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Extraction of anthocyanin pigment from grape pomace and estimation of its functional properties

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Abstract
Anthocyanins are subgroup of flavonoid and they are responsible for wide range of colours including blue, purple, violet, magenta, red, and orange of many fruits, flowers and vegetables. These anthocyanins can be used as a natural food colourants and has various health benefits such as prevention of cardiovascular disease, anticancer, antimutagenic activity. In this study, the anthocyanins from grape (*Vitis vinifera*) pomace was extracted using ethanol (50, 60 and 70%) with acidified water (0.5-2% lactic acid). The extracted anthocyanin was evaluated for its total polyphenolic content and total monomeric anthocyanin content. The result showed that acidified ethanol with 2% lactic acid yielded higher phenolic content than extraction with only ethanol solvent and maximum anthocyanin content was obtained from the 2% acidified water with ethanol of 60%. Thus, the acidified solvent can be chosen as the best method for extracting maximum anthocyanin from grape pomace. The extracted anthocyanin can be utilized as natural colourant in various food products and also as an indicator in the packaging field.

Keywords: Anthocyanins, natural colourants, ethanol, acidified water, total polyphenolic content, total monomeric anthocyanin content

1. Introduction
The study of natural colorants is an immense and vital area of research due to increase in substituting synthetic colorants which have toxic effects in humans (Chou *et al.* 2007). Anthocyanins and carotenoids are amongst the most utilized natural colorants in the food industry (International Food Information Council and Foundation US Food and Drug Administration, 2004).

The anthocyanin is derived from Greek words: “anthos” meaning flower and “kyanos” meaning blue. Anthocyanins belong to the flavonoid group because of their characteristic $C_6C_3C_6$ carbon skeleton. Anthocyanins represent the largest and the most important group of water-soluble natural pigments (Takeoka & Dao 2002) [11]. Anthocyanins are responsible for the purple, vivid blue and red color of many vegetables, fruits, and flowers (Andersen & Jordheim 2008). Anthocyanin has high potential to act as a natural food colorant to reinstate synthetic colorant (Aishah *et al*., 2013) [2].

Grapes (*Vitis vinifera* L.) are rich sources of phenolic compounds which includes flavonoids and non-flavonoids. The most abundant classes of flavonoids include the flavan-3-ols, anthocyanins and flavonols, while the non-flavonoid compound is the hydroxycinnamates (Crecelius *et al*., 2000) [6]. The most abundant anthocyanins in grapes are the glucoside forms of cyanidin (Cy), malvidin (Mv), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and pelargonidin (Pg).

Productive extraction techniques should maximize the recovery of anthocyanin and minimize the amount of adjuncts and decrease the degradation or change of the natural state of colorants. Anthocyanins are soluble in the polar solvents and generally extracted by the aqueous mixtures of some organic solvents including methanol, acetone or ethanol (Kano *et al*., 2005) [7]. Miserably, some of the solvents used (e, g. methanol/acetone) may induce toxic effect to the human health (Patil *et al*., 2009) [10]. The aim of this study is to extract anthocyanins from grape skin using ethanol as a solvent with acidified water. Also, the extracted anthocyanins were evaluated for its total polyphenolic content and total monomeric anthocyanin content to estimate their capability to act as a natural colorant.

2. Materials and Methods

2.1 Materials

2.1.1 Sources of raw materials

Grape skin (*Vitis vinifera*) purchased from local market, Chennai was identified as possible anthocyanin sources and used for the study.

2.1.2 Chemicals

Double distilled water, ethanol (AR 99%), Hydrochloric acid (LR 35%), Sodium carbonate (MW -105.99 g/mol), Folin's reagent, Potassium chloride (MW-74.55 g/mol), and Sodium acetate (MW- 82.03 g/mol). All the chemicals used in the preparation of different reagents were of analytical grade (AR) and were procured from standard approved companies.

2.2 Methods

2.2.1 Physicochemical analysis of grapes

The total soluble solids content (TSS) of grape skin was measured with an Atago type refractometer and the values were expressed as degree °Brix at 25 °C. pH was determined with Thermo electron corporation pH meter (Model, ORION 2 STAR) calibrated with pH 4 and 7 buffers.

2.2.2 Estimation of Total polyphenolic content

Total polyphenolic content was estimated by Folin ciocalteau method (Chun *et al*., 2003) [5].

