Current Research in Food Science 7 (2023) 100629

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Natural pigments (anthocyanins and chlorophyll) and antioxidants profiling of European red and green gooseberry (*Ribes uva-crispa* L.) extracted using green techniques (UAE-citric acid-mediated extraction)

Shehzad Hussain [a], Minaxi Sharma [a], Tatsiana Jarg [b], Riina Aav [b], Rajeev Bhat [a,*]

[a] *ERA-Chair for Food (By-) Products Valorisation Technologies (VALORTECH), Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, Tartu, 51006, Estonia*
[b] *Department of Chemistry and Biotechnology, Tallinn University of Technology, Akadeemia tee 15, Tallinn, 12618, Estonia*

ARTICLE INFO

Handling Editor: Professor Aiqian Ye

Keywords:
European gooseberries
Natural pigments
Anthocyanins
Antioxidant profiling
Ultrasonic-citric acid-mediated extraction
Green techniques

ABSTRACT

Green techniques to extract natural pigments are gaining prominence among consumers and food industries. This trend is predominantly due to the harmful effects imparted by commonly used synthetic dyes and the unwarranted stress created on our ecosystem. The objectives of this study were to obtain natural pigments (anthocyanins and chlorophyll) from Estonian-gown European green and red gooseberries by ultrasonic-assisted citric acid-mediated extraction method and perform antioxidant profiling (quantification via HPLC analysis). Green gooseberry extracts showed lower content of targeted compounds, with low concentrations of rutin (0.7–1.2 mg/L) and quercetin 3-glucoside (0.9–1.3 mg/L), while in the red gooseberry extracts, the amount was slightly higher (1.4–6.9 and 1.0–1.3 mg/L, respectively) with 0.6–6.8 mg/L cyanidin 3-glucoside and 0.32–0.35 mg/L peonidin 3 glucoside recorded. Further, the yield of anthocyanins ranged between 1.14–1.79 and 1.86–3.63 mg/100 g in green and red gooseberries, respectively. Total phenols ranged between 162–392 and 263–987 mg GAE/100 g in green and red gooseberry extracts, respectively. The DPPH free radicals scavenging activity showed 73–86% and 87–91% inhibition in both green and red gooseberry, respectively. Results showed significant improvements in pigment extraction with higher values obtained for targeted antioxidant compounds using conventional and UAE extraction (aqueous extract), thus confirming that green extractions are a reliable technique to obtain pigments of interest from natural sources. The results support consumers' demand and open up the avenue to explore pigments as natural colourants in food and cosmetics applications.

1. Introduction

The term 'food pigment' is extensively used to delineate a coloured substance added to food products as an additive to prevent colour loss during food processing as well as during storage. The aesthetic value of a food product depends on its colour, which is linked to visual appeal (Sharmila et al., 2019; Miranda et al., 2021). Food colourants can be categorized as synthetic dyes and natural pigments. Artificial/synthetic dyes are mainly produced from chemicals (petroleum-based chemicals), which are extensively used in food, cosmetics and pharma industries, but their application is restricted due to its potentially harmful effects on human health and on the environment (Sharma and Bhat, 2021). In developed and industrialized countries, permission to use synthetic colorants in the food industry is subject to strict legislation and a variety of toxicity tests such as immune effects, accumulation in the body, carcinogenicity, reproductive toxicity, acute and chronic toxicity etc (Amchova et al., 2015). Tartrazine, Carmoisine, Sunset yellow and Erythrosine are some the examples of synthetic food colorants used in food industry (Bachalla, 2016). Synthetic food colorants are linked with numerous health related adverse effects. Intake of food color additives has reportedly been linked to harmful effects on the liver and, kidneys (Sadar et al., 2017). Consumption of synthetic food colorants lead to reduce the amount of high density lipoprotein cholesterol (HDL-C), superoxide dismutase (SoD), glutathione secretion (GSH), and plasma immune system. On the other hand, it can also cause a significant increase in total cholesterol (LDL-C), blood glucose, lipid peroxidase and plasma urea (Dafallah et al., 2015).

