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REVIEW

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Syzygium cumini anthocyanins: recent advances in biological activities, extraction, stability, characterisation and utilisation in food systems

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Abstract

Syzygium cumini anthocyanins have become an area of great interest as biocolourants in the food industry. These anthocyanins impart a deep colour over a suitable pH range due to the high amount of anthocyanin content compared to other natural sources. An important aspect is selecting an appropriate technique where a high yield of anthocyanins can be obtained from the pulp of *S. cumini* fruit. Among various extraction techniques, ionic/ organic solvents and ultrasonication are the most employed methods due to their efficiency. These green extraction techniques are advantageous over conventional techniques due to the high recovery of anthocyanins utilising much less solvent in a shorter duration with minimal degradation. Despite that, the utilisation of recovered anthocyanins is restricted to mainly acidic (dairy) products due to their instability towards environmental parameters such as pH, light, temperature, enzymes, and metal complexes. Additionally, according to experimental studies, co-pigmentation and acylation could improve anthocyanins' stability. Being one of the most potential sources of anthocyanins, S. cumini fruits can be exploited for extraction of this biocolourant. However, there is discontinuity in the research between extraction and utilization of *S. cumini* anthocyanins as is evident from the literature survey. In this review we have summarized the research advances being executed to enhance the extraction and utilization of *S. cumini* anthocyanins using green or novel techniques and a brief account of stability analysis, characterization, and utilization.

Keywords Jamun, Anthocyanin extraction, Antioxidant, Biocolourant

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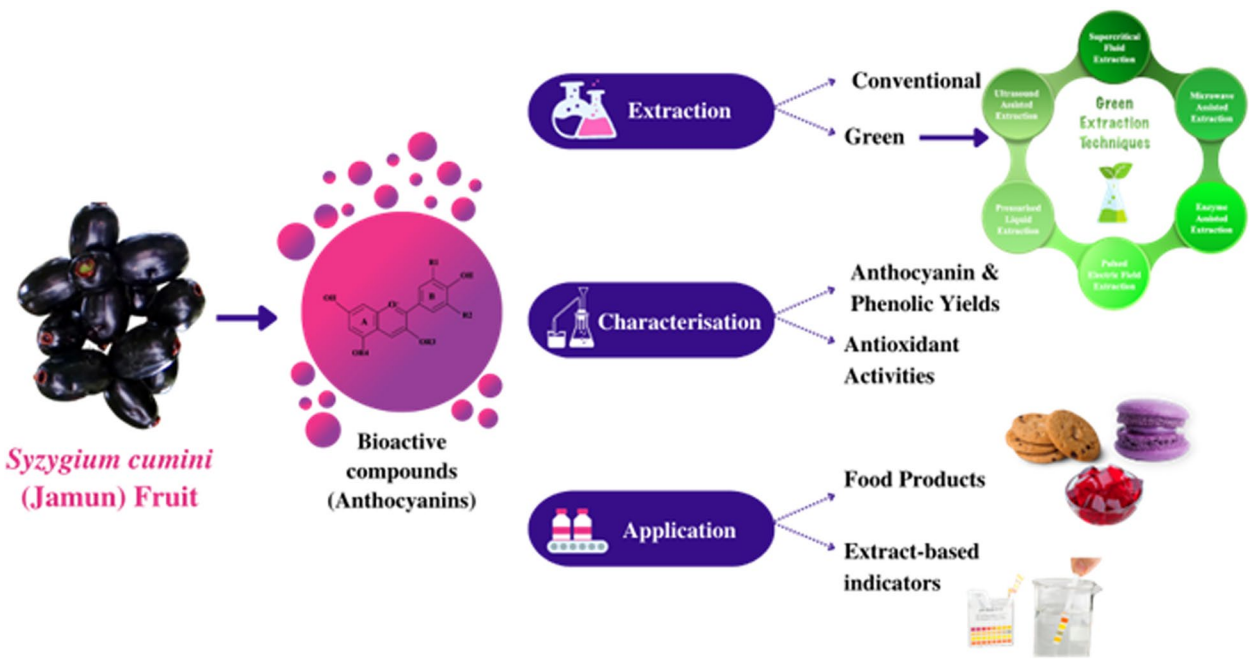
Graphical Abstract



Introduction

The colour of the food is the first thing one perceives before having/ tasting the food. In an industrial setup, while processing food items, a considerable amount of colour is lost, triggering a need for colour addition to food products (Rymbai et al. 2011). The colour of the food is a vital characteristic to indicate its safety, ripeness and appetising appearance. Synthetic colours have been a part of the food processing industry for a long time, but due to their hazardous health effects and increasing consumer awareness, their usage has been reduced (Aberoumand 2011). Natural food colours (biocolourants) are now gaining the attention of researchers and consumers as well, as they are non-hazardous to health. The colouring agents extracted from biological material are known as 'Biocolourants'. The biocolourants are mainly obtained from the parts of the plants, including flowers, fruit, peel/ skin of the fruit/ vegetable, or waste of the food processing industry. The colour of different plant parts attributes to special chemicals known as pigments like chlorophylls, carotenoids, flavonoids and betalains (Rymbai et al. 2011).

Plant pigments are a generic term used to designate many coloured molecules, classified into chlorophylls, carotenoids, flavonoids, and betalains (Chen 2015). They are essential in controlling plants' photosynthesis, growth, and development, protecting them from the damage caused by UV and visible light (Sudhakar et al. 2016). Pigments are visual signals to attract insects, birds and animals for pollination and seed dispersal (Choo, 2018). Some pigments are essential nutrients, and some serve as nutraceuticals with additional medical benefits, including preventing and treating certain diseases (Chen 2015).

The term anthocyanin was coined by Marquart in 1835, derived from the Greek words *anthos* and *kyanos* for flower and blue, designating to the blue pigment of flowers (Markakis 2012). The plant pigment 'anthocyanin' imparts the splendid natural colours (blue, purple, violet, and magenta) in several plant foods. The interest in extracting colour from anthocyanins increased after the azo dyes were banned due to health concerns and anthocyanins being considered replacements. Anthocyanins are located in the vacuole as an aqueous solution and absorb visible light conferring a variety of colours onto the media in which they occur. The chiefly occurring anthocyanins are cyanidin, petunidin, malvidin, delphinidin, and peonidin (Zhang et al. 2011). Anthocyanins are primarily found in higher plants and are present in all parts of the plant, prominently in flowers and fruits.

Syzygium cumini (Ayyanar and Subash-Babu 2012) is a tropical fruit tree commonly known as Jamun or Indian blackberry and belongs to the family *Myrtaceae*. The fruit

of *S. cumini* is an oblong, dark-purple or bluish, with pink, sweet fleshy pulp and tiny seeds and is highly perishable (Singh et al. 2019). While it is native to India, '*S. cumini*' is now found in all tropical regions (Sahu et al. 2020). Due to its therapeutic value, *S. cumini* is a significant source of nutraceuticals. The fruit and its other parts, such as leaves, bark, seed, flowers, and roots, are also abundant in various bioactive compounds like flavonoids, carotenoids, antioxidants, phenolics, and vitamins (Sahu et al., 2020). Sweet, sour, acrid, carminative, refrigerant, astringent, digestive and diuretic are the various properties of *S. cumini* (Jagetia 2017). *S. cumini* bears its fruit in the summer and rainy seasons.

