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Effect of Acidification and Types of Solvent on Anthocyanin Yield, Total Phenols, Flavonoids, Antioxidant Activity and Colour Values of Extracts from Mangosteen Pericarp (*Garcinia mangostana* L.)

G.S. Aparna[1], P.R. Geetha Lekshmi[1]

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ABSTRACT

Background: Anthocyanin is a natural pigment with potential application in the textile, pharmaceutical, cosmetic and food industries. Mangosteen (*Garcinia mangostana* L.), the 'Queen of fruits' is famous for its nutritious edible aril. The mangosteen pericarp is rich in anthocyanin and can be utilized as a natural colorant with health benefits. There is a need to standardize easier methods for the efficient extraction of anthocyanin from mangosteen pericarp so that it can be effectively utilized in food industry.

Materials: The pericarp of ripe mangosteen fruits were dried, pulverized and the anthocyanin was extracted by hot maceration with distilled water (aqueous extraction) and ethanol as solvents, acidified at different levels with citric acid (0.1% and 0.2%) and acetic acid (1% and 2%). The anthocyanin yield, total phenol, flavonoid, antioxidant activity and colour values of the concentrated extracts were determined.

Result: Acidification of the solvent medium increased the extraction efficiency of anthocyanin pigment from mangosteen pericarp. The hot maceration with 50% ethanol acidified at 2% acetic acid contributed the highest yield for anthocyanin, total phenols and flavonoids with 82.68% antioxidant activity and exhibited good colour values. This anthocyanin pigment can be utilized as a safe natural colorant for the development of functional foods.

Key words: Acetic acid, Anthocyanin, Antioxidant activity, Mangosteen pericarp, Solvent extraction.

INTRODUCTION

Plants are natural sources of antioxidants and pigments of which anthocyanins are water soluble that give red, blue and purple colour to various fruits like blueberries, blackberries, mangosteen, grapes, vegetables and flowers. Fruit peel fractions which are often wasted from consumption and food industry are rich sources of antioxidants and pigments. Mangosteen (*Garcinia mangostana* L.), an important tropical fruit of Clusiaceae family popularly known as 'Queen of Fruits' is cultivated in India and throughout the Asian countries for its nutritious arils rich in vitamins, minerals and bioactive compounds (Rohman, 2019). The mangosteen pericarp or peel changes its colour during fruit development and reaches reddish-purple colour at optimum maturity and comprises about two times the edible aril portion (Zarena and Sankar, 2012a) resulting in a high amount of biowaste. Mangosteen pericarp is rich in anthocyanins, versatile natural pigment, not only give attractive colour to food products but also enriches them with bioactive compounds and had higher antioxidant activity than the pulp (Nacsk *et al*., 2011). It is rich in flavonoids and phenols contributing to its high antioxidant activity and the extracts could be used in pharmaceutical and cosmetic industries (Lourith and Kanlayavattanakul, 2011) and has much potential application as a natural ingredient in food industries (Hiranrangsee *et al.,* 2016). Mangosteen peel extract is traditionally used to treat urinary tract and gastrointestinal infections (Ovalle-Magallanes *et al*., 2017), inflammatory and immunological diseases (Ming-Hui *et al*., 2017) and has immense potential in the functional food industry. The solvent extraction method is the most popular one to separate bioactive compounds from plant parts. Different solvents such as ethyl acetate, ethanol, methanol, acetone and hexane were used to efficiently extract the -bioactive compounds from mangosteen peel (Suttirak and Manurakchinakorn, 2014). The extraction of natural food colorants with antioxidant activity from mangosteen pericarp should be done using a safe solvent with maximum

[1]Department of Post Harvest Technology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram-695 522, Kerala, India.

Corresponding Author: G.S. Aparna, Department of Post Harvest Technology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram-695 522, Kerala, India.
Email: aparna.gs3@gmail.com

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efficiency. Hence the present study aimed to standardize easier methods for efficient extraction of anthocyanin from the mangosteen pericarp with antioxidant activity so that it can be effectively utilized for the development of functional foods by the food processing industries.