Apart from their harmful effects, artificial dyes are also sensitive to pH, light and temperature (Roriz et al., 2017). At present, natural pigments and dyes are highly looked for by the food industries and

* Corresponding author.
E-mail addresses: rajeev.bhat@emu.ee, rajeevbhat1304@gmail.com (R. Bhat).

https://doi.org/10.1016/j.crfs.2023.100629
Received 19 June 2023; Received in revised form 10 October 2023; Accepted 28 October 2023
Available online 6 November 2023

consumers since they are environmentally friendly and can easily replace or overcome the drawbacks of synthetic dyes (Gengatharan et al., 2015). Therefore, the shift from chemical origin dyes to plant-based natural pigments is significant for both human health as well as good for the environment (Sharma et al., 2022). Natural pigments including anthocyanins, betalains, and carotenoids are also widely used as colouring agents in the food industries (Sharmila et al., 2019). Fruits and vegetables can be vital source of natural pigments and antioxidants. In general, berries contain high amounts of antioxidants among all other plant based produce. For example, extracts obtained from berries have demonstrated the best antioxidant activities among a total of 92 phenolic extracts obtained from various edible plants such as fruits, berries and vegetables (Kähkönen et al., 1999; Chiang et al., 2013). Generally, anthocyanins are regarded as the leading and major group of water-soluble pigments in nature, and are accountable for the red, blue, purple and orange colours of numerous fruits and vegetables (He and Giusti, 2010; Fang, 2015). They primarily occur as acyl-glycosides or glycosides of their individual aglycone anthocyanidins. The main sources of anthocyanins are blueberries, black currants, strawberries, raspberries, cherries and purple grapes (Khoo et al., 2017). Furthermore, a wide range of antioxidant compounds are present in berries including anthocyanins, flavanols (catechins, quercetin), procyanidins, and phytoalexins. Many studies have been undertaken on berry antioxidants, however on gooseberries and their subtypes have gained least research attention (Chiang et al., 2013; Duda-Chodak and Tarko, 2007). Gooseberry is a tiny shrub (genus *Ribes* L.; *Saxifragaceae* family), which is extensively cultivated in Europe (Gentili et al., 2015). Studies have reported *p*-coumaric acid, isorhamnetin glycoside, quercetin and kaempferol to be the main antioxidant components found in gooseberry (Häkkinen et al., 1999a, 1999b; Määttä-Riihinen et al., 2004).

Extraction of pigments is usually performed by using solvents possessing similar polarity to the materials to be extracted. Hence, polar solvents can be used for the extraction of anthocyanin pigments (Mahdavi et al., 2016). In the course of a green extraction procedure, citric acid can be used to extract pigments from plant materials in combination with novel green extraction techniques such as UAE or MAE etc. Despite being a weak organic acid, metal ions found in plant tissues can be chelated by citric acid. Many pigments, including carotenoids and chlorophylls, are naturally linked to metal ions. When these metal-pigment matrices are broken down with the help of citric acid, the pigments can be extracted more easily (Novais et al., 2022). The pH of the extraction media can be changed using citric acid. Moreover, the stability and solubility of pigments can be significantly influenced by the pH of the extraction solution. In this regard, citric acid can regulate pH to provide conditions that are more favourable for the release and preservation of pigments (Zhang et al., 2023). In order to facilitate the release of pigments that are bound inside the plant cells, citric acid can facilitate the break-down of cell walls and membranes. This alteration of cell structure can improve the effectiveness of pigment extraction (Panić et al., 2019). Furthermore, being an antioxidant, the addition of citric acid in the extraction process can also help to protect pigments from oxidation-related deterioration. This may result in increased pigment yields and improved colour retention (Ngamwonglumlert et al., 2017).

