

# Antioxidant Activities of *Syzygium Cumini* and *Ardisia Elliptica* in Relation to Their Estimated Phenolic Compositions and Chromatic Properties

A. M. Siti-Azima, A. Noriham, and M. Nurhuda

**Abstract**—Plant extract serves as a good source of bioactive compounds and also natural pigment which is potential to be further applied as colourants in food and pharmaceutical products. The aim of this study is to determine antioxidant activities in *Syzygium cumini* and *Ardisia elliptica* based on FRAP, ORAC, DPPH and ABTS radical scavenging assays in relation to their total phenolic content (TPC), total flavonoids content (TFC) and anthocyanin content (tAcy). The chromatic properties based on colour density (CD), Lightness (L\*), hue angle (ho) and chroma value (c) of the selected plants were also determined to evaluate their potential as natural colourants in food. *Syzygium cumini* showed higher in FRAP value (25.66 mM TEAC), DPPH radical scavenging capacity (EC50=0.22 mg/ml) and TPC (43.64 mg GAE /g) as compared to *Ardisia elliptica* with 19.60 mM TEAC, EC50=0.24 mg/ml and 41.75 mg GAE /g respectively. *Ardisia elliptica* exhibited higher in ABTS scavenging activity (4.63 mM TEAC), ORAC value (10.95 μM TEAC), TFC (36.91 mg QE /g) and tAcy (9.97 mg cyanidin /g) as compared to *Syzygium cumini* with 4.30 mM TEAC, 10.12 μM TEAC, 17.02 mg QE /g and 8.71 mg cyanidin /g respectively. *Ardisia elliptica* showed higher in CD (1.32 AU), c (32.29) and ho values (24.68o) but lower in L\* value (50.69) as compared to the *Syzygium cumini* with 1.01 AU, 12.10, 10.02o and 75.49 respectively. FRAP, ABTS and EC50 values exhibited very strong correlation with TFC (r<sup>2</sup>= 0.974, 0.984, 0.921 respectively) and tAcy (r<sup>2</sup>= 0.953, 0.998, 0.947 respectively). They also exhibited strong correlation with TPC (r<sup>2</sup>= 0.854, 0.779, 0.749 respectively). ORAC assay exhibited weak correlation with TFC (r<sup>2</sup>= 0.357) and tAcy (r<sup>2</sup>= 0.229) but strong correlation with TPC (r<sup>2</sup>=0.713). Hence, it can be concluded that, TFC and tAcy exhibited very strong relationship with the FRAP, ABTS and DPPH scavenging activities and work as single electron transfer in their mechanism while *Ardisia elliptica* was better in their chromatic properties as compared to *Syzygium cumini*.

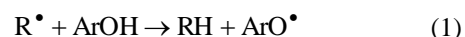
**Index Terms**—Anthocyanin, antioxidant, *ardisia elliptica*, chromatic properties, *syzygium cumini*.

## I. INTRODUCTION

Plants are world natural ornamental that gained a wide attention as a good source of natural antioxidant which is associated with health benefit and well-being. Owing to its phenolic structure plants are capable to overcome the oxidative stress in the human cell which finally led to the development of chronic disease such as tumor, cancer,

neurodegenerative, rheumatic arthritis and autoimmune disorders [1]-[3]. This nutraceutical and medicinal value lies in some phytochemical substances that produce a definite physiological action in the human body. The antioxidant properties of the phytochemical are always associated with their chromophores structure that are responsible for the bright colour of plants known as plant pigment. Anthocyanin is water soluble pigment which is responsible for red to blue chroma of plants.

Phenolic antioxidant which generically denominated as ArOH contains at least one hydroxyl group (OH) attached to the benzene ring and plays as chain breaking antioxidant [4]. Generally, there are two suggested mechanisms by which antioxidants can play their defensive role [5], [6]. In the first mechanism known as hydrogen atom donator (HAT), the free radical removes a hydrogen atom from the antioxidant (ArOH) than becoming itself a radical and terminated the oxidation process by converting free radicals to more stable products (1). In the second mechanism SET (single-electron transfer), the antioxidant (ArOH) donates an electron to the free radical becoming itself a radical cation and terminate the oxidation chain reaction by reducing the oxidised intermediates into the stable form (2).