MATERIALS AND METHODS

Extraction of anthocyanin

Mangosteen fruits of optimum commercial maturity at stage VI (Razila and Ramli, 2021) were collected from orchards of the Pathanamthitta district of Kerala, India. The experiment was conducted at the Department of Post Harvest Technology of College of Agriculture, Vellayani during august 2021. The harvested fruits without any physical damage, free from pests, diseases and physiological disorders were washed thoroughly in distilled water. The cleaned fruits were sanitized by ozonation (2 ppm for 5 minutes). The pulp and pericarp were separated manually and the pericarp were sliced into 1 to 2 cm^2 size and dried at 50°C using a cross-flow hot air drier till it attained a constant weight with moisture content less than 7%. The dried pericarp was ground to fine powder in a mechanical blender and packed in laminated aluminium pouches and stored at -20°C prior to the extraction. The anthocyanin pigment from the powdered pericarp samples were extracted using solid- liquid extraction method with distilled water (aqueous extraction) and ethanol (50% V/V) as solvent, acidified using citric acid (0.1% and 0.2%) and acetic acid (1 and 2%). Experiment treatments were T_1- Ethanol with 0.1%, Citric acid, T_2- Ethanol with 0.2% Citric acid, T_3- Ethanol with 1% Acetic acid, T_4- Ethanol with 2% Acetic acid, T_5- Aqueous extraction (distilled water) with 0.1% Citric acid, T_6- Aqueous extraction with 0.2% Citric acid, T_7- Aqueous extraction with 1% Acetic acid, T_8- Aqueous extraction with 2% Acetic acid, T_9- Ethanol (50% V/V), T_{10}- Aqueous extraction (distilled water). The ratio of solid to solvent was maintained at 1:10 and hot maceration extraction was done at 50°C for 1 hour. The solid particles from the liquid extract were separated using filter paper and the filtrate was concentrated to dryness using rotary vacuum evaporator (Heidolph) at 60°C. The extracts were stored under refrigeration at 5-7°C for further analysis.

Extraction yield

The extraction yield was calculated as percentage using following formula (Ho *et al*., 2011):

$$\text{Extraction yield (\%)} = \frac{\text{Weight of concentrated extract (g)}}{\text{Weight of pericarp powder taken for extraction (g)}} \times 100$$

The pH of uniformly concentrated extracts of anthocyanin was measured by using pocket pH tester (Hanna instruments, pHep Tester).

Total anthocyanin content (mg 100 g^{-1})

Total anthocyanin content was determined with the method of Ranganna (1997). Anthocyaninn was extracted with ethanolic HCl and measurement of colour at a wavelength of 535 nm against blank of ethanolic-HCl using a UV spectrophotometer. The values are expressed as mg anthocyanin per 100 mg.

$$\text{Total OD/100 g} = \frac{\text{OD} \times \text{Volume made up} \times 100 \times \text{Dilution factor}}{\text{Weight of sample}}$$

$$\text{Total anthocyanin (mg/100 g)} = \frac{\text{(Total OD/100 g)}}{98.2}$$

Total phenols (mg GAE100 g^{-1})

Total phenol content was estimated by using the method described by Sadasivam and Manickam (1992). One gram of the sample was extracted with 10 times the volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water (5 mL). Aliquot (0.5 mL) was taken in a test tube and made up the volume to 3 mL with distilled water followed by addition of 0.5 mL Folin- Ciocalteau reagent and 20 per cent Na_2CO_3 (2 mL) was added after 3 minutes and mixed thoroughly. The test tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 765 nm against the reagent blank. Standard curve using different concentrations of gallic acid was prepared and phenol content of the test sample was expressed as mg phenols 100 g^{-1} sample.

Total flavonoids (µg QE g^{-1})

Total flavonoid content of the fruit extracts was determined according to the colorimetric assay described by Quettier-Deleu *et al.* (2000). Rind extract (1 mL) was mixed with 4 mL of distilled water which was followed by addition of 0.3 mL of (5% w/v) $NaNO_2$. After 5 min, 0.3 mL of (10% w/v) $AlCl_3$ was added. After 6 min, 2 mL of 1 M NaOH was added and the volume was made up to 10 mL immediately by the addition of 2.4 mL distilled water. The solution was mixed vigorously and the absorbance of the solution was measured at a wavelength of 510 nm. The result was expressed in µg Quercetin equivalent/g in sample by comparison with the quercetin standard curve, which was made under the same condition.