Of the fruits' nutritional and medicinal importance, attempts have been made to extend their shelf-life, making them commercially acceptable (Ghosh et al. 2017). The fruit pulp is profoundly nutritive and contains several anthocyanins making it an excellent source of the biocolourant. It has been reported that the deep-purple colour of *S. cumini* fruit is because of anthocyanins, mainly delphinidin-3-gentioioside and malvidin-3-laminaribioside (Dagadkhair et al. 2017). The fruit of *S. cumini* has been found to consist of delphinidin, petunidin, and malvidin as the major anthocyanins (Veigas et al. 2007). Few studies have also established that *S. cumini* contains five out of the six major anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin) in their di glucoside forms (Brito et al. 2007). In recent years, *S. cumini* has been found to contain all the major anthocyanins (Fig. 1), which makes it a genuine base to exploit the fruit in extraction and optimisation studies, along with their consideration of health benefits and utilisation in food products.

The review aims to highlight some novel techniques that have been utilised for the extraction, medicinal properties and aspects regarding the stability, anthocyanin content and antioxidant activity of *S. cumini* anthocyanins. The first part includes the composition and benefits of *S. cumini* anthocyanins. In the second part, the conventional and novel extraction techniques, stability and food applications of *S. cumini* anthocyanins are discussed.

Methodology

This review follows a qualitative research design and takes a case study approach. An intensive literature search has been done using the keyword search terms such as "jamun anthocyanins", "Eugenia jambolana", "Syzygium cumini anthocyanins", "anthocyanin extraction", "green extraction of jamun anthocyanins" and "novel extraction techniques" to find out the relevant research. There are very few research papers on novel extraction studies on *S. cumini* anthocyanins due to their lower stability and perishable nature. But there was enough relevant data on the pharmacological properties of anthocyanins from *S. cumini*. The databases used were Google Scholar, NCBI, Web of Science, Science Direct, ShodhSindhu, INFED, INFLIBNET, Springer and other high-impact journals. The reference citations were done using Chegg and Mendeley, rechecking them manually. The main focus was to review the research studies conducted on extracting and utilising anthocyanins from *S. cumini*. The initial introduction gave a general overview of S. cumini, followed by the biological activities of *S. cumini* anthocyanins, their extraction, characterisation and finally, a small overview of the utilisation of *S. cumini* anthocyanins (Fig. 2).

Composition of *S. cumini*

S. cumini fruit has always been the choice of consumers due to its high nutritional value, as it is rich in carbohydrates, minerals and vitamins, as shown in Table 1. *S. cumini* is the only fruit with minimum calories as it has no traces of sucrose and contains glucose and fructose as primary sugars. *S. cumini* is also an essential source of antioxidants, particularly anthocyanins (Kapoor et al. 2015).

The central part of the fruit is water, ash, protein and sugar, with acidity represented by sulphuric and malic acids. The fruit also contains calcium, magnesium, phosphorus, iron, sodium, potassium, copper, sulfur, chlorine, vitamin A, thiamine, riboflavin, niacin, ascorbic acid, chlorine and folic acid in 100g edible portion (Eswarappa & Somashekar, 2020). *S. cumini* is also found to be enriched with phytochemicals, such as tannins, glycosides, sterols, alkaloids, flavonoids and carbohydrates among others (Table 2).

Biological activities of *S. cumini* anthocyanins

In various traditional systems of medicine, '*S. cumini*' is an important medicinal plant as it is effective in no. of diseases (Swami et al. 2012). Conventionally, all the parts, including fruit, leaves, seeds, and bark of the plant, are used and recognised in folk/ ayurvedic medicine. The benefits of anthocyanins obtained from *S. cumini* fruit pulp are discussed in Fig. 3. *S. cumini* has shown high antioxidant activity when compared with non-traditional fruits, mainly due to the anthocyanin components (Singh et al., 2019).

Anthocyanins, a significant component of the human diet, are perpetually consumed through food or their derived products and a few dark-coloured vegetables. It is prevalent for fruits and vegetables to exhibit diverse biological activities (Bowen-Forbes et al. 2010).