Several methods have been established based on these two mechanisms. Oxygen radical antioxidant capacity (ORAC) assay and total reactive antioxidant potential (TRAP) assay are categorised under the HAT while ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferric thiocyanate (FTC) assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+) scavenging assay are categorised under SET [7]-[9].

*Syzygium cumini* Skeels (syn. *Eugenia jambolana* Lam., *Eugenia cumini* Druce) belongs to the family of Myrtaceae is widely found in subcontinent of Asia and other tropical country. It is commonly known as Keriang dot or Keriang Aceh by the Malaysian, Java plum by the Americans and Jamun or Jambul by the Indian. The berry is oblong in shape with dark purple skin and whitish aril. The major anthocyanin being found in the berries are cyanidin-diglucoside, ptunidin-diglucoside, delphinidin-diglucoside and malvidin-diglucoside [10]. It has been reported to possessed several bioactivities against

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diabetic [11] and cancer [12]. *Ardisia elliptica*'s fruit are roundish berries that turn shiny black when ripen, and resemble the eye ball of chicken; hence it is known as Mata Ayam (Chicken Eyes) among the Malaysian [13]. In Borneo it is known as Kayu lundu, Limjong, Merjima, Pis-pis, Sarusup, Semporna, Serusop, Surusup, Tapiok, Tursop laut. It is also commonly known as Buah Letus, Cempenai, Mata Pelanduk, Mempenai, Daun Bisa Hati, Penah, Periah, Shoebutton *Ardisia* and Seashore *Ardisia*. *Ardisia elliptica* leaves have been medicinally used as antiobiotic and antiviral [14]. Hence in this work the study on the antioxidant activities in *Syzygium cumini* and *Ardisia elliptica* based on FRAP, ORAC, DPPH and ABTS radical scavenging assays in relation to their estimated phenolic compositions which are total phenolic content (TPC), total flavonoids content (TFC) and anthocyanin content (tAcy) were determined. The chromatic properties based on colour density (CD), Lightness ( $L^*$ ), hue angle ( $h^\circ$ ) and chroma value ( $c$ ) of the selected plants were also determined to evaluate their potential as natural colourants in food.

## II. MATERIALS AND METHODS

### A. Preparation of Raw Material

Freshly ripened *Ardisia elliptica* and *Syzygium cumini* was collected and obtained from Santan, Perlis, Malaysia. The fruits were washed, sealed pack and stored at  $-20^\circ\text{C}$  for further used.

### B. Extraction Procedure

The raw materials were stirred in the 100 mM citrate buffer (pH 3.0) for 10 minutes at  $100^\circ\text{C}$ . The ratio of plant parts to citrate buffer is 1:4 (v/v). The extraction procedure was conducted twice to maximize the yield. The extracts were filtered by using Buchner funnel and concentrated by using rotary evaporator at  $60^\circ\text{C}$  and 100 mbar. The concentrated filtrate was lyophilised and kept in amber bottle at  $-20^\circ\text{C}$  prior to further analysis.

### C. Antioxidant Activities

Radical scavenging activity of the plant extracts were conducted based on the method developed by [8]. The result was expressed as  $\text{EC}_{50}$  (Efficient Concentration at 50% scavenging activity). Ascorbic acid was used as a standard in comparison with the extracts.

The antioxidant capability of the extract based on ferric reducing antioxidant power (FRAP) was determined according to the method described in [7]. The readings of the blue (ferrous tripyridyltriazine) complex were measured by Perkin Elmer UV-Vis spectrophotometer at  $\lambda_{\text{max}}=593$  nm. The linear standard calibration curve ranging from 0-100 mM Trolox with  $r^2=0.999$  was established. The final results were expressed in mM TEAC / g of fresh extract weight in which TEAC is denoted for Trolox Equivalent Antioxidant Capacity.

