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ORIGINAL ARTICLE

Green extraction of anthocyanins from *Syzygium cumini* fruit pulp using aqueous glycerol through ultrasound-assisted extraction

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Abstract

Aqueous glycerol is a proposed green extractant for anthocyanins and polyphenols as an alternative to conventional solvents. The aim of this study was to investigate the potential use of aqueous glycerol to extract anthocyanins from Syzygium cumini fruit pulp with high yields. The ultrasound-assisted extraction process was also examined to enhance the extraction yield. The application of ultrasound-assisted extraction along with glycerol as a modifier remarkably enhanced the extraction of anthocyanins compared to the conventional extraction. Aqueous glycerol (70%) was screened against conventional solvents (ethanol, methanol and water), where glycerol showed promising outcomes. The optimised ultrasonication time and extraction temperature (25 °C for 5 min) were selected based on our previous study of NADESs. The results showed that glycerol gave the highest amount of anthocyanin content (201.05 mg C3G/100 g fresh weight) compared to conventional solvents for retrieving anthocyanins from *S. cumini* fruit pulp. Besides the anthocyanin content, the extract yield, phenolic content, and antioxidant activities using DPPH and FRAP were also determined. Glycerol showed a higher phenolic recovery (0.9 mg GAE/g dry sample), resulting in higher antioxidant activity (DPPH activity-73.35% inhibition). Therefore, the application of UAE with aqueous glycerol provides accessibility and enhanced anthocyanin extraction efficiency, thus fulfilling the green and sustainable approach to anthocyanin extraction.

Keywords *Jamun* · Anthocyanins · Aqueous glycerol · Green extraction · *Syzygium cumini* fruit pulp

Abbreviations

UAE	Ultrasound-assisted extraction
NADESs	Natural deep eutectic solvents
C3G	Cyanidin-3-glucoside
TAC	Total anthocyanin content
TPC	Total phenolic content
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
TPTZ	2,4,6-Tri-pyridyl-s-triazine
TY	Total yield

1 Introduction

There is an increasing interest in using natural food colourants to prepare jams, cakes, juices and other food items. Food products with vibrant colours have an edge on the sensory scale, which plays a critical role in the product's palatability [25]. However, the food industry has been utilising synthetic food colours for a long time due to their stability, efficiency and cost-effectiveness [3]. However, the safety of synthetic food colours has been questioned in recent years. The research for better and safer alternatives has led to the recovery of plant bioactive compounds, a great source of food colours [18]. These plant bioactive pigments include chlorophylls, carotenoids, betacyanins and flavonoids. Anthocyanins, a class of flavonoids, are considered to have a wide colour range and are currently being explored and validated as a promising alternative to synthetic colours [12].

Syzygium cumini (*S. cumini*) is one of the underutilised fruits of the Myrtaceae family cultivated in subtropical and tropical regions [33]. Previous studies indicate that the pulp of *S. cumini* fruit is a rich source of major anthocyanins named malvidin, cyanidin, petunidin, delphinidin, and peonidin [48, 52, 56]. The fruit is also a rich source of antioxidant compounds where the anthocyanins may act similarly [6]. Despite its commercial use in juice processing, there is limited research data on anthocyanin extraction from

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S. cumini [47, 54]. However, it is a trending research topic [17], D. [29], N. [30, 46].

Applying a suitable extraction technique is a crucial factor to consider for enhancing the recovery of bioactive compounds [50]. Conventional extraction techniques have lately been associated with many disadvantages, such as longer extraction times, lower stability, thus higher degradation, and use and consumption of organic solvents [44]. The use of solvents such as methanol for the extraction processes and the extracts are not preferred in food products due to their toxicity, flammability and environmental pollution [15]. These solvents and techniques, therefore, don't align with the concept of "green extraction processes" [26].

Glycerol has been acknowledged as a promising alternative for being a green and high-performing extraction solvent [1]. As glycerol is a by-product of the biodiesel industry, it is readily available due to the high production of biodiesel [35]. It has been identified as one of the green solvents attributed to its biodegradability, natural origin, and safety [55]. Glycerol is also considered a cheaper alternative to conventional solvents. It also lowers the cost of the overall extraction process as the number of unit operations is reduced, for there is no requirement to separate the solvent from the extract [37, 45]. The solvent seems highly attractive because it has a high polarity and boiling point [40]. Glycerol, being highly polar, has the potential for targeted recovery of anthocyanins, where there is minimal chance of other components being extracted. Water/glycerol systems have been considered effective in anthocyanin recovery due to a lowered dielectric constant, aiding in increased pigment diffusion with the solvent [34, 39]. Compared to other organic solvents, such as ethanol and methanol, glycerol is a non-toxic solvent with considerable extraction efficiency [23].