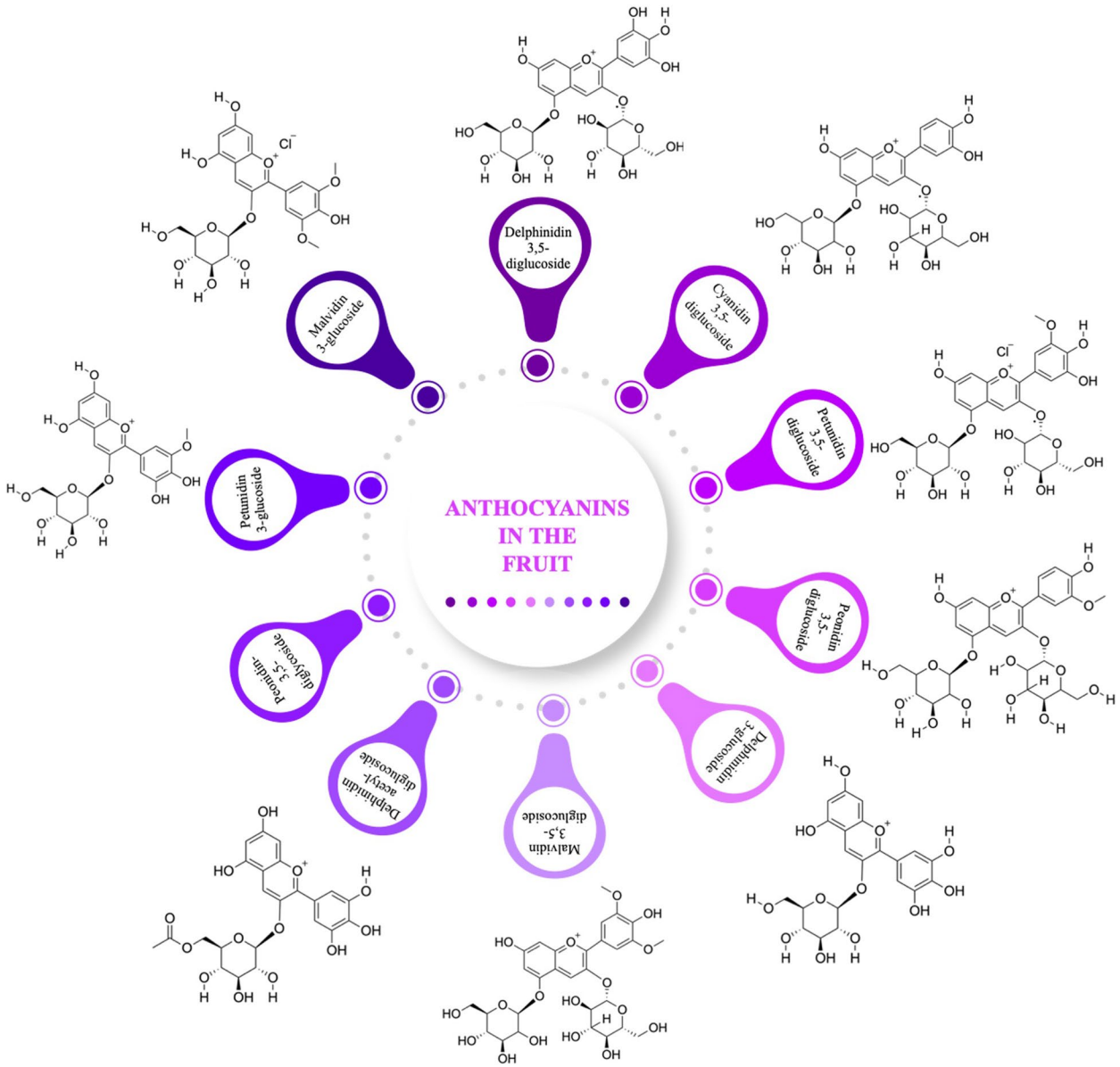


Fig. 1 Anthocyanins present in *S. cumini*

Antioxidant activity

The antioxidant activity of anthocyanins is dependent on the aglycone part (Kähkönen & Heinonen 2003). Antioxidants can reduce ROS formation by radical scavenging, redox-active transition metals' deactivation, and by inducing specific signalling pathways to cell defence modulation (Qin & Hou 2016). This cell-damaging activity by ROS has been associated with the pathogenesis of various diseases, such as type 2 diabetes, atherosclerosis, and cancer (Bakuradze et al. 2019). The presence of powerful antioxidant compounds in the '*S. cumini*' fruit makes it an excellent nutraceutical fruit. The antioxidant compounds present in *S. cumini* are ascorbic acid, anthocyanins, and total phenols (Kapoor et al. 2015). According to Kapoor et al. (2015), the antioxidant activities of freeze-dried and hot air-dried *'S. cumini'* powder were 88.34% and 83.53%, respectively. According to the study of Mitra et al. (2013), it is reported that there was minimal loss of anthocyanins due to the absence of liquid water when freeze-dried. The scavenging activity of five major anthocyanidins from *S. cumini* pulp can be arranged in a descending order: Dp > Pt > Cy > Pe > Mv with an EC_{50} 15.5, 21, 45, 46, 61, and 65 µg/ ml. Du et al. (2008) investigated the antioxidant activity of mulberry anthocyanins by the DPPH assay free

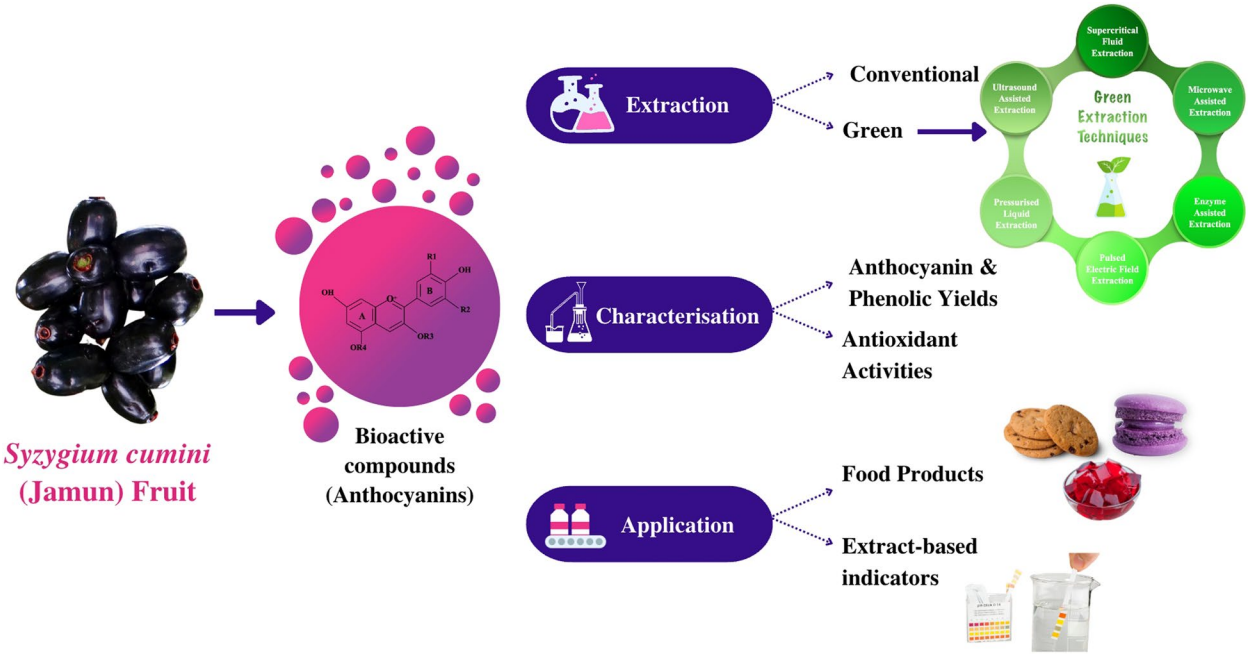


Fig. 2 *S.cumini* anthocyanin extraction, characterization and application

radical scavenging method. They found the radical scavenging rates of cyanidin glycosides, cyanidin 3-glucoside, cyanidin 3-galactopyranoside, cyanidin 3-arabinoside, cyanidin 3-rutinoside, cyanidin 3,5-diglucoside and cyanidin 3-xylosyl-glucoside-5galactoside against DPPH radical (0.1mM) were 32%, 25%, 26%, 25%, 21%, and 22% at the same molar concentration of samples (17 µM).

Table 1 Proximate composition of freshly picked '*S. cumini*' fruit per 100 grams of the edible portion (Swami et al., 2012)

Components	Weightage
Water	80.80%
Protein	0.81
Sugar (fructose & glucose)	12.70
Acidity (sulphuric, malic)	0.63%, 0.88%
Moisture	85.8 gm
Ether extract	0.15 gm
Crude fibre	0.3 gm
Nitrogen	0.129 gm
Ash	0.32 gm
Calcium	8.3 mg
Phosphorus	16.2 mg
Iron	1.62 mg
Carotene	0.004 mg
Thiamine	0.008 mg
Riboflavin	0.009 mg
Niacin	0.290 mg
Total ascorbic acid	5.7-18 mg

Antidiabetic

Diabetes mellitus is caused by insulin deficiency, thus disturbing the endocrine system and carbohydrate metabolism (Alberti & Zimmet 1998). It is known that plants possess pancreatic a-amylase inhibitors forming a critical aspect in the treatment of diabetes on withdrawal of starch indigestion (Eichler et al. 1984). Gajera et al. (2017) prepared methanolic and aqueous extracts of all parts of the Jamun plant and tested them for antidiabetic functionality associated with phenolic constituents. The screening for a-amylase inhibitors was done using the porcine pancreatic a-amylase (PPA), and an inhibition assay was carried out by the di-nitro salicylic acid (DNSA) method. The methanolic extracts of pulp (16.1%) of a medium to small *S. cumini* fruit had a higher antidiabetic potential compared to aqueous extracts but lower than the kernel (92.6%) methanolic extract. The methanolic pulp extracts of a very small *S. cumini* fruit exhibited maximum (53.8%) antidiabetic activity in comparison to other-sized fruits. The small-sized fruits were further examined for IC_{50} (indicates fruit extract concentration showing ≥50% inhibition on PPA activity) values to check for a-amylase inhibition activity. The methanolic extracts of the pulp of *S. cumini* fruit had the highest IC_{50} value of 270 mg/ ml, thus being a moderate option for antidiabetic activity (Gajera et al. 2017). The aqueous extract of *S. cumini* fruit showed reduction in blood glucose levels by approximately 20% where the serum insulin levels enhanced in normal and diabetic rats

Table 2 Phytochemical profile of *S. cumini* fruit (pulp and skin)

