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Effect of Ethanol Concentration on Total Monomeric anthocyanins, Total Phenolic Compound and Antioxidant Activity in *Syzysium cumini* L.(Java Plum) Fruits.

Tanwarat Kajsongkram[1*], Saowalux Rotamporn[1], Sirinan Thubthimthed[1]

[1] *Expert Centre of Innovative Herbal Products (InnoHerb), Thailand Institute of Scientific and Technological Research, 35 Moo 3 Technopolis, Khlong 5, Khlongluang, Pathumthani 12120, Thailand*

* Corresponding author: Tel. +66(0)25779108; Fax. +66(0)25779110; E-mail address: tanwarat@tistr.or.th

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Introduction

Java plum (*Syzygium cumini* L.), also known as jambu, jamblon, jambula, jamboola, Java plum, jamun, jaam/kalojaam, jamblang, jambolan, blackplum, Damsonplum, Duhat plum, Jambolan plum, or Waa in Thai, belongs to the *Myrtaceae* family and has a small, egg-shaped purple fruit when ripe, with the pulp surrounding a single large seed. Several different phenolic classes have been reported in the fruits in high amounts. Among these compounds, anthocyanins are one of the main polyphenols present, especially in the peels of the fruits. Anthocyanins are the largest and most important group of water-soluble and vacuolar pigments in nature. They are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium cation. Anthocyanins have been used as a source of colors and phytonutrients over the last years. Many health benefits have been associated with anthocyanins, including reduction of oxidation stress, prevention of coronary heart disease, obesity and diabetics, and anticancer activity. Due to its health benefits, we will study the preparation process of anthocyanin-rich extract as the raw material for medical and health supplement purpose.[3]

The aim of this study is to investigate the effect of ethanol concentration on the total anthocyanins content)TAC(, total phenolic contents)TPC(and free radical scavenging)DPPH)1,1-diphenyl-2-picrylhydrazyl(method(of Java plum)*Syzygium cumini* L.(fruits. The ethanol concentration has been used as 90%, 70%, 50%, 30% and 0%v/v ethanol

Methods

plant material

Java plum (*Syzygium cumini* L.) fruits were obtained from the Kanchanaburi province, Thailand. The fruits were stored at -20 ºC and only the edible portions were separaeted and dried using freeze dryer before extraction

Anthocyanin-rich extract preparation

The dried powder of Java plum (*Syzygium cumini* L.) fruits (1.5 g.) were extracted with 50 ml of 5 ethanol concentrations by using sonicator for 5 min. The extraction were studied at the concentration of 90%, 70%, 50%, 30%, 0%, v/v ethanol. The supernatants were filtered through a Whatman No.1. and concentrated by using a rotary evaporator at 40 ºC under vacuum condition.

Sample preparation

The Java plum fruit (JPF) extract 0.25 g was weighted and extracted with 50 ml water by sonicator for 5 minutes and filtered through a Whatman No.1 filter paper. The supernatants were evaporated to dryness by using a rotary evaporator at 40 ºC under vacuum condition. Then the filtrate was transferred to 50 ml volumetric flasks and the volume of each was adjusted to 50 ml with 0.01%-HCl-acidified-water . Extracts were then kept at −80 ºC until further analyzed.

Determination of total anthocyanin content (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 was measured by the pH-differential method.[3] Anthocyanin-rich 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.[6] Absorbance was measured at 765 nm. TP was expressed as milligrams of gallic acid equivalent per 100 g dry weight.

The DPPH free radical-scavenging activity of each sample was determined.[1] 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

Effect of ethanol concentration on total monomeric anthocyanin content in Java plum (Syzygium cumini L.) fruits.

The total monomeric anthocyanin content (TAC) in JPF extracts could be determined using visible spectrophotometry. Figure 1. showed TAC in 5 ethanol concentrations varied from 796.98-1115.63 mg/100g dryweight .The TAC of 70% v/v ethanol extracts from anthocyanin-rich extracts showed the highest as 1115.63 mg/ 100 g dry weight. The increase of the ethanol concentration in the extraction solution up to 70% resulted to increase TAC. The TAC of extracts significantly decrease at 90% v/v ethanol. In previous research in *Benitaka cultivar grapes* study, it has similarly results which showed solvents containing 70% ethanol in water leads to extract the higher TAC when comparing with 60% and 80% v/v of ethanol.[7]

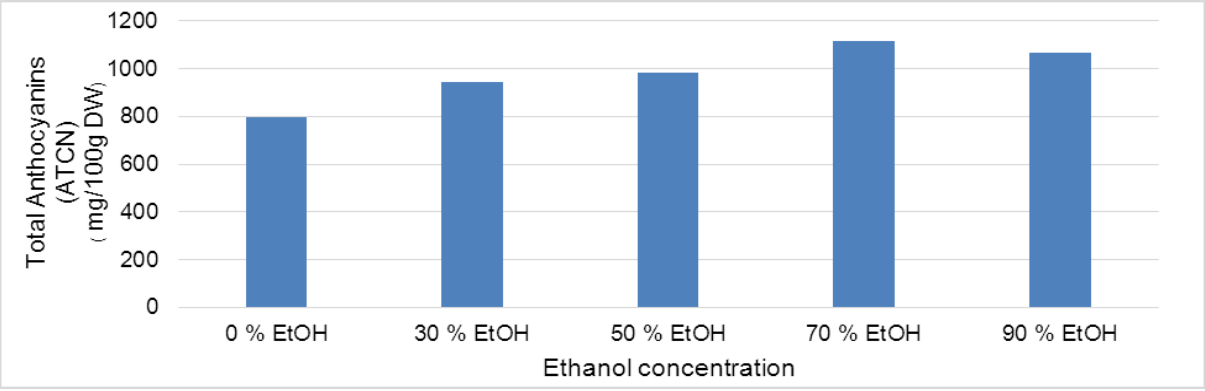

Figure 1. Total monomeric anthocyanin content)mg anthocyanins/100 g dry weight(of JPF extracts.