Ultrasonication has been utilised to improve anthocyanin extraction processes in various fruits and waste [14]. Ultrasonication effectively accelerates the chemical reactions through cavitation that causes a structural change internally into the food matrices [13, 57]. Ultrasonic pre-treatments can accelerate extraction, reduce processing times and save energy [21]. On an industrial scale, glycerol can prove to be a safer option due to its lower vapour pressure, and in combination with ultrasonication, it may help in energy-efficient methods [53]. The previous experiments also showed that using ultrasonication with green solvents caused the internal structural changes of food matrices, thus increasing the recovery of anthocyanins and phenols [43]. Yet the research is limited to fully assessing the potential for efficient recovery of anthocyanins utilising energy efficient, time-saving and cost reducing technique and solvent combinations, specifically from the fruit pulp of *S. cumini*.

The present study uses ultrasound-assisted extraction to enhance recovery and screen conventional solvents (water, ethanol and methanol) against aqueous glycerol. A comparative analysis was conducted of the extracts for various parameters such as antioxidant activity, total anthocyanin and phenolic content and total yield. The main objective is to find an alternative green solvent to extract anthocyanins from the *S. cumini* fruit pulp.

2 Materials and methods

2.1 Experimentation material

The fruit of *S. cumini* was collected from a local vendor in the Patiala region of Punjab, India. The extraction solvents glycerol, ethanol and methanol were purchased from the Loba Chemie Pvt. Ltd. company. Distilled water for the extraction was collected from the distillation apparatus setup in the lab. The pulp of *S. cumini* fruit was separated from seeds, dried and powdered proportionately before use. The anthocyanin content and other assays were determined spectrophotometrically using a UV–Vis spectrophotometer (Shimadzu Scientific Instruments, Japan).

2.2 Ultrasound-assisted anthocyanin extraction using glycerol and comparative analysis

The whole process has been summarized in the figure. *S. cumini* dried-powdered pulp was weighed (2 g) and placed in a conical flask (solid-solvent ratio, 1: 20). An amount of solvent (40 ml) was added in accordance. The viscosity of the solvent is a critical factor that directly affects the extraction process. The high viscosity of solvents can hinder the diffusion process, leading to lower recovery of anthocyanin or phenolic compounds [16]. Multiple studies have reported that glycerol is a viscous solvent, so 70% aqueous glycerol was used. Higher concentrations would not extract higher yields of anthocyanins [32, 34, 37, 39]. The extraction temperature plays a critical role in the recovery of anthocyanins. Anthocyanins are temperature-sensitive compounds; thus, a lower temperature yields higher anthocyanins, which may further differ based on solvents or extraction techniques [26, 41]. The temperature (25 °C) for the anthocyanin extraction from the sample was kept constant for all the solvents. The pre-treatment of ultrasonication was given for 5 min, and then extraction was performed for 2 hours on an incubator shaker at the mentioned temperature. A comparative study was designed to investigate the influence of green solvent (glycerol) over conventional solvents (water, ethanol, methanol) in the anthocyanin recovery from the fruit pulp of *S. cumini*. The

extracts were then collected and filtered, where filtrate was used to perform further assay procedures.

2.3 Evaluating extract yield

To calculate the extract yield of the sample, the extracts were dried in a hot-air oven to free the extract from the solvent. The total yield (TY) was calculated using the dried crude extract using the formula given in Eq. 1. And expressed as a percentage (%). All measurements were conducted in triplicates.

$$\mathrm{TY}(\%\mathrm{db}) = \frac{\text{extract weight} \times 100}{\text{weight of sample}}$$

2.4 Determining total phenolic content (TPC)

The total phenolic content was determined using the Folin–Ciocalteau method Swer et al. (2018) adopted with slight modifications. To 0.2 ml diluted sample, 2.5 ml of Folin–Ciocalteau reagent was added and incubated for 5 min at room temperature. Then 2 ml sodium carbonate (7.5 g/100 ml) was added, mixed and incubated at room temperature for 2 h. After incubation, the mixture was shaken, and the absorbance was measured at 754 nm against distilled water as blank in UV–Vis spectrophotometer.

