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COMPARATIVE STUDIES ON ANTIOXIDANT ACTIVITIES, TOTAL PHENOLICS AND ANTHOCYANIN CONTENT OF FOUR NATIVE FRUITS

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INTRODUCTION

Anthocyanins are the ubiquitous water-soluble pigments found in most flowers and fruits, and are responsible for their impressive red and blue colours. Anthocyanins have been predominantly found in nature as glycosides of polyhydroxy and polymethoxy derivatives of 2-phenyl-benzopyryliurn or flavylium salts (Fig. 1). Individual members are differentiated by the number of hydroxyl and methoxyl groups of the B-ring, by the number of sugar units attached to the aglycone and their position of attachment, and by the nature and number of aliphatic or aromatic acids attached to the sugar residues These plant pigments exist in the form of glycosides and the most common sugar moieties are glucose, galactose, rhamnose and arabinose. Cyanidin derivatives are the most abundant anthocyanins in plants. As a component of the human diet, anthocyanins are known to have health promoting activities because of their high antioxidant properties *in vitro* and *in vivo*. In recent years several studies have shown that anthocyanins display a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic. The objective of this study was to investigate antioxidant activity (DPPH assay), total phenolics and anthocyanin content of 4 anthocyanin-rich fruits: *Antidesma thwaitesianum* (Buni fruit), *Syzygium cumini* (Java plum), *Lepisanthes fruticosa* (Luna nut) and *Morus alba* (Mulberry). The results would be useful as basic information for finding sources of anthocyanin-rich raw materials with high antioxidant activities and also useful as medical and health supplement.

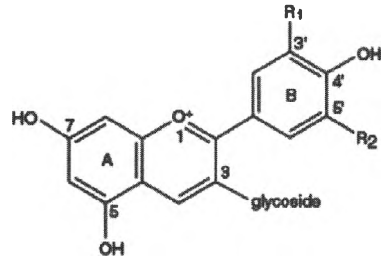

Figure 1 Chemical structure of anthocyanin

MATERIALS AND METHODS

Materials All fresh fruits were collected from different parts of Thailand. They were graded, washed and crushed into small pieces.

Sample preparation The fresh samples (10 g.) were extracted in 50 ml aqueous acid acetone by sonicator for 5 min. (3 times) and filtered through a Whatman No.1 filter paper. The supernatant was collected and partitioned with chloroform in separatory funnel. The upper aqueous layer, containing the acetone/water mixture was collected while the chloroform/acetone layer was carefully discarded. Residual acetone and chloroform were removed from the anthocyanin extract by using a rotary evaporator at 40 °C under

vacuum condition. Extracts were taken to 50 ml in volumetric flask by 0.01%-HCl-acidified-water. Extracts were then kept at −80 °C until further analyzed.

Determination of total monomeric anthocyanins (TAC), total phenolics (TP) and antioxidant activities (DPPH assay)

Materials Absolute methanol, acetone, chloroform, hydrochloric acid, potassium chloride, sodium acetate, Folin reagent and sodium carbonate were reagent grade (Lab Scan, Ireland). DPPH (2,2-diphenyl-l-picryhydrazyl) and Trolox (hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Fluka (Germany).

Apparatus UV-Vis absorbance of TAC and TP was measured on a UV-Vis spectrophotometer UV-2450 (SHIMADZU, Japan). For DPPH assay, absorbance was recorded on a Microplate reader (GENios plus, Austria) with Magellan software.

Method The total monomeric anthocyanin content (TAC) was measured by the pH-differential method (Giusti and Wrolstad, 2001). Anthocyanin extracts were prepared in 0.025 M potassium chloride buffer, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5 to measure the absorbance of the colored oxonium and the colorless hemiketal form by comparison of the absorbance value at 520 nm using spectrophotometer. The calculated values from the pigment as cyanidin-3-glucoside, MW = 449.2 and €=26,900, were compared and reported as the monomeric anthocyanin content.

Total phenolics (TP) were measured by the Folin–Ciocalteu (FC) method (Singleton and Rossi, 1965). Absorbance was measured at 765 nm. TP was expressed as milligrams of gallic acid equivalent per 100 g fresh weight.

The DPPH free radical-scavenging activity of each sample was determined (Brand-Williams et al, 1995). Briefly, a 50% v/v of aqueous methanolic DPPH solution (607 μM) was prepared. The initial absorbance of the DPPH was measured at 517 nm and did not change throughout the period of assay. The aliquots (20 μl) of Trolox and each sample (with appropriate dilution if necessary) were added to 180 μl of aqueous methanolic DPPH solution. Discoloration was measured at 517 nm after incubation for 30 min at 30 °C in the dark. Measurements were performed at least in triplicate. The percentage of DPPH (%DPPH) was calculated as:

$$\% \text{ DPPH} = (Ac - As) \times 100 / Ac$$

where Ac is the absorbance of the control, and As is the absorbance of the sample. IC50 values were calculated to denote the concentration of a sample required to decrease the absorbance at 517 nm by 50%.

RESULTS AND DISCUSSION

In this study, free radical scavenging activity, TP and total TAC of 4 native fruits were investigated and shown in Figures 2, 3 and 4. For DPPH assay, free radical scavenging activity of *Antidesma thwaitesianum* (Buni fruit), *Syzygium cumini* (Java plum), *Lepisanthes fruticosa* (Luna nut), and *Morus alba* (Mulberry) was revealed as 32461.25, 4344.47, 3557.47 and 1317.20 μmoles Trolox equivalents/100 gram fresh weight, respectively. Among these four fruits, *Antidesma thwaitesianum* (Buni fruit) showed the highest activity.