Antioxidant activity (%)

Total antioxidant activity of anthocyanin extract was determined using 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The scavenging effect on DPPH free radical was measured according to the procedure described by Shen *et al.* (2010) and was expressed as per cent DPPH as shown in the following equation:

$$\text{\% inhibition of DPPH} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where,

A_0= Absorbance of DPPH solution without sample.

A_1= Absorbance of the test sample after 30 minutes.

Colour analysis

Colorimetric data were taken using a Hunter Lab Color Flex EZ (USA). After standardizing the instrument with different coloured tiles, the samples were placed in a transparent quartz container and reading where recorded in triplicates. The color values were express as CIE Lab* coordinates. L*, a* and b* attributes were directly determined by HunterLab colorimeter. Chroma (C*) and hue angle (H^0) were calculated from a*, b* values with the formulas $(a^{*2}+ b^{*2})^{1/2}$ and tan inverse (b*/a*) respectively.

Statistical analysis

One way analysis of variance with Duncan's multiple range test applied using agricolae package in R Studio (version 1.3-5 2021.06.2-06).

RESULTS AND DISCUSSION

Concentrated anthocyanin extracts exhibited characteristic dark red colour and biochemical properties of the extracts were also analyzed. As the anthocyanins are stable in acidic medium, the extraction was performed in acidified solvent using citric acid and acetic acid under the optimal condition and the pH values of the extracts ranged from 3.60 to 5. The highest yield (28.57%) was observed for the treatment T_4 (Ethanol with 2% acetic acid) which was statistically superior over all other treatments. The lowest (13.94%) yield was observed in the aqueous extraction (T_{10}) where distilled water is used as the solvent without acidification (Fig 1).

The total anthocyanin content of mangosteen rind extracts varied significantly with different levels of acidification and type of solvents and it varied from 132.43 to 294.73 mg 100 g^{-1} among the treatments. The extraction with ethanol acidified at 2% acetic acid (T_4) showed significantly higher total anthocyanin content of 294.73 mg 100 g^{-1}. Anthocyanin extracted with ethanol acidified using acetic acid exhibited higher anthocyanin content than ethanol with citric acid. Similar reports have been published by researchers on different plants (Gerardi *et al*., 2015; Espinosa-Acosta *et al*., 2018). Acetic acid is a weak acid and it will not damage anthocyanin even during evaporation. The Aaqueous extracts showed significantly lower total anthocyanin content compared to ethanol (50%v/v) extracts. The anthocyanin content extracted from fresh and dried mangosteen pericarp was reported as 23.54±0.31 and 20.83±0.96 mg Cyn-3-Glu 100 g^{-1}, respectively by Hiranrangsee *et al.* (2016).

The total phenol content, total flavonoids and antioxidant activity in terms of DPPH radical scavenging assay of anthocyanin extract from mangosteen rind in different solvent systems are depicted in Table 1. The ethanol extracts exhibited higher phenols, flavonoids and antioxidant activity than the water extracts. Mangosteen peel extract exhibited higher total phenolics, total anthocyanin contents and strongest antioxidant capacity compared to other pigmented plant samples such as *Ardisia colorata, Clitoria ternatea* and *Syzygium cumini* was reported by Azima *et al.* (2017). The extraction using ethanol with 2% acetic acid (T_4) recorded the highest total phenol content of 1549.55 mg GAE 100 g^{-1} and there was no significant difference with treatment T_3 (Ethanol+Acetic acid 1%). Aqueous extract of anthocyanin (T_{10}) recorded the lowest phenolic acid content of 1241 mg GAE 100 g^{-1}. Ethanolic extraction (50%) for an extraction time of 30 min yielded the highest concentration of anthocyanins and total phenols from *Hibiscus sabdariffa* (Roselle) calyces (Ortega and Beltran, 2014).