The results were calculated based on the calibration curve of gallic acid, and the results were expressed as Gallic acid equivalents (mg GAE/100 g).

2.5 Total anthocyanin content (TAC)

The total anthocyanin content of the *S. cumini* anthocyanins was determined spectrophotometrically using the method given by Giusti & Wrolstad [19] with slight modifications. The aqueous aliquots of test samples were separately diluted with potassium chloride buffer (pH 4.5) and sodium acetate buffer (pH 1.0) and allowed to equilibrate for 1 h at room temperature. The absorbance was measured simultaneously at 530 and 700 nm against a blank (distilled water). The total anthocyanin content expressed as cyanidin-3-glucoside was calculated using the following equation:

$$\mathrm{AC}(\text{mg C3G/L}) = \frac{\mathrm{A} \times \mathrm{M.W.} \times \mathrm{DF} \times 1000}{\varepsilon \times \mathrm{L}}$$

where, A is the absorbance which is finalised after finding the difference in absorbances at 530 and 700 nm in different buffers, M.W. is the molecular weight of cyanidin-3-glucosides (449.2 g/mol), DF is the dilution factor, ε is the molar absorptivity of cyanidin-3-glucoside (26,900 L/cm mol), and 1 is the cuvette path length (in cm).

2.6 DPPH antioxidant assay

DPPH radical scavenging assay was performed following the method of Brand-Williams et al. [11]. An aliquot of the extract solution was diluted 1:4 times before estimation to obtain a reading within the spectrophotometer's linear range. Briefly, 0.1 ml of the diluted sample was treated with 3.9 ml of 0.1 mm methanolic DPPH solution and allowed to stand for 30 min in the dark at 37 °C. The absorbance was recorded at 517 nm immediately against methanol as blank. Per cent inhibition of the DPPH radical by the sample was calculated using the following equation:

$$\text{DPPH radical scavenging}(\%) = \frac{A_{\text{blank}} - A_{\text{sample}} \times 100}{A_{\text{blank}}}$$

where, A_{sample} = absorbance of DPPH on reaction with the sample extract, A_{blank} = absorbance of DPPH with methanol as blank instead of sample.

2.7 FRAP antioxidant assay

The ferric reducing antioxidant power (FRAP) assay was performed according to the method given by [9] with minor modifications. The sample extract was diluted 1:100 times with distilled water to obtain a reading within the linear range of the spectrophotometer at 593 nm. Briefly, 0.1 ml of the diluted sample was added to 3 ml of FRAP reagent consisting of acetate buffer (300 mM, pH 3.6), 2,4,6-Tripyridyl-s-Triazine-TPTZ (0.031 mg in 10 ml 40 mM HCl) and ferric chloride (20 mM) in the ratio of 10:1:1. After 4 min the absorbance was recorded at 593 nm against FRAP as blank. The results of absorbance values directly indicated the sample's antioxidant activity.

All the experiments were conducted with three replications and the results reported are mean values recorded (Fig. 1).

3 Results and discussion

3.1 Total extraction yield

The pulp of *S. cumini* fruit was subjected to four different solvent treatments with varying extraction yields. The yields of extracts using the different solvents are indicated in Fig. 2. Among the solvents used, the maximum extraction yield of 31.9% was obtained for glycerol. The results show that the extract yield is influenced by the solvent adopted for the extraction. The water/glycerol mixture, under similar conditions applied for other solvents, was able to solubilise a higher amount of pigment, leading to a higher extraction