S. No.	Metabolite	Identified compounds	Pharmacological properties	References
1.	Glycosides and Vitamin A & C	Cyanidine diglycosides, riboflavin, thiamine, folic acid, nicotinic acid	Antidiabetic	Agarwala et al. (2019)
2.	Flavonols	myricetin-3-*O*-glucuronide, myricetin-3-*O*-galactoside, myricetin-3-*O*-glucoside, myricetin-3-*O*-rhamnoside, myricetin-3-*O*-pentoside, laricitrin-3-*O*-galactoside, laricitrin-3-*O*-glucoside, syringetin-3-*O*-galactoside, syringetin-3-*O*-glucoside	Anticancer	Qamar et al. (2022)
3.	Flavanonols	DHQ-dihexoside-1, DHQ-dihexoside-2, DHQ-dihexoside-3, MDHQ-dihexoside, MDHQ-dihexoside, DHM-dihexoside-1, DHM-dihexoside-2, DHM-dihexoside-3, DHM-dihexoside-4, DHM-dihexoside-5, DHM-dihexoside-6, MDHM-dihexoside-1, MDHM-dihexoside-2, MDHM-dihexoside-3, MDHM-dihexoside-4, MDHM-dihexoside-5, MDHM-dihexoside-6, DMDHM-dihexoside-1, DMDHM-dihexoside-2, DMDHM-dihexoside-3, liquiritigenin Flavan-3-ols: catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin 3-*O*-gallate, catechin 3-*O*-gallate, epigallocatechin 3-*O*-gallate, gallocatechin 3-*O*-gallate	Anticancer	Asanaliar and Nadig. (2020)
4.	Tannins	galloyl-glucose, 3galloyl-glucose-1, 2galloyl-glucose, 3galloyl-glucose-2, 3galloyl-glucose-3, 3galloyl-glucose-4, 4galloyl-glucose-1, 4galloyl-glucose-2, 5galloyl-glucose-1, 5galloyl-glucose-2, 5galloyl-glucose-3, 6galloyl-glucoside-1, 6galloyl-glucoside-1, castalagin, vescalagin, (2) HHDP-glucose-1, (2) HHDP-glucose-2, G-(2) HHDP-glucose-1, (2) HHDP-glucose-2, (2) G-HHDP-glucose-1, (2) G-HHDP-glucose-2, (2) G-HHDP-glucose-3, (3) G-HHDP-glucose, trisgalloyl-HHDP-glucose-1, trisgalloyl-HHDP-glucose-2	Anti-hyperlipidemic	Agarwala et al. (2019)
5.	Phenolic acid	quinic acid, gallic acid, chlorogenic acid, caffeic acid	Anti-inflammatory	Dagadkhair et al. (2017)
6.	Coumarins	umbelliferon, scopoletin	Anti-hyperlipidemic	Swami and Kalse (2020)
7.	Terpenoid	rosmanol	Anti-hyperlipidemic	Swami and Kalse (2020)

(Ayyanar et al. 2013). In the same study, the fruit extract of S. cumini has also been reported to decrease the risk of development of atherosclerosis in diabetic patients as it reduces the action of free radicals.

Anti-cancer activity

Berries, a magnificent bioactive polyphenolic source, are reported to play an essential role in cancer prevention, including carcinogen-induced DNA damage inhibition, oxidative DNA damage protection, and signalling pathways modulation (Aqil et al. 2014). The berry bioactives also help in the regulation of transcription and growth factors, inflammatory cytokines, and tumour angiogenesis (Kausar et al. 2012). Anthocyanins (appropriately anthocyanidins) have the potential to block the cell cycle at various stages by affecting the cell cycle regulator proteins (e.g., p21, p27, p53, cyclin A, cyclin D1, etc.), leading to inhibition of cell proliferation (Wang et al. 2008). A study by Aqil et al. (2014) reported that anthocyanidins from *S. cumini* pulp extract had shown strong anticancerous activity with an IC_{50} value of 59 ± 4 mg/ ml against human non-small-lung cancer cells (A549). Wang et al. (2008) reported the selective inhibition of growth and stimulation of apoptosis of a highly tumorigenic rat oesophageal epithelial cell line (RE-149-DHD) compared to its low tumorigenic precursor line (RE-149).

Hepatoprotective

The liver plays a key role in the body's metabolism and detoxification of substances entering the human body, but the factors such as viral infections, alcohol consumption and liver injuries leading to liver diseases induce a great concern. The antioxidant property of anthocyanins has been reported to benefit liver health and provide hepatoprotective effects (Hou et al. 2013). The ethanolic extracts of *S. cumini* pulp, when administered, showed protective activity in rats against paracetamol-induced hepatotoxicity. The pulp extract protected rat hepatocytes against carbon tetrachloride-induced *in vitro* toxicity (Das and Sarma 2009). Donepudi et al. (2012) reported that *S. cumini* fruit extract lowered serum ALT levels up to 60%, thus being effective in the treatment of the hepatocellular injury.

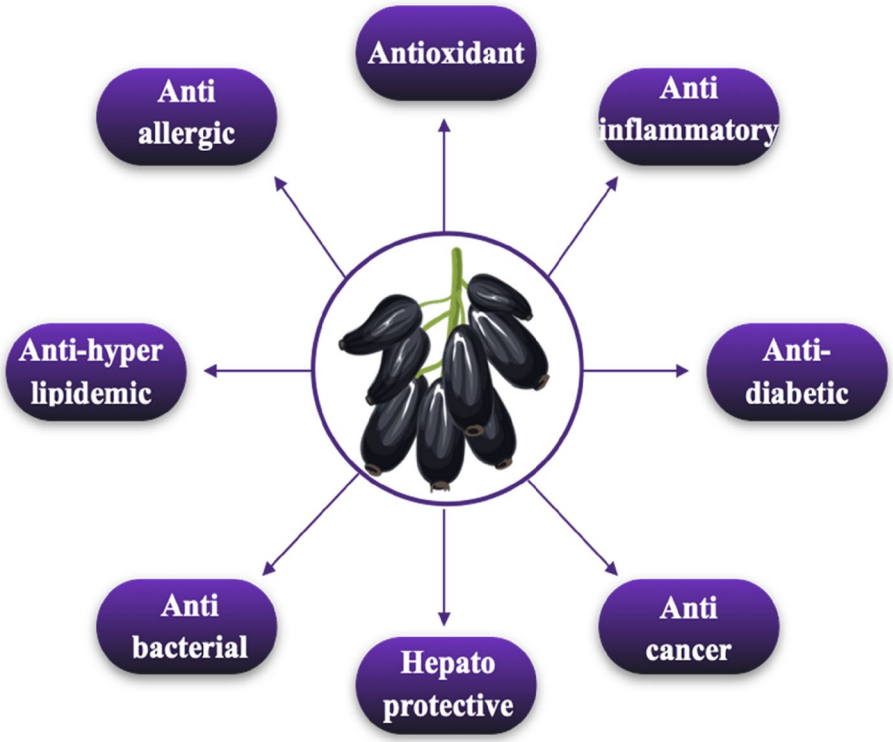


Fig. 3 Various biological activities of *S. cumini* anthocyanins (Sahu et al., 2020, Jagetia, 2017, Swami et al., 2012)

Other

Anthocyanins can destroy the cell wall of food-borne pathogens, thus exhibiting antibacterial activity (Ma et al. 2019). Anthocyanins can be considered antibiotic substitutes reducing the harm caused by them. The ethanolic extracts of *S. cumini* pulp were found to show antimicrobial activity against various Gram-negative bacteria like *P. aeruginosa* and *S. flexneri* and Gram-positive bacteria like *S. aureus* and *B. subtilis* (Jagetia 2017).

Anthocyanidins exhibit anti-inflammatory activities which are structure dependent. They possess the ability to inhibit cyclooxygenase-2 (COX-2) expression in lipopolysaccharide (LPS)-activated RAW 264 cells or inhibit inducible nitric oxide (iNOS) protein and mRNA expression in LPS-activated murine J774 macrophages (Hou et al., 2005). Cyclooxygenase is a critical enzyme that plays a role in inflammation. (Arya et al. 2018) reviewed that *S. cumini* fruit extract helped protect against cholestatic liber injury and inflammation in mice. (Donepudi et al. 2012) established with experimental studies that *S. cumini* fruit phytochemicals lowered hepatic inflammation and also reduced oxidative stress, thus defending the hepatocellular damage in mice.