2.2.3 Estimation of Total Monomeric Anthocyanin (TMA)

Potassium chloride and Sodium acetate were used for estimating TMA at 520 nm and 700 nm using UV-Visible spectrophotometer.

3. Result and Discussion

3.1 Effect of solvents on the extraction of TPC and TMA from grape skin by acidified ethanol extraction method

Extraction efficacy of ethanol solvent with acidified water containing lactic acid was compared for total phenolic content and total monomeric anthocyanin in the crude extract of grape skin extract. As shown in table.2 total phenolic content and total monomeric anthocyanin using ethanol acidified solvent had higher affinity of extraction than with only ethanol solvent.

The total phenolic content of grape skin extract had high significant yield due to acidified solvents which degraded the cell membrane and dissolved phenolic components and stabilized them simultaneously. Compared to different treatments, acidified ethanol (70% ethanol + 2% lactic acid) extracted total phenolic content of 2473.03 mg GAE/100g from grape skin extract using acidified ethanol (70% ethanol + 2% lactic acid) compared to other treatments. The TPC was higher than the result presented by Carrera *et al*. (2011) who reported TPC values of 1287 mg GAE/100g for grape skin during ripening.

The TMA content of grape skin extract increased with increase in lactic acid concentration. Table.2 shows that treatment T_{12} extracted more anthocyanin content than other combinations. A significant increase of TMA content (411.34mg/100g) was observed in grape skin extract and this was higher than the result obtained by Paun *et al*. (2022) who reported a TMA content of 168.26mg C3G/100g in their study using 50% ethanol and 0.01% HCl. It was observed that there was gradual increase in the extraction of anthocyanins from T_1 to T_{12}. This could be due to the lactic acid that stabilizes the pigments as obtained by

Patil *et al*. (2009) [10]. However, the anthocyanin content decreased grape skin extract when the ethanol and lactic acid concentration is high. The decrease in the anthocyanin content was due to acylated anthocyanins which might be degraded (hydrolysis reaction) and in case of 3-monoside anthocyanins the glycoside bonds could be destroyed (Kapasakalidis *et al*., 2006) [8]

Acidification with lactic acid helps to maintain a low pH, which makes it easier to produce flavylium chloride salts from simple anthocyanins which increase the effectiveness of anthocyanin extractions. The flavylium cation form of anthocyanins is usually extracted under cold conditions with methanol or ethanol containing a tiny amount of acid, with the goal of getting the red colour stable in a highly acid media flavylium cation form as deliberated by

(Arab *et al.*, 2011) [1].

3.2 Effects of solvents on physico-chemical properties of grape skin extract by acidified ethanol solvent method

Physicochemical properties such as pH and TSS of grape skin extract were determined and the results are given in table 3. There was a highly statistical difference in the pH and TSS of grape skin extract as the lactic acid concentration increased. It was observed that the pH was gradually decreasing from 5.79 to 1.61 for grape skin extract. This was due to the increase in the lactic acid concentration in the acidified solvent. This was in accordance to the findings by Mohamed *et al.* (2016) who also reported that the pH decreased on increasing the acid concentration.

The TSS of the grape skin extract (8.50 °Brix) increased gradually on increasing the ethanol and lactic acid concentration. There was high statistical difference in the TSS of extract. The acidified solvents degraded the cell membrane and dissolve soluble solids into the extract thereby, increasing the TSS content.

Table 1: Treatment combinations of the solvents

Treatments	Solvent ratio
T1	70% Ethanol
T2	50% Ethanol + 0.5% lactic acid
T3	60% Ethanol + 0.5% lactic acid
T4	70% Ethanol + 0.5% lactic acid
T5	50% Ethanol + 1% lactic acid
T6	60% Ethanol + 1% lactic acid
T7	70% Ethanol + 1% lactic acid
T8	50% Ethanol + 1.5% lactic acid
T9	60% Ethanol + 1.5% lactic acid
T10	70% Ethanol + 1.5% lactic acid
T11	50% Ethanol + 2% lactic acid
T12	60% Ethanol + 2% lactic acid
T13	70% Ethanol + 2% lactic acid

Table 2: TPC and TMA of grape skin (*Vitis vinifera*)