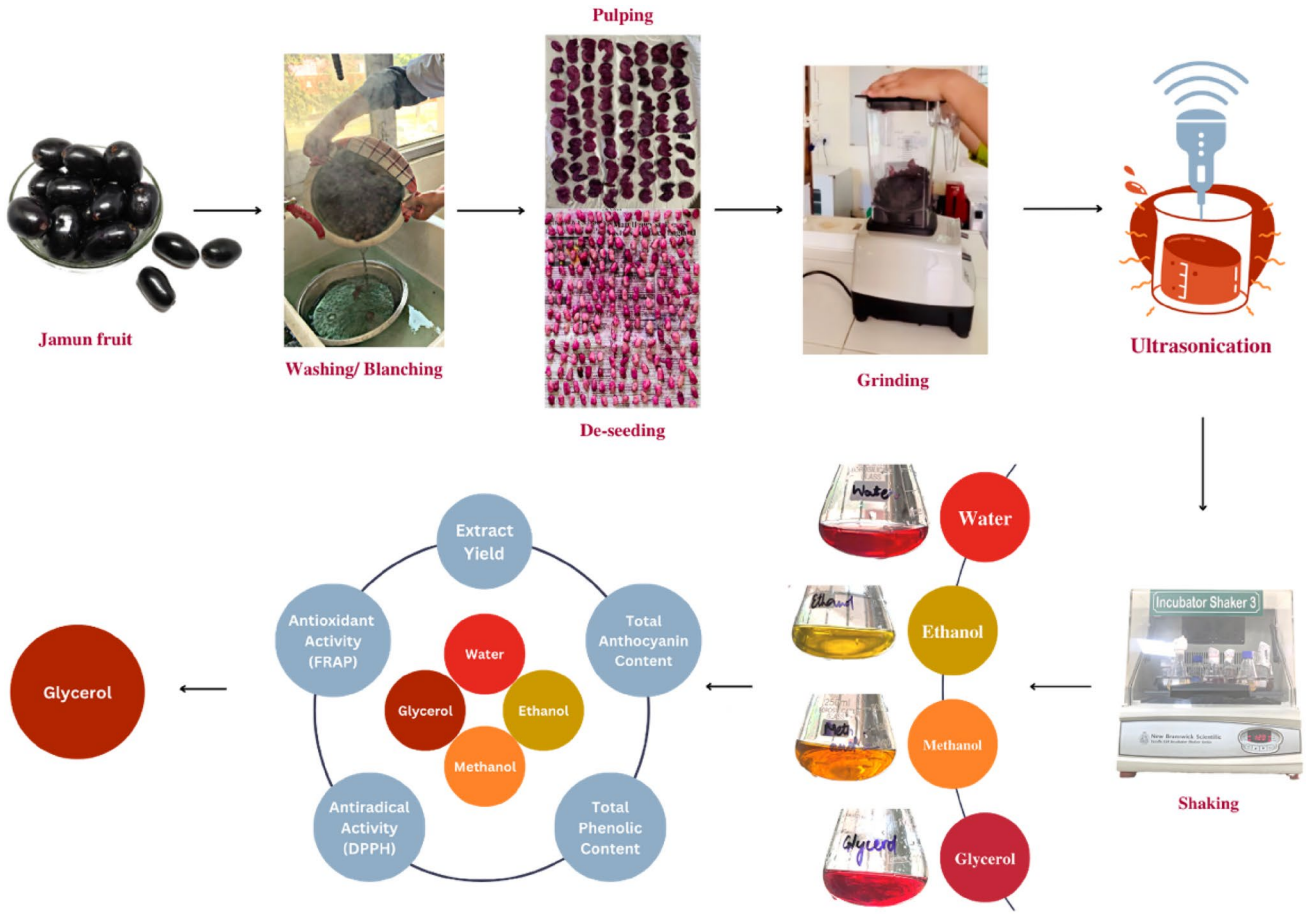

Fig. 1 Extraction of anthocyanins from *Syzygium cumini*

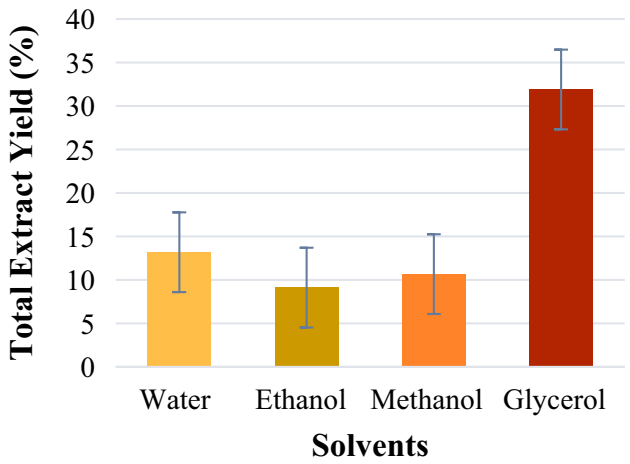

Fig. 2 Total extract yield with different solvents

yield. This may be attributed to two main factors: firstly, the viscosity of the glycerol solution was lowered with the addition of water, facilitating the solvent penetration into the solid particles of the sample; secondly, the higher polarity of the extraction system assisted in efficient solute–solvent complex transfer into the extract [5]. Moreover, the higher extraction yield could also be associated with the multiple hydrogen bonding networks formed by glycerol and water, which facilitate the extraction of anthocyanins. As glycerol is a highly polar solvent and anthocyanins are also a group of polar flavonoids, their extraction must be prominent with the solvent [58]. Another critical factor in increased yield may be the higher solvent-to-solid ratio, which increases the mass transfer ratio, thus resulting in a higher diffusion rate and maximum extraction yield [28]. A similar study by Özkan et al. [41] reported a 10–15% higher extraction yield with aqueous glycerol compared to ethanol and butanediol from gülfatma flowers. Glycerol is considered to be highly beneficial in maintaining the stability of anthocyanins, which may be due to the multi-hydroxyl group structure of the solvent, thus retaining the higher amount of anthocyanins [20]. Glycerol, via hydrogen bonds, is known to form a cage-like structure in interaction with water, contributing to higher anthocyanin recovery [16]. The extraction yield also depends on extraction conditions, where ultrasonication pre-treatment plays an important role. It is an effective way

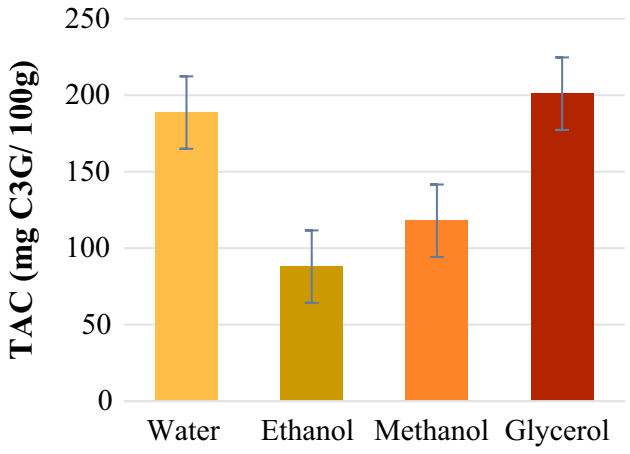

Fig. 3 Total anthocyanin content yield with different solvents