Multiple studies have also reported the cardioprotective and anti-hyperlipidemic activity of flavonoids and anthocyanins. (Chhikara et al. 2018) investigated the lipid-lowering activities of different parts of *S.cumini,* which showed a satisfactory reduction in serum lipid levels. *S.cumini* have also exhibited cardioprotective effects in isoproterenol-induced myocardial infection in rats.

Extraction of *S. cumini* Anthocyanins

Anthocyanins could either be extracted using conventional solid-liquid extraction methods or with the help of green extraction methods (Table 3). Lately, green techniques have gained significant attention from researchers due to their many advantages, including high time efficiency, lower energy input, reduction in solvent usage, and high yields of targeted compounds (Damayanti et al. 2020).

"Green extraction techniques are the ones that enable lowered energy consumption, permit the use of alternate solvents and sustainable natural products ensuring safe and high-quality extract/ product" (Chemat et al. 2012). With the application of green extraction as an innovative initiative, certain principles have been identified to guide researchers in all dimensions of solid-liquid extraction (Chemat, Abert-Vian, et al. 2019a, 2019b). It is considered that even if the primary treatment is the same, the modification could help the procedure to be greened (Armenta et al. 2019). This fundamental approach towards greening the extraction process is based on

Table 3 Techniques applied for the *S. cumini* anthocyanin extraction

Extraction Method	Solvent	Solid: liquid ratio	Extraction conditions	Results		References
				Anthocyanin concentration	**Optimum conditions**	
Temperature-Controlled Water Bath	Distilled water	1:10 – 1:15	Extraction temperature (40-60 °C); time (20-100 min)	10.58 mg/100 g at optimised conditions	Extraction temperature (44 °C); extraction time (93 min); solid:liquid ratio (1:15)	Maran et al. (2015)
MAE	DES	1:48	Extraction time (0-240s); extraction power (100-400W)	8.197 mg of C3G g^{-1} at 400W power for 240s		Sharma and Dash (2022)
UAE	DES	1:48	Temperature (40-70 °C); extraction time (150 min); ultrasonication power (100W)	8.525 mg of C3G g^{-1} at 70°C ultrasonication temperature		Sharma and Dash (2022)
BUE	Acidified aqueous ethanol	1:15	Ultrasonication power (135W); ultrasonication temperature (30-40 °C)	55 mg C3G g^{-1}	Ultrasonication time (40 mins.); ultrasonication temperature (30 °C); ethanol concentration (70% v/v)	Sabino et al. (2021)
PUE	Acidified aqueous ethanol	1:15	Ultrasonication power (5000W/L);	60.5 mg C3G g^{-1}	Ultrasonication power (5000W/L); ultrasonication time (10 min); ethanol concentration (80% v/v)	Sabino et al. (2021)
PLE	Acidified water and acidified aqueous solutions of ethanol, methanol, acetone	1:6	Pressure (11,721 kPa); rinsing time (5min); extraction time for each cycle (10min); and purge with nitrogen flow (200 s)	10-45 mg C3G g^{-1}	Temperature (90 °C); ethanol concentration (80% v/v); extraction cycles (2)	Sabino et al. (2021)
$SCCO_2$	Ethanol (99.9% purity)	1:50	Extraction pressure (100-200 bar); temperature (40-60 °C); co-solvent flow rate (1-3g/ min)	231.28 ± 0.76 mg/100 g for TMAC at optimised conditions	Extraction pressure (162 bar); temperature (50 °C); solvent flow rate (2g/ min)	Maran et al. (2014)

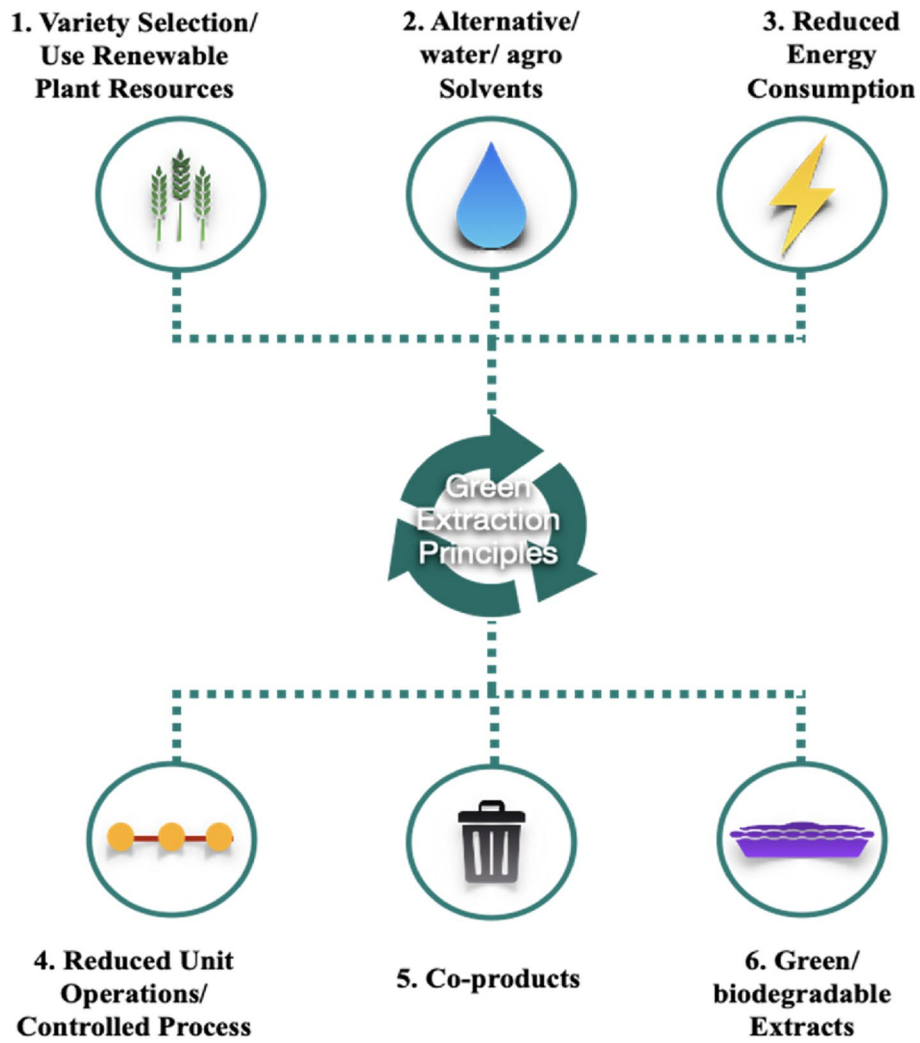


Fig. 4 Six principles of green extraction

the six principles (fig. 4) where i) reduction in over-utilisation of plant sources is promoted, or plant breeding using biotechnological processes is considered, ii) use of bio-based solvents is considered to meet the ends, iii) application of energy-efficient process by optimisation thus leads to reduced energy consumption covering overall related concerns, iv) sustainable processes are used to renew all the co-products by creating them as further valuable products, v) efficient processes developed lowering the unit operations yet providing value, vi) the extracts should be natural considering the use of water as solvent or removal of the solvents at the end although this point still being controversial (Chemat et al. 2019a, 2019b); Armenta et al. 2019).

The extraction method substantially affects the yield, purity, stability, and composition of bioactive compounds/ anthocyanins. There are various green extraction techniques utilised in extracting bioactive compounds, especially pigments.