The highest total flavonoids was recorded as 38.46 µg QE g^{-1} for the extraction treatment T_4 (Ethanol+2% Acetic acid) which was followed T_2 (Ethanol+ 0.2% Citric acid) with 36.30 µg QE g^{-1}. Anthocyanin extracted with distilled water without acidification (T_{10}) recorded the lowest total flavonoid content of 22.353 µg QE g^{-1} Ngawhirunpat *et al.* (2010) reported that water extract of mangosteen hull recorded the

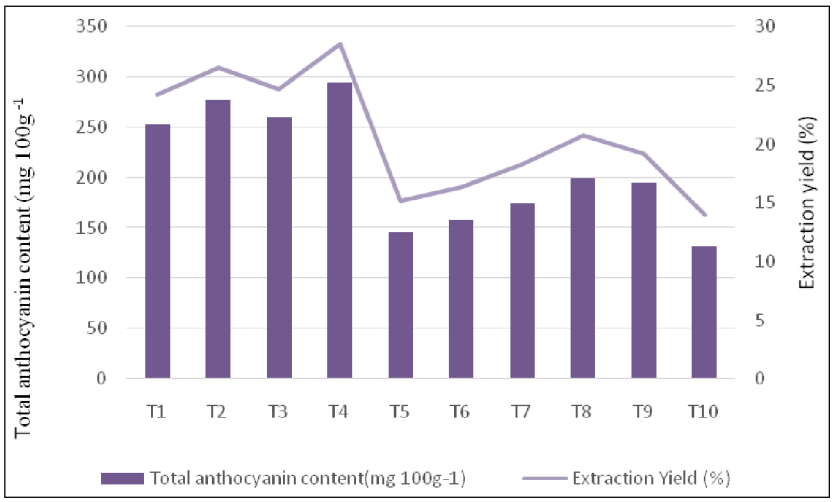


Fig 1: Total anthocyanin content and extract yield of anthocyanin extracts of mangosteen pericarp.

highest total flavonoid when compared with methanol and hexane extract.

Among the anthocyanin extracts the highest antioxidant activity of 82.68% was also observed for the acidified (2% Acetic acid) ethanolic extraction, the treatment T_4. The antioxidant activity of mangosteen extracts is associated with the bioactive compounds present, mainly phenolics, because of their ability to scavenge free radicals (Zarena and Sankar, 2012b). A correlation study conducted between total phenolic content and antioxidant activity of mangosteen peel revealed that the samples with high total phenolic content exhibited higher antioxidant activity (Amin and Lee, 2005). Fugal *et al*. (2006) studied colour extraction from mangosteen pericarp and reported that ethanol (50%) extracted colour recorded the good antioxidant activity mainly due to the presence of xanthones.

L*, a* and b* attributes were directly determined by Hunter Lab colorimeter and C* (Chroma) and H° (hue angle) were calculated subsequently (Table 2) and values were found significantly different statistically. The L* value for the treatments varied from 9.53 to 25.44 and the pigment extracts with ethanol as solvent, were expressed with darker colour and lower Lightness (L*) values compared to water extracts. The a* and b* colour coordinates regulate the chroma (color intensity) and Hue angle (H°) of a sample and the values in the range of 0 to 60 yield shades of red to yellow or 300 to 360 which have the shades of pink to red as described by (Torskangerpoll and Anderson (2005). Hue° values of the anthocyanin extracts obtained from mangosteen peel using different solvent system ranged from 24.16 to 37.74 which indicated that all were in the red region (Table 2). The present finding is in line with the reports by Yenrina *et al.* (2016) where Hue values are grouped according to colour and Hue° of 18 to 54 come in the red region.

The color intensity (chroma) presented values closer to 100 indicates pure color (depth of the color) (Netravathi *et al.,* 2022). Chroma values of mangosteen peel extracts varied from 17.06 to 34.30 in different solvent systems where the treatment T_4 (Ethanol with 2% Acetic acid) recorded a chroma value of 22.72. A red sample with varied dilution strengths from pink to red will have the same hue angle but higher chroma values. Chroma increases with pigment concentration to a maximum and then decreases as the colour darkens as reported by Wrolstad (2005).

Table 1: Total phenols, total flavonoids and antioxidant activity of anthocyanins extracts from mangosteen pericarp.