Various conventional and non-conventional extraction methods are used for the extraction of anthocyanins and other natural pigments from plant-based raw materials. The selection of the extraction process is mainly based on the shelf life and stability of the compounds. In some cases, prior to applying the extraction methods, it is important to remove other compounds such as protein, lipids and contaminants from the sample matrix that may obstruct the extraction of anthocyanins and other natural pigments (Tan et al., 2022). Decoction, maceration, soaking, percolation and Soxhlet extraction are some examples of conventional extraction methods, which are currently used in natural dye industries because these extraction methods have low costs of instrumentation and maintenance (Goti and Dasgupta, 2023). However, despite their wide usage, these conventional extraction methods also have some limitations such as high energy consumption, use of harmful and expensive organic solvents, use of huge volumes of solvents, lower yield, use of high temperature and long extraction time, which all can deteriorate antioxidants and other compounds of interest (Belwal et al., 2018). Hence, novel green extraction methods such as high-pressure extraction, supercritical fluid extraction, pulsed electric field extraction, ultra-sonication and microwave-assisted extraction techniques are preferred over conventional extraction methods (Wani et al., 2021).

Some of the green extraction techniques may require higher initial equipment costs as compared to conventional extraction techniques; however, these methods have advantages in terms of lower energy requirements, lower solvent usage, and possibly safer and more sustainable practices (Chemat et al., 2019). In addition, the cost-effectiveness and objectives of the extraction process will determine how cost-effective a certain extraction method actually is. For instance, solvent-based conventional extraction techniques often need costly equipment and procurement and disposal of solvents can be expensive.

Within this background, the current study was designed to extract, investigate and characterize anthocyanins and other antioxidant compounds in Estonian-origin, European type of red and green gooseberries. A citric acid-mediated green extraction in combination with ultrasonic-assisted extraction was employed for the anthocyanins extraction.

2. Material and methods

2.1. Chemicals and reagents

Folin-Ciocalteau reagent, Gallic acid, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma Aldrich. Citric acid, for green extraction, was purchased from Fischer Scientific, Leicestershire (UK), and ethanol for conventional extraction was procured from Merck (Darmstadt. Germany). All the solvents and reagents used for this study were of analytical grade.

2.1.1. HPLC materials and reagents

All HPLC-grade organic reagents, solvents, and reference standard materials were obtained from commercial suppliers: acetonitrile (Fisher Chemical), formic acid (Honeywell, Fluka), caffeic acid (Acros Organics), daidzin (Acros Organics), quercetin 3-glucoside (PhytoLab), procyanidin B1 (PhytoLab), procyanidin B2 (PhytoLab), malvidin 3-galactoside chloride (PhytoLab), malvidin 3-glucoside chloride (PhytoLab), petunidin 3-glucoside chloride (PhytoLab), peonidin 3-glucoside chloride (PhytoLab), delphinidin 3-galactoside chloride (PhytoLab), delphinidin 3-glucoside chloride (PhytoLab), hyperoside (MCE), cyanidin 3-galactoside chloride (PhytoLab), cyanidin 3-glucoside chloride (PhytoLab), cyanidin 3-arabinoside chloride (PhytoLab), myricetin (Sigma-Aldrich), rutin trihydrate (Sigma-Aldrich), (+)-catechin hydrate (Cayman Chemical Company), chlorogenic acid (MP Biomedicals), astragalin (MedChemExpress), phlorizin hydrate (Tokyo Chemical Industry), L-epicatechin (BLDpharm), 2,4-dihydroxybenzoic acid (BLDpharm).

2.2. Sample preparation

Ripened and matured gooseberries (red and green) were purchased from the local fresh fruit and vegetable market in Tartu, Estonia. Berries were washed with clean potable water and frozen prior to freeze-drying. Freeze-dried samples were ground to attain a homogeneous fine powder. The ground samples in powder form were vacuum packed and kept at −20 °C to protect them from oxidation and light, until analysis.

2.2.1. Sample preparation for determination of anthocyanins and antioxidants

The extracts were filtered through an RC 0.45 μm membrane syringe filter. The sample extracts were diluted twice with water pH 1.5,

adjusted with citric acid.

2.3. Anthocyanin extraction

2.3.1. Conventional extraction

Freeze-dried ground gooseberries samples were mixed with extraction solvents, water and ethanol (75%). The extraction was facilitated with continuous shaking for 10, 20 and 30 min. The obtained extracts were filtered and centrifuged at 3000×*g* at 4 °C for 20 min using a refrigerated centrifuge (Nüve NF 800 R, Turkey).