Conventional Solvent Extraction

Conventional solvent extraction is a commonly carried out solid-liquid extraction technique where diffusion of the solvent takes place with the solid food matrix. In this technique, mass transfer of molecules takes place, which may be opposed by the plant structure but can be enhanced by reduced particle size, optimum temperature and acidified solvent pH (Cissé et al. 2012). The particle contact time, solvent-solid ratio, temperature and size of the particle play an important role in yielding higher anthocyanin content. The most commonly used solvents in anthocyanin extractions are methanol and ethanol. (Maran et al. 2014) conducted a solvent optimisation experiment for anthocyanin extraction from *S.cumini,*

replacing methanol with ethanol due to less toxicity. The use of 20% ethanol with 1% acetic acid resulted in the highest anthocyanin content of 763.80 mg/ 100ml. The highest extraction yield of *S. cumini* anthocyanin using water as solvent has been reported at a temperature of 44 °C over a time of 93 min with a solid-liquid ratio of 1:15 g/ml (Maran et al. 2014). However, these conventional techniques yield higher results but have certain disadvantages, due to which novel extraction technologies were explored by researchers. Conventional extraction techniques were said to be easier in preparation but time-consuming with low efficiency. Also, the extraction has to be done at higher temperatures leading to the degradation of anthocyanins.

Microwave Assisted Extraction

(M. Sharma & Dash 2021)(M. Sharma & Dash 2021) Microwave-assisted extraction (MAE) is an effective technique providing benefits over conventional processes as microwave power aids ionic conduction mechanisms and dipolar rotation, stimulating the molecules (Alara et al. 2021). The solvent contact time with the plant tissue enables the penetration of solvent into the matrix, thus releasing the anthocyanin components. It is also reported that microwave-assisted extraction is advantageous as it is less time-consuming, requires minimal solvent usage and provides higher yields. An experimental study on microwave-assisted extraction of *S. cumini* anthocyanin estimated the effect of independent variables on TAC (Total Anthocyanin Content). They reported that the highest effect starting from microwave power followed by liquid-solid ratio and extraction time had great effects (M. Sharma & Dash 2021). The study also reported that lower microwave power with a longer duration of extraction resulted in a higher yield of TAC.

Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE) produces mechanical waves using ultrasound energy. These waves constitute a series of compression and rarefaction cycles that induce collapsing cavitation bubbles. These collisions lead to the fragmentation of the sample cellular matrix, thus solubilising the bioactive component in the solvent, thus aiding in the release of the bioactive pigments. Multiple studies have reported a higher rate of anthocyanin extraction due to increased mass transfer rate, solvation, and substrate porosity within the plant cell. The solvents utilised may either be conventional solvents such as methanol, ethanol, or green solvents (deep eutectic solvents, ionic solvents) and enzymes. The results from ultrasound-assisted anthocyanin extraction from *S. cumini* pulp revealed that a maximum anthocyanin yield of 8.525 mg C3G/ g was obtained at a sonication temperature of 70°C over a 150 min extraction (Sharma & Dash 2022).

Pressurised Liquid Extraction

Pressurised liquid extraction (PLE), also called accelerated solvent extraction (ASE), is a technique utilising conventional solvents for extraction but under high temperature (50-200 °C) and pressure (500-3000 psi). The solvent diffusion is improved, facilitating the cell rupture of the solid matrix; thus, the lowered requirement of solvent is reduced and improved extraction rate and time. Pressurised liquid extraction is an emerging green technique in comparison to traditional extraction methods. The experimental study by Sabino et al. (2021) reported that on the extraction of anthocyanins from *S. cumini* fruit extract, the highest TAC obtained was after two extraction cycles at a temperature of 90 °C with an ethanol concentration of 80% v/v.

Supercritical carbon dioxide Extraction

Supercritical carbon dioxide ($SCCO_2$) extraction is a novel technology for the extraction of bioactive compounds, which may or may not cause any environmental damage and also aims to shorten the processing time. In a supercritical state, carbon dioxide behaves like both liquid and gas due to high density and diffusivity (Arumugham et al. 2021). In this process, carbon dioxide diffuses into the entire plant matrix, thus acquiring the bioactive compounds. As the technique does not require high temperatures for pigment extraction, hence is now the most widely used technique to extract valuable bioactive compounds. In combination with supercritical extraction, other extraction methods such as UAE, pressurised solvent extraction, subcritical water extraction can also be used which may further help in reducing the cost of production thus increasing the extraction efficiency at the same time (Essien et al. 2020). Maran et al. (2014) reported the optimum conditions of *S. cumini* anthocyanin extraction by utilising a supercritical fluid method where extraction pressure of 162 bar with a co-solvent flow rate of 2.0 g/ min at 50 °C temperature yielded higher anthocyanins with TMAC of 231.13 mg/ 100g.

Pulsed Electric Field

Pulsed electric field (PEF) is a non-thermal technology which uses moderate to high electric field strength and works in two ways: i) in batch mode (100-300 V/ cm) and ii) in continuous mode (20-80 kV/ cm). PEF speeds up the chemical reactions enhancing the solvent solubility and electroporation, enhancing the cell membrane permeability. The target food is placed between the electrodes, and a high-voltage electric field punctures the cell

membrane of the food matrix, which opens the protein channels creating hydrophilic pores (Ranjha et al. 2021). It prevents anthocyanin degradation and is a key factor in inhibiting the microbial spoilage of food products (Yang et al. 2016). PEF has been reported to improve the anthocyanin yield at moderate and high-intensity treatments. The pulsed electric field, in comparison to conventional extraction, yielded higher anthocyanins from *S. cumini* fruit extract, where the electric field strength was kept moderate (Chand & Prince, 2018). The study reported that anthocyanin yield, antioxidant activity and colour of the extracted anthocyanins from *S. cumini* fruit had a higher value than the untreated sample.

Characterisation of *S. cumini* Anthocyanins

To carry out anthocyanin and antioxidant activity analysis, the pH differential method and DPPH assay are the commonly applied techniques. Estimation of anthocyanin content is the primary goal which attracts the attention of researchers, thus providing valuable information on the importance of bioactive compound extraction from a selected fruit or vegetable. Determination of antioxidant activity on the other hand provides a value acquisition when proposing the product to a consumer so that it is acceptable by them.

Total Anthocyanin Content

The attractive purple colour of *S. cumini* is due to the presence of anthocyanin pigments, an important subgroup of flavonoids showing antioxidant properties (Gaibor et al. 2022). Anthocyanins can get degraded easily depending on the extraction technique, food processing, and storage conditions. So, the total anthocyanin content varies among different plants and may range from as low as 20 mg/ 100 g to as high as 600 mg/ 100 g. However, all six major anthocyanins (delphinidin, peonidin, petunidin, malvidin, cyanidin, and pelargonidin) have been found in *S. cumini* fruit pulp (Schulz et al. 2016). As the anthocyanin absorptivity is highly dependent on the pH and direct methods for the anthocyanin content determination can incur errors due to interference of other metabolic compounds; thus, to determine the anthocyanin content, an indirect method like the pH differential method is applied (Joshi & Preema 2017). The pH differential method is extensively used to examine the quality of fresh and processed fruits and vegetables. It determines the anthocyanin content by measuring the absorbance change at two different pH values where monomeric anthocyanins undergo a reversible structural transformation as a function of pH. Ghosh et al. (2017) used the pH differential method to determine the total anthocyanin content of *S. cumini* pulp and reported it to be 195.58 mg/100g. Kapoor et al. (2015) noted total anthocyanin content was higher in freeze-dried samples (305.47 mg/100g) than in hot air-dried samples (259.89 mg/100mg). Aqil et al. (2014) reported total anthocyanins from the dry extract of hydrolysed and non-hydrolysed fruit pulp to be 45.7 mg/ gm (4.6%) and 47.1 mg/ gm (4.7%), as shown in Fig. 5. According to spectrophotometric analyses of *Syzygium cumini* extract, Veigas et al. (2007) reported their anthocyanin content to be 216 mg/ 100 ml, equivalent to 230 mg/ 100 g fruit on a dry weight basis.