The ABTS $\cdot^+$  scavenging capacity test was carried out according to the method described by [15]. The samples were measured spectrophotometrically by Perkin Elmer UV-Vis spectrophotometer at  $\lambda_{\text{max}}=734$  nm and regression  $r^2=0.998$

was obtained. The final results were expressed in mM TEAC / g of fresh extract weight.

Oxygen Radical Absorbance Capacity (ORAC) assays was conducted using the procedures outlined by the reagent provider (Zen- Bio, Inc.; Research Triangle Park, NC). The samples were measured at 530 nm by microplate reader Bio-Tek Instruments, Inc provided with Gen 5 software.

### D. Total Phenolic Content

Total phenolic content was evaluated based on the Folin-Ciocalteu colorimetric method developed by [16]. The absorbance was measured by Perkin Elmer UV-Vis spectrophotometer at  $\lambda_{\text{max}}=765$ nm. Quantitative measurements were conducted based on a Gallic acid standard curve ranging from 0-0.5 mg/ml with  $r^2=0.999$ . The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g of fresh weight extract.

### E. Total Flavonoids Content

Aluminium chloride colorimetric method described by [17] was used to estimate total flavonoid content in the plant extracts. The absorbance of orange colour of the aluminium complex was measured by using Perkin Elmer UV-Vis Spectrophotometer at the visible range with  $\lambda_{\text{max}}=415$  nm. A series of quercetin standard were prepared and a linear standard calibration curve with  $r^2=0.998$  was established. The results were expressed as quercetin equivalent (QE) in mg/g of fresh weight extract.

### F. Total Anthocyanin Content and Colour Properties

Total anthocyanins in the samples were measured spectrophotometrically according to the method described by [18]. The colour density (CD) was conducted based on the method described by [19].

### G. Statistical Analysis

Analyses of data were obtained from triplicate samples. Statistical analyses were conducted using Statistical Analysis System 9.1.3 software package [20]. Two sample t-tests were used to compare the mean values. Values were expressed as means  $\pm$  standard deviations. A correlation matrix was calculated between all independent variables to examine co-linearity.

## III. RESULTS AND DISCUSSIONS

DPPH scavenging activity is a kinetic antioxidant method which based on the reduction of DPPH $\cdot$  free radical into DPPH $_2$  by the action of antioxidant [21]. The DPPH $_2$  is a stable organic radical which exhibit violet colour in alcohol solution with an absorption peak in the visible range at  $\lambda_{\text{max}}=518$  nm, which disappear in the presence of antioxidant. The capacity of the compounds to scavenge the DPPH radical has been used as the magnitude of the antioxidant capacity [22]. The  $\text{EC}_{50}$  values required to scavenge 50% radical were calculated from the regression equation prepared from series concentration of extracts. The lower  $\text{EC}_{50}$  indicate the better DPPH radical scavenging activity. *Ardisia elliptica* extracts exhibited higher in  $\text{EC}_{50}$  value (0.24 mg/ml) of DPPH radical scavenging activities as compared to the  $\text{EC}_{50}$  value (0.22 mg/ml) of *Syzygium cumini* extracts (Table I). This indicated

that *Syzygium cumini* extracts is 1.09 times greater in scavenging effect than *Ardisia elliptica* extracts. *Syzygium cumini* presented 1.83 times lower scavenging effect of ascorbic acid while *Ardisia elliptica* exhibited 2.00 times lower scavenging effect as compared to the ascorbic acid. *Ardisia elliptica* exhibited higher ORAC value (10.95  $\mu\text{M}$  TEAC) as compared to *Syzygium cumini* (10.12  $\mu\text{M}$  TEAC).

FRAP assay is based on the ability of the antioxidant to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  in the presence of TPTZ, forming an intense blue  $\text{Fe}^{2+}$ -TPTZ complex with an absorption maximum at 593 nm [23]. Based on the FRAP assay as shown in the TABLE I, *Syzygium cumini* extracts (25.66 mM TEAC) showed a significantly higher ( $p < 0.05$ ) in FRAP value as compared to the *Ardisia elliptica* extracts (19.60 mM TEAC). Conversely, in ABTS scavenging activities *Ardisia elliptica* extract showed better ABTS scavenging activity as compared to *Syzygium cumini* extract with ABTS value 4.63  $\mu\text{M}$  TEAC and 4.30  $\mu\text{M}$  TEAC respectively. *Syzygium cumini* is insignificantly higher ( $p < 0.05$ ) in TPC (43.64 mg GAE / g) but significantly lower ( $p < 0.05$ ) in TFC (17.02 QE / g) and tAcy (8.71 mg cyanidin equivalent / g) as compared to *Ardisia elliptica* extracts (TPC=41.75, TFC=36.91, tAcy=9.97) (Table I).

