Kaur, C., & Kapoor, H.C. (2001). Review: antioxidants in fruits and vegetables the millennium health. *International Journal of Food Science and Technology, 36*: 703–725.