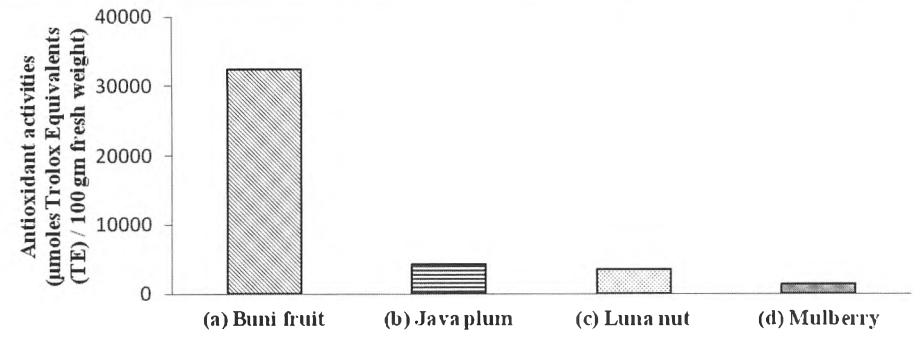

Figure 2 The DPPH free radical scavenging activity of (a) *Antidesma thwaitesianum* (Buni fruit), (b) *Syzygium cumini* (Java plum), (c) *Lepisanthes fruticosa* (Luna nut), (d) *Morus alba* (Mulberry) (in µmoles Trolox equivalents / 100 gm fresh weight

The TP differed among different types of fruits and the TP values were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight. There were significant differences among these tested fruits. The TP of *Antidesma thwaitesianum* (Buni fruit), *Syzygium cumini* (Java plum), *Lepisanthes fruticosa* (Luna nut), and *Morus alba* (Mulberry) were found to be 816.29, 741.79, 692.63 and 296.07 mg GAE/g fresh weight, respectively. *Antidesma thwaitesianum* (Buni fruit) contained the highest total phenolics.

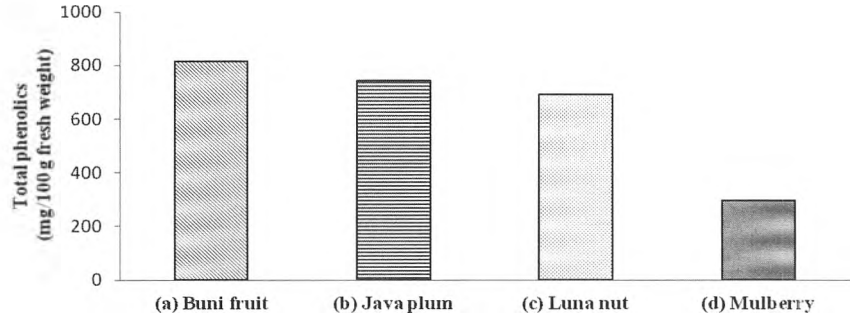

Figure 3 The total phenolics of (a) *Antidesma thwaitesianum* (Buni fruit), (b) *Syzygium cumini* (Java plum), (c) *Lepisanthes fruticosa* (Luna nut), (d) *Morus alba* (Mulberry) (in mg / 100 gm fresh weight)

Total anthocyanin contents in *Antidesma thwaitesianum* (Buni fruit), *Syzygium cumini* (Java plum), *Lepisanthes fruticosa* (Luna nut), and *Morus alba* (Mulberry) were 259.83, 285.25, 122.01 and 220.30 mg/100g fresh weight, respectively. *Syzygium cumini* (Java plum) had the highest anthocyanin contents.

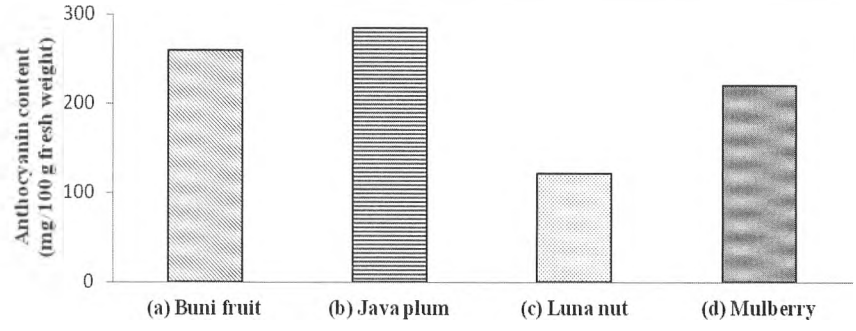

Figure 4 Total anthocyanin content of (a) *Antidesma thwaitesianum* (Buni fruit), (b) *Syzygium cumini* (Java plum), (c) *Lepisanthes fruticosa* (Luna nut), (d) *Morus alba* (Mulberry) (in mg / 100 gm fresh weight)

CONCLUSION

In this study, four fruits native to Thailand were investigated for their antioxidant activity, total phenolics and total anthocyanin content. The result showed that *Antidesma thwaitesianum* (Buni fruit) contained the highest in anthocyanins and antioxidant activity, and *Syzygium cumini* (Java plum) contained the highest in total phenolics. The results would be useful as basic information in the screening for sources of anthocyanin-rich raw materials with high antioxidant activities for medical and health supplemental purposes.

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