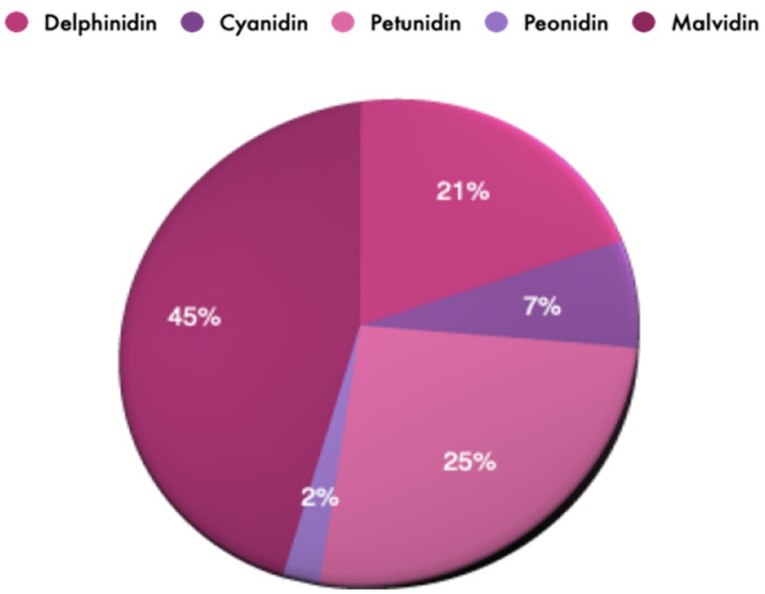


Fig. 5 Relative (%) anthocyanidin content in hydrolysed pulp extract (Aqil et al., 2012)

Chemical profiling of *S. cumini* anthocyanin

The chemical profiling of extracted anthocyanins from the fruit of *S. cumini* has not been explored extensively. The identification of anthocyanins has been carried out after the fruit pulp incorporation into the food products but not from the crude anthocyanin extracts. Therefore, there is a scarcity of literature regarding the chemical profiling or identification of anthocyanin compounds. There are only very few studies available which are further discussed. Sabino et al. (2021) identified the anthocyanins from *S. cumini* extracts with the highest concentration by UPLC-ESI-QTOF-MS, which were diglucosides of delphinidin, petunidin and malvidin where bath ultrasound and probe ultrasound extraction provided the highest proportion of major anthocyanins. Santos et al. (2013) identified all the six major anthocyanins through TLC and ESI-Q-TOF-MS extracted by acidified solvent extraction.

Antioxidant activities

Apart from the anthocyanin's pigmentation effects, they have the ability to prevent the oxidation of lipids in various lipid environments. As anthocyanins exist in various forms (hydrated, isomeric, protonated, deprotonated) depending on the pH, they play an important role in the antioxidant activity of anthocyanins. The structural factors of anthocyanins determine their radical scavenging activity and metal chelating capability (Kähkönen & Heinonen 2003). Further, the selection of extraction techniques for anthocyanins is an important factor in deciding the antioxidant capacity of the anthocyanins. There is a number of antioxidant assays that assess the potentiality of anthocyanins to prevent the naturally occurring oxidation process. DPPH assay is a spectrophotometric technique used for the determination of the total antioxidant capacity of the sample (Tena et al. 2020). The assay assesses the capacity of the free radical ($DPPH^*$) to react with hydrogen donor (AH^+). The disadvantage of DPPH is the complexibility of analysis where other substances at the same wavelength may interfere. FRAP assay is a colorimetric technique that determines the total antioxidant activity of anthocyanins based on the reduction of a dye named 2,4,6-tripyridyl-s-triazine (TPTZ) with ferric chloride hexahydrate ($FeCl_3.6H_2O$) under acidic conditions. FRAP assay generates reproducible results (Tena et al. 2020). ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assay is the most recent technique, which is based on decolourisation. In this, a stable radical is produced by the reaction of blue/green ABTS chromophore with potassium persulfate. The limitation of DPPH can be overcome by the ABTS assay as it can determine the antioxidant activity from a substance mix by distinguishing between additive and synergistic effects (Frond et al. 2019).

Stability of *S. cumini* anthocyanins

Anthocyanins are considered stable when their flavylium cation cannot transform into carbonyl pseudo bases and chalcones, which are colourless forms (Ghareaghajlou et al. (2021); Chen, 2015). Anthocyanins are unstable but show the greatest stability under acidic conditions (Fennema 1996). The stability of anthocyanins varies according to their chemical structure and concentration in food. This varying pattern further influences the final product's health benefits and anthocyanin content (Arruda et al., 2021). The following are the most critical factors affecting the stability of anthocyanins:

pH

Due to the ionic nature of anthocyanin molecules, the colour of anthocyanins is dependent on the pH of the solution. They are said to have lower bioavailability because of their sensitivity to changing pH (Mahdavi et al., 2014). Anthocyanins are stable at lower pH (<3, red form) and start degrading at increasing pH (Joshi & Preema 2017). The effect of pH on the stability of *S. cumini* anthocyanins was investigated by (Jampani et al. 2014) at different pH, and it was observed that the anthocyanins degraded with the increased pH (1-5). Sharma et al. (2016) reported that anthocyanins in *S. cumini* were stable at pH 1 over three days at 30 °C but the anthocyanin content reduced to 78% at pH 3.1. When the anthocyanin molecules are in a solution, there is the presence of equilibrium between the cationic form (coloured) and pseudo base (colourless) (Wahyuningsih et al. 2017). The equilibrium of these is directly influenced by pH (Wahyuningsih et al. 2017).

The anthocyanins are red in acidic solutions, violet or purple in neutral solutions and blue in alkaline pH (fig. 6). Anthocyanins can also act as pH indicators. Anthocyanins can be used as food colourants due to their variant pH (Wahyuningsih et al. 2017).

Temperature

Temperature significantly affects the stability of anthocyanins in foods. There is the destruction of pigments if heated for a longer duration at a steady temperature (Ghareaghajlou et al. 2021). The *S. cumini* anthocyanins showed a degradation effect when the pulp extracts were incubated at different temperatures ranging from 30 to 80 °C (Jampani et al. 2014). The higher the temperature, the higher the degradation of anthocyanins (100 to 25%). It is reported that methylated, glycosylated, or acylated anthocyanins are more stable than highly hydroxylated

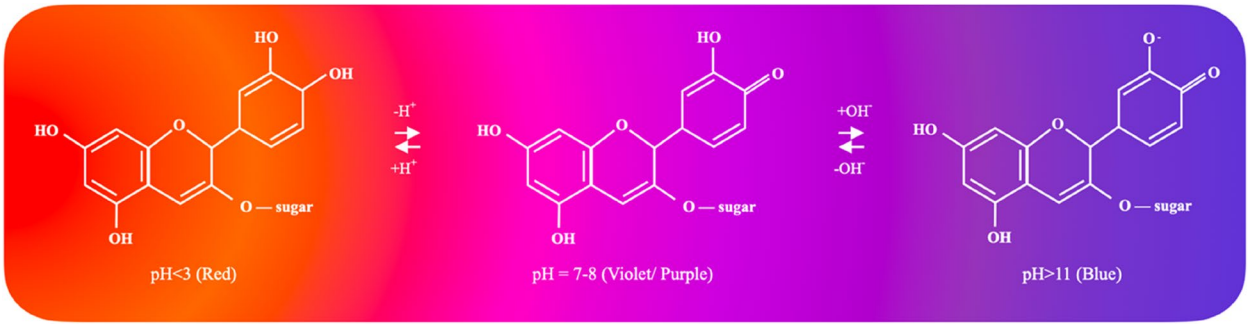


Fig. 6 The equilibrium of anthocyanin and the coloured form

anthocyanidins (Fennema 1996). Also, prolonged exposure to heat can cause the browning of anthocyanins (Maccarone et al., 1985). Not only during drying or heating, but the storage temperature for anthocyanins should also be taken care of. (Wadibhasme et al. 2020) developed a fortified *S. cumini* instant drink mix where they observed a decrease in anthocyanin content as they increased the inlet temperature. Kapoor et al. (2015) established that the antioxidant activity of the *S. cumini* fruit pulp alters due to thermal degradation during processing. Sharma & Dash (2022) reported that as the temperature increased, the anthocyanin content of the *S. cumini* fruit extract decreased.