2.3.2. Citric acid mediated UAE-assisted extraction

The citric acid-mediated ultrasound-assisted extraction (CA-UAE) was carried out in an ultrasonic device (Digital Sonifier® S450 CE, Branson Ultrasonics Co., Danburry, USA) as a green extraction method. The accurately weighed (5 g) ground samples of gooseberries were mixed with extraction solvents: water and ethanol (sample to solvent ratio of 1:10). The sample mixture was then kept in an ultrasonic chamber and run at an amplitude of 40% for 10, 20 and 30 min. The temperature was controlled and maintained under 30 °C by using condensation accessories connected to the ultrasonic extractor. After CA-UAE, all the samples were centrifuged (at 3000×*g* for 20 min), filtered and stored (at −20 °C) for further analysis. In this study, we used citric acid (concentration 0.3%) to adjust to pH 2.5 for stabilizing (to prevent degradation of anthocyanins as the structure of anthocyanins is more stable at low pH) the anthocyanins and facilitate better extraction. The experimental design for the extraction of anthocyanins is elucidated in Table 1.

2.4. Anthocyanin determination

The anthocyanin content was investigated using the pH-differential method of Giusti and Wrolstad (2001). Briefly, 0.5 mL of sample was assorted with 9.5 mL each of potassium chloride (KCl) buffer (pH 1.0) and sodium acetate ($C_2H_3NaO_2$) buffer (pH 4.5). After mixing, it is incubated at room temperature (in dark) for 20 min. The absorbance values were taken at 520 and 700 nm. Then the anthocyanins content was calculated according to Eq. (2):

$$\text{Anthocyanin content}\left(\frac{\text{mg}}{\text{g}}\right) = \left[(\text{A520} - \text{A700})_{\text{pH1.0}} - [(\text{A520} - \text{A700})_{\text{pH4.5}}\right] x\text{V}x\text{n}x\text{M} / \varepsilon x\text{m}x\text{b}\Big) \quad (2)$$

where V, n, M, ε, m, and b represent the volume, dilution factor, molar mass (449.2 g/mol), molar absorptivity (26,900 L/mol·cm), dry weight, and cuvette thickness (1 cm), respectively.

Table 1
Experimental design for the extraction of anthocyanins from green and red gooseberries.

Samples	Extract	Extraction time (min.)
Green and Red gooseberries	E-CE	10
		20
		30
	E-UAE	10
		20
		30
	W-CE	10
		20
		30
	W-UAE	10
		20
		30

E-CE, Conventional extraction with ethanol; E-UAE, Ultrasound-assisted extraction with ethanol; W-CE, Conventional extraction with water; W-UAE, Ultrasound-assisted extraction with water. All the treatments were extracted at pH 1.5 adjusted using citric acid.

2.5. Chlorophyll estimation

Chlorophyll content was estimated using the method provided by Minguez-Mosquera, et al. (1991). Briefly, 3 g of accurately weighed sample was dissolved in the cyclohexane. Final volume of 10 mL was made. Chlorophyll content was calculated from the absorption value of the green gooseberry sample solution at 670 nm and specific coefficient for pheophytin a, Eo = 613 using Eq. (3):

$$C = \left(A_{670}x10^6\right) / (613 \text{ x } 100 \text{ x } d) \quad (3)$$

2.6. Total phenolic content (TPC) assay

Total phenolics of the samples were investigated by using the standard method (Singleton and Rossi, 1965; Dudonne et al., 2009) with slight modifications. A standard curve of gallic acid was plotted at different concentrations (10–100 μg/mL) to obtain the regression equation. According to the methodology, 400 μL of gallic acid (different concentration for standard curve) were assorted with 2 mL of Folin-Ciocalteu reagent (freshly prepared, 0.2 N). After mixing, 1.6 mL of 7.5% (*w/v*) sodium carbonate (Na_2CO_3) solution was added and vortexed for 2 min. The samples and standards were incubated in dark for 2 h at room temperature. Absorbance was recorded at 765 nm using spectrophotometer. All the analyses of samples and standards were done in triplicate and the TPC was denoted as mg GAE (Gallic acid equivalent)/100 g of the extracts using regression equation (R = 0.9937) attained from standard curve.