Effect of ethanol concentration on total phenolics content in Java plum (Syzygium cumini L.) fruits.

Measurement of the TPC in JPF extracts showed that the increase of ethanol in the extraction solution (from 0% to 70%) resulted to increase TPC of the extracts but TPC of the extracts decrease at 90% of ethanol (Figure 2). The TPC of JPF extracts at 70% ethanol was 1718.29 mg GAE/100g dry weight.

From the literature review, the red grape pomace study show that the solvents containing 70% and 50% ethanol in water leads to extracting of higher content of total anthocyanins, as compared to 10% and 30% ethanol, and to methanolic solutions.[5]

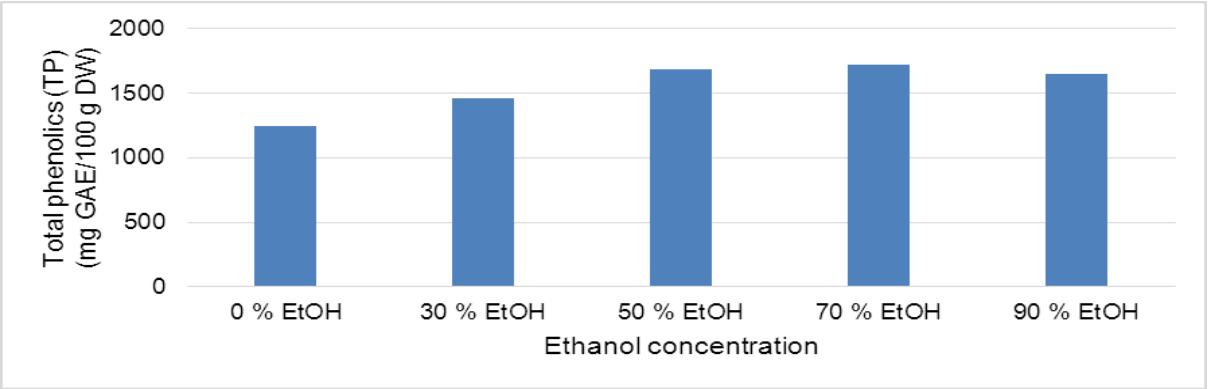

Figure 2. Total phenolics content (TPC))mg gallic acid equivalents (GAE)/100g dry weight.(of JPF extracts

Effect of ethanol concentration on radical scavenging properties in Java plum (Syzygium cumini L.) fruits.

The DPPH radical model has been widely used to valuate the antioxidant activity of fruit and vegetable extract. The method is based on the the reaction that hydrogen-donating antioxidants reduce violet DPPH free radical to yellow DPHH-H, a non-radical form.[4] The reduce amount of DPPH absorption at 517 nm indicated the radical-scavenging ability of antioxidants.

Figure 3 displays DPPH radical-scavenging activity of the anthocyanins from of JPF extracts. The results show that the radical-scavenging activities of antioxidants increased with the increment of ethanol concentrations (from 0% to 70%) but the free radical scavenging activity of the extracts decrease at 90% of ethanol. The free radical scavenging activity(IC_{50}) of JPF extracts at 70% ethanol was 0.46 mg/ml.

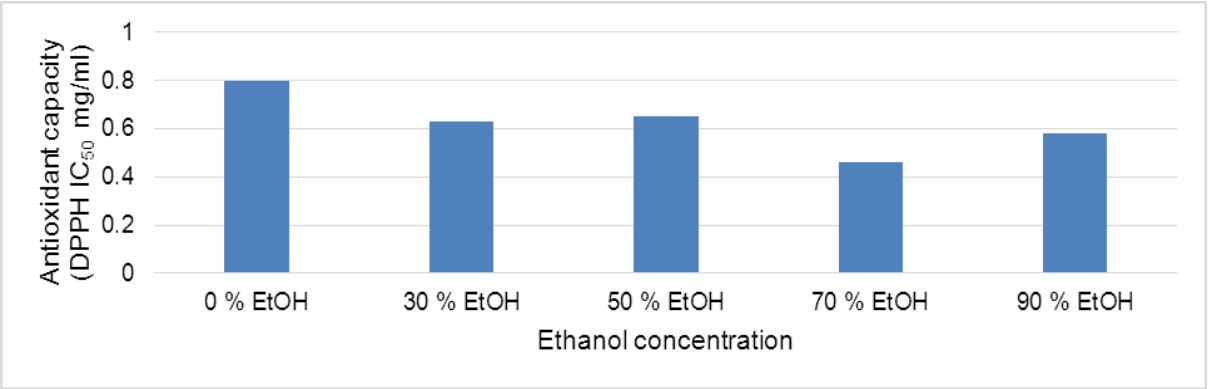

Figure 3. The free radical scavenging activity, IC_{50})mg/ml) of JPF extract

Conclusion

The performed studies indicate that Java plum *(Syzygium cumini L.)* fruits are a rich source of anthocyanins , phenolic compounds, and also possess a significant antioxidant activity. The best results regarding anthocyanins content, total phenolics content and antioxidant activity were obtained at extraction with 70% ethanol.

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