TABLE I: ANTIOXIDANT ACTIVITIES AND ESTIMATION OF PHENOLIC COMPOSITIONS IN SYZYGIUM CUMINI AND ARDISIA ELLIPTICA FRUITS

| Antioxidant activities and phenolic compositions    | Plant samples                 |                                 |
|---|-------------------------------|---------------------------------|
|   | <i>Syzygium cumini</i> fruits | <i>Ardisia elliptica</i> fruits |
| <sup>b</sup> FRAP mM (TEAC)                         | 25.66 $\pm$ 1.40 <sup>A</sup> | 19.60 $\pm$ 0.53 <sup>B</sup>   |
| <sup>a</sup> EC <sub>50</sub> (mg/ml)               | 0.22 $\pm$ 0.00 <sup>A</sup>  | 0.24 $\pm$ 0.00 <sup>A</sup>    |
| <sup>b</sup> ABTS (mM TEAC)                         | 4.30 $\pm$ 0.05 <sup>B</sup>  | 4.63 $\pm$ 0.09 <sup>A</sup>    |
| <sup>b</sup> ORAC ( $\mu\text{M}$ TEAC)             | 10.12 $\pm$ 1.29 <sup>A</sup> | 10.95 $\pm$ 1.63 <sup>A</sup>   |
| Total phenolic content (mg GAE / g extract)         | 43.64 $\pm$ 4.40 <sup>A</sup> | 41.75 $\pm$ 3.50 <sup>A</sup>   |
| Total flavonoids content (mg QE / g extract)        | 17.02 $\pm$ 1.10 <sup>B</sup> | 36.91 $\pm$ 2.37 <sup>A</sup>   |
| Total anthocyanin content (mg cyanidin / g extract) | 8.71 $\pm$ 0.14 <sup>B</sup>  | 9.97 $\pm$ 0.05 <sup>A</sup>    |

Note: Analysis of data were obtained from triplicate sample Values were means  $\pm$  SD

<sup>AB</sup>Means with different capital letter within the row were significantly different at ( $p < 0.05$ )

<sup>a</sup> EC<sub>50</sub> for Ascorbic acid were 0.12  $\pm$  0.00

<sup>b</sup> TEAC is Trolox equivalent antioxidant capacity

The colour properties of *Ardisia elliptica* extract and *Syzygium cumini* extract at pH 3.0 were evaluated to determine the potential natural colourant in food. *Ardisia elliptica* extract exhibit a higher CD,  $c$ , and  $h^{\circ}$  but lower in  $L^*$  as compared to the *Syzygium cumini* extract (Table II). Based on the CD and  $L^*$  *Ardisia elliptica* demonstrated a better intensity than *Syzygium cumini*. Higher in  $c$  and  $h$  indicating that *Ardisia elliptica* is more saturated/vivid with redder in colour as compared to *Syzygium cumini*. Thus, *Ardisia elliptica* has better potential as red colourant as compared to *Syzygium cumini*.

The correlation analyses were conducted in this research to determine the relationship between the phenolic composition and four antioxidant activities studied which are FRAP, DPPH, ABTS and ORAC as well as their antioxidative mechanism. From the results obtained in TABLE III TFC and tAcy showed a very strong correlation with FRAP, EC<sub>50</sub> and ABTS while TPC exhibited strong correlation with FRAP,