Other

Light is an essential parameter in the stability of anthocyanins. Although light is necessary for anthocyanin production, it also can increase the degradation of bioactive compounds (Enaru et al. 2021). It is observed that when in the dark, the degradation of anthocyanins is to a limited extent than when kept in light (Amogne et al. 2020). It has been recognised that long exposures of the pigments to light accelerate their degradation. Light, on the other hand, also affects the antioxidant activity of anthocyanins. Shwetha and Preetha (2016) reported that *S. cumini* anthocyanins degraded at five times the speed even in the encapsulated form when subjected to light, thus having a negative effect on colour stability.

Anthocyanins can form metal complexes if o-dihydroxyl groups are present, thus shifting from red to stable blue and violet pigmentations (Joshi & Preema 2017). Co-pigmentation is another process where pigments form complex/ molecular associations with other colourless compounds or metal ions thus affecting the colour intensity of the extracts (Enaru et al. 2021). Various studies have reported that in order to improve the stability of anthocyanins, they should be co-pigmented with other substances. The anthocyanin-enriched Sephadex extract of *S. cumini* anthocyanins showed noticeable degradation, which could be associated with loss of stabilising factors such as metal pigmentation complex and co-pigmentation during the process of enrichment of anthocyanins (Sharma et al. 2016).

Applications of *S. cumini* anthocyanins

In the range of natural food colorants; blue, pink or purple is undoubtedly the greatest challenge as the sources are very limited or the extracted pigment has low stability. The colours that create novelty and have a pleasurable fragrance and taste with health-promoting properties are highly accepted by consumers. *S. cumini* is not just a fruit but also possesses various medicinal properties more than that, it can be manipulated for its anthocyanin capacities, making it a potential future biocolorant substituting synthetic colourants. The anthocyanin pigment extraction has been carried out from the pulp of *S. cumini* fruit using novel extraction techniques. To the best of our knowledge, there is no evidence that the anthocyanin pigment after extraction has been utilised as a colorant in food products. On the other hand, the pulp of the fruit has indirectly been incorporated into various food products where the effect of the anthocyanin pigment cannot be unseen. Some studies have mentioned the development of color with increased concentrations in the food products like flatbread and drink mix. Raza et al. (2015) extracted bioactive components from the fruit and seed of *S. cumini*, where it was concluded that the seed exhibited no anthocyanins, but the pulp or the skin of the fruit contained the highest anthocyanins. *S. cumini* anthocyanins have not directly been used as a colorant, but the pulp of the fruit has been incorporated into various food products where the quality assessment due to the presence of anthocyanins has given the best results.

Thus the pulp of the *S. cumini* fruit has been utilised in making processed foods like instant drink mix, chapattis/ flatbread, squash, cake, dairy desserts and various others. There is a scarcity of literature where anthocyanins,

after extraction, have been utilised in food products from the pulp of *S. cumini*. There are studies of different fruits Kapoor et al. (2015) supplemented unleavened flatbread (Indian chapatti) with *S. cumini* pulp (5, 10, and 15%) and observed that with increasing supplementation levels, the color of the flatbread became darker in blueness due to the presence of anthocyanins but also providing softness to the chapatti thus making it highly acceptable. Bhatt et al. (2020) developed a squash from the extract of *S. cumini* fruit pulp where the juice with a concentration of 35% was considered best on the basis of sensory analysis, also showing storage stability of at least 6 months under ambient and refrigerated temperature conditions. Wadibhasme et al. (2020) developed a powdered instant drink mix on the fortification of *S. cumini* fruit containing bioactive compounds with a better shelf life. As the drink mix was prepared at a low temperature making it more healthy and nutritive with an attractive colour and flavour.

Many natural pigments, including *S. cumini* anthocyanins, have the potential to be used as a pH-based indicator of food quality in packaging systems because of changing colour expressions with changing pH (Ghareaghajlou et al. 2021). As *S. cumini* is an unconventional fruit and of low economic value, only a few studies have been reported on the use of *S. cumini* anthocyanins as indicators and biocolorant. There is no evidence that the anthocyanin extract from *S.* cumini could have been utilised as a biocolorant in food products, but a few studies are available for its use as a pH indicator. Talukder et al. (2020) developed an extract-based indicator for monitoring the quality of chicken patties during storage producing reliable responses indicating its potential applications in food packaging systems for the assessment of food quality and safety. Merz et al. (2020) developed a colorimetric indicator film based on chitosan, polyvinyl alcohol, and *S. cumini* anthocyanins by casting method. They concluded that these indicator films could be used for monitoring the freshness of seafood products. Though anthocyanins based indicators are in trend these days for safe food packaging systems, the use of anthocyanins as colourants in food products is a long way to go where multiple research studies have been conducted in developing a technique for pilot scale operations and increasing the stability of the anthocyanins.

The primary limiting factor for using anthocyanins as biocolorants is their stability which few studies have suggested being increased by finding alternatives for their recovery methods. A study by do Carmo Brito et al. (2017) tracked the colour changes of the *S. cumini* anthocyanin extracts for the first time by monitoring CIELAB parameters. They suggested that *S. cumini* anthocyanins are a promising potential source for their application as a natural pigment in the food industry. Coelho Leandro et al. (2021) experimented the recovery of *S. cumini* anthocyanins by adsorption-desorption in laponite platelets. They discovered that L^*, a^*, b^* values remained constant for the analyses period of 14 days thus indicating the potential use of *S. cumini* anthocyanins as a biocolorant in food products further helping to preserve the redness of various fruits. It can be concluded that there is a scope for utilising *S. cumini* anthocyanins as biocolorants or natural food colors having no or little harmful effects.

Conclusion

In recent years, green extraction has been on trend to optimise the extraction processes by research scientists. The extraction of anthocyanin pigments is lately undertaken due to its low stability. Also, anthocyanins are challenging to utilise in food products where they may lose colour and are stable with acidic foods. Extraction optimisation and utilisation studies have been conducted mainly with exotic or common fruits in the market. *S. cumini* is an unconventional tropical fruit limited to the Indian subcontinent and adjoining regions of Southeast Asia. Although fresh produce of *S. cumini* is utilised directly in smoothies and various drinks, it cannot be stored for longer durations due to its instability. Anthocyanins are an essential constituent of *S. cumini* and are responsible for the fruit's typical sensory and health benefits. Some studies have been conducted on the extraction of anthocyanins from *S. cumini*, including green extraction processes but have not been exploited yet to the full extent. Almost all green techniques have been explored in the case of *S. cumini* anthocyanin extraction, but there are no extensive research papers on them. There are no multiple studies to establish a single best technique that works best for *S. cumini* anthocyanins. Also, the extracted anthocyanins are highly susceptible to degradation in terms of pH, light, temperature, processing and storage, thus requiring interference to conduct stability studies and develop a pure and stable *S. cumini* anthocyanin product. Currently, there is a requirement for novel and eco-friendly extraction techniques, and applying a combination of green techniques is recommended for future studies.

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Authors' contributions
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