2.7. DPPH· radical scavenging activity determination

The DPPH· radical scavenging activity of green and red gooseberry extracts was determined spectrophotometrically by following the method of Brand-Williams et al. (1995) as described by Velázquez et al. (2003) with minor changes. In brief, an aliquot (40 μL) of properly diluted extracts was assorted with 200 μL DPPH solution (0.02 mg/mL). Samples were then kept for 15 min at room temperature followed by absorbance measurement at 517 nm. The radical scavenging activity is expressed in mg equivalent Trolox per g of sample (mg Trolox equivalent/g).

2.8. HPLC analysis of targeted compounds

HPLC-UV-MS analysis was performed on Agilent 1200 Series System, consisting of G1322A degasser, G1311A quaternary pump, G1329A autosampler, G1316A thermostated column compartment, G1365B multiple wavelength detector (MWD) and G6125B single quadrupole mass detector (MSD, mass accuracy ± 0.13 Da). Macherey-Nagel Nucleoshell RP18 column (150 × 3.0 mm, 2.7 μm) was employed for the separation of analytes.

Sample preparation was carried out using Radwag MYA 11.4Y microbalances (accuracy 0.006 mg) and calibrated automatic pipettes of 20–200 μL and 100–1000 μL. The extracts were filtered through an RC 0.45 μm membrane syringe filter.

2.8.1. HPLC-MWD-MS method

Elution conditions were developed by optimization of the described HPLC-DAD method (Anastasiadi et al., 2016). The samples were analysed on Macherey-Nagel Nucleoshell RP18 column (150 × 3.0 mm, 2.7 μm) using eluents A – 3% formic acid and B – acetonitrile in a 42-min gradient from A:B 95:5 (v/v) to A:B 10:90 (v/v). The flow rate was set at 0.5 mL/min, column temperature at 30 °C, and injection volume at 10 μL. The analytes were followed at 260 nm, 280 nm, 320 nm, 360 nm and 520 nm. Additional peak identification was carried out by ESI-MS in

scan mode with typical spray chamber settings, positive polarity, fragmentor voltage of 100 V and *m/z* 100–2000 mass range. The peaks were identified based on their retention time, specific UV–Vis absorption and MS signal.

2.8.2. Quantification of anthocyanins and antioxidants

Anthocyanins and other targeted antioxidant compounds were quantified via calibration graphs. For calibration, a sequence of reference standard solutions of known concentrations were analysed, and corresponding peak areas were plotted against analyte concentration with intercept of linear regression line set at 0. The content of each analyte, X_i (mg/L) was determined using Eq. (1):

$$X_i = \frac{S_i \bullet f_i \bullet 1000}{k_i} \quad (1)$$

where S_i – peak area of the analyte on the chromatogram of test solution, mAU•s;

k_i – corresponding calibration curve slope ($y = kx$, intercept set at 0);

f_i – dilution factor.

All samples were analysed in replicates ($n = 3$) and the results obtained presented as mg/L in the liquid extract.

2.8.3. Calibration solutions

Stock solution of reference standard mixture: 0.02–0.5 mg/ml individual reference standard in water: methanol mixture (pH 1.5, adjusted with citric acid). Calibration solutions in the range of concentrations of 0.00009–0.5 mg/ml were prepared by subsequent dilution of the stock solution. The calibration was performed for 2 independent parallels of solution series.

2.9. Colour measurement

The colour pattern of anthocyanin extracts was measured by the method described by Sharma et al. (2022). The L*, *a**, and *b** values were taken by using the X-Rite spectrophotometer (Grands Rapids, MI, USA) and the variation in colour values (*L**, *a**, *b**, and ΔE^*) were calculated with respect to their solvents medium of each. The L*, *a** and *b** values are meant for lightness, greenness to redness, and blueness to yellowness, respectively. All colour measurements were recorded in triplicates. The colour difference was calculated using the following Eq. (4) (previously suggested by a study conducted by Pauli, 1976).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (4)$$

2.10. Statistical analysis

All samples were analysed in replicates (n = 3) and results obtained are presented as mean ± standard deviation (SD). The results were statistically analysed by Multivariate Analysis of Variance (M-ANOVA) with Duncan's multiple range *post-hoc* test ($P < 0.05$) using IBM® SPSS® (Statistics version 22.0).