EC<sub>50</sub> and ABTS. The correlations strength demonstrated that flavonoids (flavone and flavonols) and anthocyanins play a major role in FRAP, DPPH and ABTS antioxidant activities of *Syzygium cumini* and *Ardisia elliptica* as compared to the phenols. Besides, a very strong correlation between FRAP with TFC ( $r^2=0.960$ ) and tAcy ( $r^2=0.953$ ), ABTS with TFC ( $r^2=0.992$ ) and tAcy ( $r^2=0.997$ ) and EC<sub>50</sub> with TFC ( $r^2=0.984$ ) and tAcy ( $r^2=0.998$ ) but low correlation between ORAC with tAcy and TFC manifest that the flavonoids and anthocyanin works as single electron transfer (SET) rather than hydrogen atom donator (HAT). Previous literature reported by [24] indicated that flavonoids owing to the subclass flavonol that has combination of 3-hydroxy group, 2, 3-double bond and 4-oxo function in the C ring and the catechol group in the B ring as their functional group in their chemical structure namely quercetin, myricetin, kaempferol, rutin, galangin and fisetin explained the reason behind reactivity towards reducing capacity of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and the strong correlation between FRAP assay and flavonoids. In addition, [25] reported that only myricetin and quercetin exhibit strong reaction with  $\text{Fe}^{3+}$  and the possible complexation between flavonoids and  $\text{Fe}^{3+}$  occurs between the 5-hydroxyl and the 4-oxo groups. References [17] and [26] reported very strong correlation between FRAP assay and anthocyanin and supported the finding in this research.

TABLE II: CHROMATIC PROPERTIES OF AQUEOUS EXTRACTION OF SYZYGIUM CUMINI AND ARDISIA ELLIPTICA FRUITS

| Plant samples                   | Colour density (CD)          | Lightness ( $L^*$ )           | Chroma -city ( $c$ )          | Hue angle ( $h^{\circ}$ )     |
|---------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <i>Syzygium cumini</i> fruits   | 1.01 $\pm$ 0.03 <sup>B</sup> | 75.49 $\pm$ 1.01 <sup>A</sup> | 12.10 $\pm$ 0.66 <sup>B</sup> | 10.02 $\pm$ 0.92 <sup>B</sup> |
| <i>Ardisia elliptica</i> fruits | 1.32 $\pm$ 0.05 <sup>A</sup> | 32.29 $\pm$ 1.76 <sup>B</sup> | 50.69 $\pm$ 0.44 <sup>A</sup> | 24.68 $\pm$ 1.02 <sup>A</sup> |

Note: Analysis of data were obtained from triplicate samples

Values were means  $\pm$  SD

<sup>AB</sup>Means with different capital letter within the column were significantly different at ( $p < 0.05$ )

TABLE III: CORRELATION BETWEEN ANTIOXIDANT ACTIVITIES OF SYZYGIUM CUMINI AND ARDISIA ELLIPTICA FRUITS WITH THEIR PHENOLIC COMPOSITIONS

| $r^2$ | FRAP  | EC <sub>50</sub> | ABTS  | ORAC  |
|-------|-------|------------------|-------|-------|
| TPC   | 0.854 | 0.779            | 0.749 | 0.713 |
| TFC   | 0.974 | 0.984            | 0.921 | 0.357 |
| tAcy  | 0.953 | 0.998            | 0.947 | 0.229 |

#### IV. CONCLUSIONS

Based on the antioxidant activities being tested, *Syzygium cumini* extract exhibited higher in FRAP value and DPPH radical scavenging activity as compared to the *Ardisia elliptica* extract while *Ardisia elliptica* exhibited higher in ABTS radical scavenging activity and ORAC value as compared to the *Syzygium cumini*. Pearson correlation coefficient ( $r^2$ ) showed that flavonoids (flavones and flavonols) and anthocyanins exhibited very strong relationship with SET antioxidant activities which are FRAP and ABTS scavenging activity and weak relationship with HAT antioxidant activity which is ORAC indicating that flavonoids and anthocyanin favour the SET mechanism

rather than HAT. On the other hand, phenolic (phenols) showed a strong correlation with four antioxidant activities assays being tested. *Ardisia elliptica* exhibited significantly higher in chromatic properties as compared to *Syzygium cumini* and demonstrated that *Ardisia elliptica* has better potential in food applications as natural colourant as compared to *Syzygium cumini*.

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