Treatment	TPC (mg GAE/100g)	TMA (mg/100g)
T_1	$892.77^{a} \pm 0.22$	$219.34^{a} \pm 0.24$
T_2	$936.23^{b} \pm 0.30$	$230.39^{b} \pm 0.21$
T_3	$990.19^{c} \pm 0.26$	$239.14^{c} \pm 0.20$
T_4	$1095.75^{d} \pm 0.21$	$253.39^{d} \pm 0.23$
T_5	$1177.00^{e} \pm 0.03$	$278.38^{e} \pm 0.23$
T_6	$1647.09^{f} \pm 0.05$	$283.34^{f} \pm 0.15$
T_7	$1717.42^{g} \pm 0.20$	$304.32^{g} \pm 0.22$
T_8	$2006.54^{h} \pm 0.14$	$328.32^{h} \pm 0.19$
T_9	$2065.54^{i} \pm 0.32$	$337.41^{i} \pm 0.22$
T_{10}	$2220.92^{j} \pm 0.14$	$347.52^{j} \pm 0.23$
T_{11}	$2382.55^{k} \pm 0.27$	$364.18^{k} \pm 0.22$
T_{12}	$2438.55^{l} \pm 0.29$	$411.34^{m} \pm 0.25$
T_{13}	$2473.03^{m} \pm 0.10$	$397.95^{l} \pm 0.27$
F Value	7757440.67**	78163.82**

@Average of six trials
**Statistically highly significant ($P \leq 0.01$)
Means bearing various superscripts in the same column differs highly significantly ($P \leq 0.01$).
TPC- Total Phenolic Content, TMA- Total Monomeric Anthocyanin, GAE- Gallic Acid Equivalent

Table 3: Physico-Chemical properties of grape skin (*Vitis vinifera*)

Treatment	pH	TSS
T_1	$5.79^{e} \pm 0.01$	$8.50^{a} \pm 0.008$
T_2	$2.81^{d} \pm 0.02$	$9.20^{b} \pm 0.01$
T_3	$2.80^{d} \pm 0.02$	$9.80^{c} \pm 0.01$
T_4	$2.80^{d} \pm 0.02$	$10.20^{d} \pm 0.01$
T_5	$2.06^{c} \pm 0.01$	$10.81^{e} \pm 0.01$
T_6	$2.05^{c} \pm 0.01$	$11.42^{f} \pm 0.01$
T_7	$2.03^{c} \pm 0.01$	$11.61^{g} \pm 0.01$
T_8	$1.75^{b} \pm 0.01$	$11.81^{h} \pm 0.008$
T_9	$1.74^{b} \pm 0.01$	$12.04^{i} \pm 0.01$
T_{10}	$1.73^{b} \pm 0.01$	$12.22^{j} \pm 0.01$
T_{11}	$1.63^{a} \pm 0.01$	$12.43^{k} \pm 0.01$
T_{12}	$1.63^{a} \pm 0.01$	$12.61^{l} \pm 0.009$
T_{13}	$1.61^{a} \pm 0.01$	$12.82^{m} \pm 0.01$
F Value	4523.99**	13141.78**

@Average of six trials
**Statistically highly significant ($P \leq 0.01$)
Means bearing various superscripts in the same column differs highly significantly ($P \leq 0.01$).
TSS- Total Soluble Solids

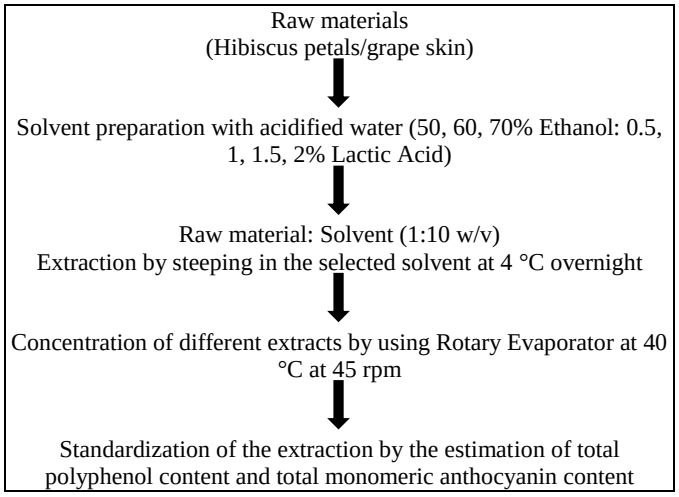

Fig 1: Flow chart for standardization of extraction of crude anthocyanin

4. Conclusion

It could be concluded that adding acid to the extraction solvent has a significant effect on stabilizing anthocyanins, resulting in an improvement in extraction efficiency. The extracted anthocyanin can be used as a natural colourant in several food products and thereby improving their functional properties.

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