to accelerate the diffusional process, thus contributing to higher and more efficient recovery of anthocyanins [10]. According to a study by Jovanović et al. [24], who extracted anthocyanins from bilberry fruits using ultrasound with NADES solvent, report a positive influence of sonication for a shorter time, yielding higher anthocyanins.

3.2 Total anthocyanin content

Figure 3 presents the results of anthocyanin content in the extracts obtained with different solvents from the pulp of *S. cumini* fruit. Anthocyanin content in the pulp of *S. cumini* fruit varied from 87.94 mg C3G/100 g (ethanol) to 201.05 mg C3G/100 g (glycerol). In the case of solvents, the highest anthocyanin concentrations were noted for water (188.69 mg C3G/100 g) and glycerol (201.05 mg C3G/100 g) at 25 °C. In the presented experiment, glycerol has been identified as the best extraction system for anthocyanins. According to Kowalska et al. [32], water-glycerol extraction systems increased the content of anthocyanins compared to pure water in black chokeberry and elderberry extracts. In the given studies, the authors demonstrated extraction efficiency. The results show that a 70% aqueous glycerol mixture provided a very satisfactory extraction yield in anthocyanins higher than water, ethanol, and methanol. A similar pattern was observed in a study by Soares et al. [51], where the anthocyanin content yield (49.8 mg/g) for aqueous glycerol solution along with ultrasonication was 28.7% higher than water. In agreement with our results, a study of anthocyanin extraction from frozen blueberry honeysuckle by Kaniewska et al. (2013) indicated that water/glycerol extraction systems prove to be better solvents than water. The better efficiency of water-alcohol extraction solvent may result from the affinity of anthocyanins to polar solvents. Glycerol enhances the solubility of more polar flavonoids, resulting in higher recovery [1]. It is also suggested that the intermediate concentration of the solvent, which is around 70% (v/v), is the best condition for the higher recovery of anthocyanins [36].