3. Results and discussions

Green techniques such as ultrasonic-assisted extraction coupled with citric acid-mediated extraction were used for estimation of antioxidant activity/compounds (TPC and $DPPH^{\cdot}$), and colour attributes of European green and red gooseberries grown in Estonia. These techniques were further synergized with conventional extraction method (CE; with ethanol and water) for extracting anthocyanins and for estimation of chlorophyll, antioxidants and for evaluating the colour properties. Results obtained for each studied parameter are discussed in the text below.

3.1. Anthocyanins

Table 2 shows the results of anthocyanin content in green and red gooseberry extracts obtained by conventional and UAE extraction methods. Accordingly, significant ($P < 0.05$) differences in the content of anthocyanin were observed, since these colorants are accountable for red and blue colour in the fruits. Conventional extraction with ethanol (E-CE-20) showed the highest anthocyanin content (3.63 mg/100 g) in red gooseberries, while the least content of anthocyanin (1.14 mg/100 g) was observed in green gooseberry extract (E-UAE-20). Maximum extraction yield of anthocyanin content was observed in E-CE in both green and red gooseberries.

Significant differences ($P < 0.05$) were observed in green and red gooseberries anthocyanin content obtained from conventional and UAE extraction with water and ethanol. Anthocyanin content in red gooseberries ranged from 3.36 to 3.63 mg/100 g of E-CE, 1.86–2.48 mg/100 g of E-UAE, 2.26 to 2.56 mg/100 g of W-CE and 2.32 to 2.44 mg/100 g of W-UAE. A similar trend was also recorded for the anthocyanin content of green gooseberries, where anthocyanin content was ranging from 1.66 to 1.79 mg/100 g of E-CE, 1.14 to 1.67 mg/100 g of E-UAE, 1.16 to 1.56 mg/100 g of W-CE and 1.31 to 1.44 mg/100 g of W-UAE.

Comparable results of anthocyanin content have been reported by previous researchers, both in green and red gooseberries (*Ribes glossularia*) (Pantelidis et al., 2007), however they reported higher content of anthocyanin in red gooseberry. Määttä-Riihinen et al. (2004) studied 18 Scandinavian berry species for their phenolic and anthocyanin content, however in their study anthocyanins were not detected in both green and red gooseberry. The difference in content of anthocyanin maybe due to different cultivar being investigated. UAE method has capability of enhancing the extraction of anthocyanins to a specific level when it is compared to solvent extraction method. On the other hand, mechanical effects and the cavitation generated by ultrasound could damage the anthocyanin structure during the UAE process (Tan et al., 2022). Therefore, the ultrasonic conditions must be strictly monitored during UAE process.

Table 2
Anthocyanins and chlorophyll in green and red gooseberries extracts obtained from conventional and green extraction methods.

Extract	Anthocyanins (mg/100 g)		Chlorophyll (mg/kg)
	Green Gooseberries	Red Gooseberries	Green Gooseberries
E-CE-10	1.66 ± 0.08^{h}	3.36 ± 0.19^{b}	2.24 ± 0.03^{c}
E-CE-20	1.66 ± 0.19^{h}	3.63 ± 0.10^{a}	2.85 ± 0.04^{b}
E-CE-30	1.79 ± 0.11^{fg}	3.55 ± 0.02^{a}	3.13 ± 0.01^{a}
E-UAE-10	1.67 ± 0.07^{gh}	1.86 ± 0.02^{f}	1.32 ± 0.01^{d}
E-UAE-20	1.14 ± 0.04^{l}	2.20 ± 0.12^{e}	1.13 ± 0.07^{ef}
E-UAE-30	1.38 ± 0.14^{jk}	2.48 ± 0.04^{c}	1.18 ± 0.04^{e}
W-CE-10	1.16 ± 0.05^{l}	2.26 ± 0.04^{e}	0.41 ± 0.03^{i}
W-CE-20	1.47 ± 0.04^{ij}	2.47 ± 0.04^{c}	0.46 ± 0.02^{i}
W-CE-30	1.56 ± 0.05^{hi}	2.56 ± 0.05^{c}	0.45 ± 0.02^{i}
W-UAE-10	1.31 ± 0.02^{k}	2.32 ± 0.02^{de}	0.54 ± 0.01^{h}
W-UAE-20	1.32 ± 0.04^{k}	2.32 ± 0.04^{de}	0.97 ± 0.01^{g}
W-UAE-30	1.44 ± 0.01^{ijk}	2.44 ± 0.01^{cd}	1.12 ± 0.02^{f}