Treatments	Total phenols (mgGAE 100 g^{-1})	Total flavonoids (µg QE g^{-1})	Antioxidant activity (%) inhibition
T_1- Ethanol+Citric acid (0.1%)	1500.49^{c}	34.11^{d}	80.10^{bc}
T_2- Ethanol+Citric acid (0.2%)	1538.23^{b}	36.30^{b}	80.57^{ac}
T_3- Ethanol+Acetic acid (1%)	1540.94^{ab}	35.40^{c}	81.13^{ab}
T_4- Ethanol+Acetic acid (2%)	1549.55^{a}	38.46^{a}	82.68^{a}
T_5- Distilled water+Citric acid (0.1%)	1316.45^{h}	23.56^{h}	73.47^{ef}
T_6- Distilled water+Citric acid (0.2%)	1357.34^{g}	25.82^{g}	75.85^{d}
T_7- Distilled water+Acetic acid (1%)	1388.68^{f}	25.16^{g}	75.15^{de}
T_8- Distilled water+Acetic acid (2%)	1401.0^{e}	29.05^{f}	76.67^{d}
T_9- Ethanol (50%)	1443.43^{d}	32.08^{e}	78.78^{c}
T_{10}- Distilled water	1241.12^{i}	22.35^{i}	72.42^{f}

Values with the same letter in each column are not significantly different ($p<0.05$), as separated by Duncan's multiple range test.

Table 2: L*, chroma and hue angle (H°) values of anthocyanin extracts from mangosteen pericarp.

Treatments	Lightness (L*)	Chroma*	Hue angle (H^0)
T_1- Ethanol+Citric acid (0.1%)	16.42 ± 1.04^{de}	33.08 ± 1.91^{ab}	27.59 ± 2.04^{d}
T_2- Ethanol+Citric acid (0.2%)	12.02 ± 2.60^{f}	30.54 ± 2.60^{c}	25.63 ± 1.37^{ef}
T_3- Ethanol+Acetic acid (1%)	10.65 ± 0.92^{fg}	22.23 ± 1.10^{e}	26.16 ± 0.99^{de}
T_4- Ethanol+Acetic acid (2%)	9.53 ± 1.01^{g}	22.72 ± 1.52^{e}	24.16 ± 0.85^{f}
T_5- Distilled water+Citric acid (0.1%)	19.42 ± 1.58^{c}	34.30 ± 1.28^{a}	37.74 ± 1.04^{b}
T_6- Distilled water+Citric acid (0.2%)	18.02 ± 0.92^{cd}	11.01 ± 1.52^{h}	36.47 ± 0.60^{b}
T_7- Distilled water+Acetic acid (1%)	19.58 ± 2.19^{c}	17.06 ± 1.43^{g}	37.19 ± 0.62^{b}
T_8- Distilled water+Acetic acid (2%)	15.74 ± 0.93^{e}	19.39 ± 2.06^{f}	33.35 ± 1.00^{c}
T_9- Ethanol (50%)	23.01 ± 0.89^{b}	31.62 ± 1.31^{bc}	34.69 ± 2.19^{c}
T_{10}- Distilled water	25.44 ± 1.10^{a}	28.44 ± 3.02^{d}	40.67 ± 1.02^{a}

Values with the same letter in each column are not significantly different ($p<0.05$), as separated by Duncan's multiple range test.

CONCLUSION

The study was conducted with the objective to standardize easier methods for efficient extraction of anthocyanin from the mangosteen pericarp so that it can be effectively utilized as food colourant with antioxidant activity for the development of functional foods. The anthocyanin was extracted from dried and powdered pericarp of ripe mangosteen fruits by hot maceration with distilled water (aqueous extraction) and ethanol (50% V/V) as solvents, acidified at different levels with citric acid (0.1% and 0.2%) and acetic acid (1% and 2%). The ratio of solid to solvent was maintained at 1:10 and extraction was done at 50°C for one hour. Based on yield, anthocyanin content, total phenols, flavonoids, antioxidant activity of the extracts, anthocyanin extracted with ethanol acidified using 2% acetic acid was recorded as the best solvent system and can be further utilized as a natural food colorant.

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Conflict of interest: None.

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