3.3 Total phenolic content

Figure 4 shows the results for total phenolic content, which was approximately equal for water as a solvent compared to glycerol. The TPC was recorded with the maximum value of 0.998 mg GAE/g dry sample (water), while glycerol led to an equally efficient phenolic extraction of 0.9 mg GAE/g dry sample. Similar to water and methanol, the aqueous glycerol solvent system's polarity helped to influence the solvent's affinity towards extracted compounds, thus facilitating the anthocyanin extraction at a suitable ratio [8]. A similar study by [1] explored the efficacy of aqueous glycerol for polyphenolic extraction from rice bran, where they concluded that increasing glycerol volume increased the yield of total phenols. The difference in the polyphenolic extraction efficiency may be due to the varied functionality of glycerol with different plant materials. In a study by [2], acidified glycerol treatments were given to waste orange peels where the yield of total polyphenols achieved was approximately 30% higher than the conventional extraction techniques in a significantly shorter processing time. The glycerol's viscosity may have slowed the diffusion of phenolics in water/glycerol mixtures compared to pure solvents [42].

3.4 Antioxidant activities (DPPH and FRAP)

The results of the DPPH assay have shown that the higher anthocyanin content is proportionate to the antioxidant capacity, but this is not a general principle [49]. To ascertain the stated fact, the anthocyanin extracts of each solvent

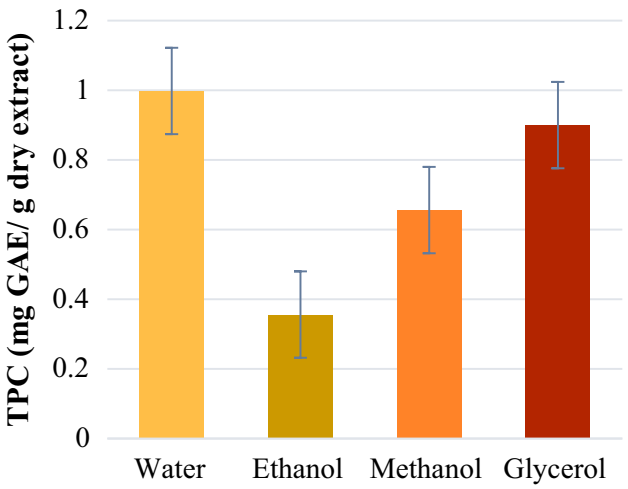

Fig. 4 Total phenolic content with different solvents

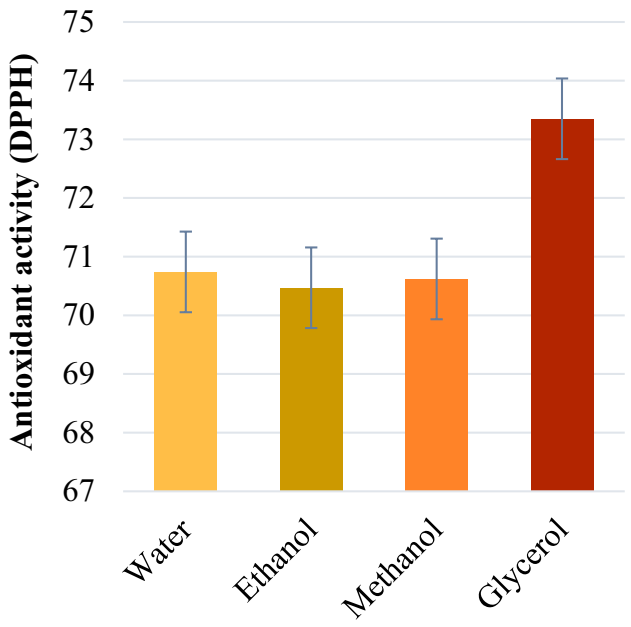

Fig. 5 DPPH radical scavenging activity with different solvents

were assayed with two antioxidant tests: antiradical activity (DPPH) and reducing power (FRAP).

As can be seen, the experimental values of antiradical activity in terms of the DPPH assay of *S. cumini* pulp extract are presented in Fig. 5. The DPPH free radical scavenging activity of glycerol (73.35% inhibition) was significantly higher than the conventional solvents. The radical scavenging activity for the conventional solvents was similar (water–70.74, ethanol–70.47 and methanol–70.62% inhibition). The water-glycerol extract displayed a higher antiradical activity, which is proportionate to the higher phenolic and anthocyanin content. These findings concurred with our previous study with NADES (in the communication), where the antiradical activity was proportionate to anthocyanin content. This outcome strongly demonstrated that the antioxidant activity of the extracts varies with the type of solvent used given the same conditions. Anis & Ahmed [4] observed similar results for antiradical activities of phenols and flavonoids from *Rumex hastatus* extracted with water-glycerol extraction systems, where the highest antiradical activity (90.62%) was obtained with the higher phenolic content. Increasing solvent polarity promotes higher antioxidant extraction [22]