E-CE-10; conventional extraction with ethanol for 10 min, E-CE-20; conventional extraction with ethanol for 20 min, E-CE-30; conventional extraction with ethanol for 30 min, E-UAE-10; ultrasonic-assisted extraction with ethanol for 10 min, E-UAE-20; ultrasonic-assisted extraction with ethanol for 20 min, E-UAE-30; ultrasonic-assisted extraction with ethanol for 30 min, W-CE-10; conventional extraction with water for 10 min, W-CE-20; conventional extraction with water for 20 min, W-CE-30; conventional extraction with water for 30 min, W-UAE-10; ultrasonic-assisted extraction with water for 10 min, W-UAE-20; ultrasonic-assisted extraction with water for 20 min, W-UAE-30; ultrasonic-assisted extraction with water for 30 min. Chlorophyll is estimated only in green gooseberry.

3.2. Chlorophylls

Pigments such as chlorophylls are accountable for the green colour in plants and are found in plant chloroplast allied with carotenoids, lipoproteins and lipids. These natural pigments, whose structure is alike to haemoglobin in the human and animal blood, is crucial beside daylight for capturing energy for the photosynthesis process, playing a crucial role in plants (Turkiewicz et al., 2020; Mishra et al., 2011).

Chlorophyll content of green gooseberry extracts obtained by conventional and UAE extraction was analysed and absorbance was compared at 670 nm (Table 2). The extraction yield of chlorophyll content showed an inclining trend, E-CE > E-UAE > W-UAE > W-CE. Conventional extraction with ethanol (E-CE-30) showed the highest content of chlorophyll (3.13 mg/kg) in green gooseberries. While the lowest content of chlorophyll was recorded in conventional extraction with water. Significant differences ($P < 0.05$) were observed in chlorophyll content of conventional and UAE extraction with water and ethanol (2.24 to 3.13 mg/kg of E-CE, 1.13 to 1.32 mg/kg of E-UAE, 0.41 to 0.46 mg/kg of W-CE and 0.54 to 1.12 mg/kg of W-UAE). These variations in chlorophyll values may be due to extraction time, effect of ultrasonic waves and processing temperature generated during UAE.

Earlier, Karabagias et al. (2013) have reported on the chlorophyll content of olive oils extracted from different olives cultivars from different locations. According to their study, chlorophyll content ranged between 0.09 and 4.45 mg/kg. Jeana Gross (1983) also reported the chlorophyll content in *Ribes* fruit ranging from 34.0 mg/kg for unripe (green) and 34.0 mg/kg for half ripe (green-red) (chlorophyll *a*) and 11.2 μ/g. for unripe (green) and 9.0 μg/g for half ripe (green-red) for chlorophyll *b*. However, to our knowledge, there are not many studies reporting on the extraction of chlorophyll content from gooseberries using conventional green extraction methods.

3.3. Identification and quantification of major anthocyanins by HPLC method

Anthocyanins belong to flavonoid class of compounds and are water soluble pigments commonly found in plant fruits, flowers, leaves and stems (Tan et al., 2022). In nature, anthocyanins are generally present in two main forms; aglycones and glucosides. Amongst them, glycosylated anthocyanins aglycons and glucosides had greater solubility in water, while aglycones has higher solubility in ethanol (Pérez et al., 2021).