As stated earlier, despite the consolidated concept that higher phenolic content is accompanied by proportionally higher antioxidant capacity, several other investigations highlighted that a correlation between phenolic content and antioxidant activity is not always significant [38]. The differences in anti-radical activity and reducing power may reflect differences in the total amount of polyphenols and interactions amongst them, which may affect the antioxidant activity of the extracts. It has been reported that anthocyanins account for a major part of antioxidant capacity, followed by other phenolic compounds [59]. The results (Fig. 6) showed a proportionate decrease in the reducing power of the extracts between the solvents as opposed to their phenolic content. The water-glycerol extract showed a lower reducing power when compared to the conventional solvents, where the antiradical activity was higher for the same. There is enough significance in the interactions between antioxidant constituents of the extracts, where the higher reducing and antiradical activities may be displayed by the polar fractions.

The differences between the antiradical activity and reducing power may be attributed to the synergism/antagonism mechanism reflecting the differences in interactions amongst the polyphenols, thus affecting the antioxidant activity of extracts [27, 31, 42]. Similar results were observed for antiradical activity and reducing power by Philippi et al. [42] while extracting polyphenols from eggplant peel using aqueous glycerol aided with ultrasonic treatments. The reducing power was quite lower compared to antiradical activity, where the difference between the results for glycerol and ethanol was insignificant. A significant difference between the results of DPPH and FRAP was observed for the phenolic extraction of lotus seedpod using aqueous glycerol, where antiradical was higher for ultrasound with glycerol but reducing power was higher for water bath incubation with glycerol [7].

Summarising the results of the presented study, the efficient extraction of anthocyanins can be alternatively carried out by glycerol as a solvent. It is clear from the results that a higher yield of anthocyanins was obtained compared to the conventional solvents.

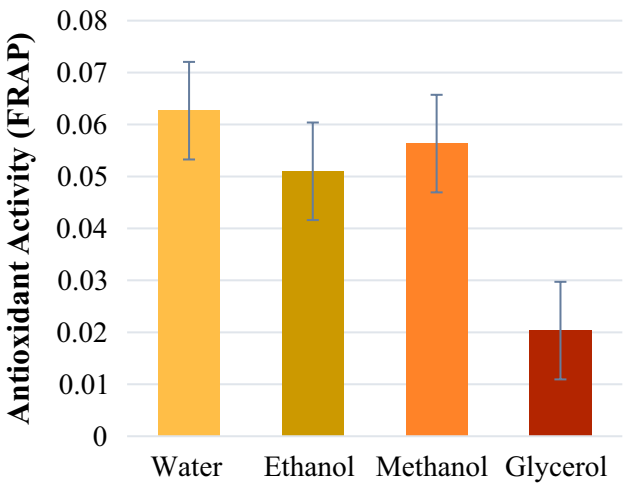

Fig. 6 FRAP reducing power assay with different solvents

4 Conclusion

Anthocyanins, being vacuolar pigments, may not be completely extracted with the conventional techniques of solvent extraction, where the sample may not be able to completely disperse into the substrate, thus making them unavailable for extraction. Using a green extraction process as a pre-treatment, such as ultrasonication, may help reduce the utilisation of organic solvents. Green solvents such as glycerol are natural substances without toxicity, making them a suitable candidate for separating bioactive compounds from food matrixes. This study has validated the efficiency of recovering phenolic compounds, that is, anthocyanins, from *S. cumini* fruit pulp. For a potential industrial application, such a procedure would be desirable as the production costs would be significantly lowered, and the extracts would not require further processing. They could be directly incorporated into food products. Pre-treatment techniques, such as ultrasound, would lower the time taken, thus increasing the efficiency of the whole process. This investigation may be regarded as the first step in applying an integrative process development approach that provides alternatives for recovering anthocyanins from the pulp of *S. cumini* fruit. However, the scalability of the extraction methods needs to be explored for commercial success of this method.

Author contributions Darshanjot Kaur: Writing and experimentation. Ovais Shafiq Qadri: Conceptualization, review, and correspondence.

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Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest statement On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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