Spectral characteristics and chromatography technique were used for identification and quantification of anthocyanins in green and red gooseberry extracts. The results of HPLC analysis are provided in Table 3. In Fig. 1, the HPLC chromatogram of (a) reference standard mixture and (b) gooseberry extracts are shown. The analysed samples proved to contain anthocyanins and antioxidants, as well as other unidentified compounds. Accordingly, as per the results of HPLC analysis, peaks 1, 2, 3 and 4 were identified as rutin, quercetin 3-glucoside, cyanidin 3-glucoside and peonidin 3-glucoside, respectively. All analytes were detected at 260 nm (Fig. 1), however, compounds 3 and 4 provided the best response at 520 nm, which was further chosen for their quantification. Berries with deep colours are rich source of antioxidant compounds and anthocyanins (McDougall et al., 2005; Heinonen et al., 1998).

As shown in Table 3, green gooseberry extracts had the lowest content of compounds of interest with low concentrations of rutin (0.7–1.2 mg/L) and quercetin 3-glucoside (0.9–1.3 mg/L) being detected. In the red gooseberry extracts their amount was slightly higher (1.4–6.9 mg/L and 1.0–1.3 mg/L, respectively), besides, 0.6–6.8 mg/L cyanidin 3-glucoside and 0.32–0.35 mg/L peonidin 3-glucoside were found. The data obtained allows to conclude that extraction of pigments from green and red gooseberries performed using conventional and UAE extraction with water to have exhibited higher yield of targeted compounds such as cyanidin 3-glucoside, peonidin 3-glucoside, rutin, and quercetin 3-glucoside. Conventional extraction technique typically provided higher yields of anthocyanins and other antioxidants, compared to ultrasound-assisted extraction.

3.4. Gooseberry antioxidants

3.4.1. Total phenolic content (TPC)

Results of TPC of green and red gooseberry extracts are shown in Table 4. Significant ($P < 0.05$) differences in the TPC were observed among different extracts. Highest TPC (987.29 mg/100 g) was recorded in the red gooseberries, while the lowest content (161.88 mg/100 g) was found in conventional extraction with water (W-CE-10).

Significant ($P < 0.05$) differences among extracts (UAE extraction with water and ethanol) as well as green and red gooseberries were also observed. Higher content of TPC was found in red gooseberries as compared to green gooseberries. In red gooseberries, TPC ranged from 263.13 to 987.29 mg/100 g. While in green gooseberries, it was from 161.88 to 391.90 mg/100 g. Among different extracts, conventional extraction with ethanol showed highest TPC in red gooseberries followed by UAE extracts with ethanol. In detail, TPC in red gooseberries ranged from 771.88 to 987.29 mg/100 g in E-CE extract, 559.79 to

Table 3
Anthocyanins and antioxidants determined in the extracts by HPLC-MWD-MS.

Extract	Red Gooseberries (mg/L)				Green Gooseberries (mg/L)	
	Cyanidin 3-glucoside	Peonidin 3-glucoside	Rutin	Quercetin 3-glucoside	Rutin	Quercetin 3-glucoside
E-CE-10	0.63 ± 0.01	–	3.7 ± 0.1	1.1 ± 0.1	0.69 ± 0.02	0.94 ± 0.02
E-CE-20	0.9 ± 0.1	–	3.4 ± 0.1	1.06 ± 0.02	0.84 ± 0.02	0.98 ± 0.01
E-CE-30	0.81 ± 0.03	–	3.4 ± 0.04	1.02 ± 0.03	0.8 ± 0.1	0.9 ± 0.1
E-UAE-10	–	–	6.7 ± 0.1	1.3 ± 0.1	0.87 ± 0.03	0.97 ± 0.02
E-UAE-20	–	–	6.9 ± 0.1	1.4 ± 0.1	0.90 ± 0.01	0.96 ± 0.03
E-UAE-30	–	–	6.0 ± 0.2	1.31 ± 0.01	0.86 ± 0.04	1.27 ± 0.04
W-CE-10	5.6 ± 0.1	0.326 ± 0.006	1.43 ± 0.03	1.09 ± 0.01	1.04 ± 0.03	0.86 ± 0.03
W-CE-20	6.4 ± 0.1	0.340 ± 0.002	1.49 ± 0.01	1.18 ± 0.02	1.2 ± 0.1	0.9 ± 0.1
W-CE-30	6.6 ± 0.1	0.354 ± 0.004	1.55 ± 0.02	1.23 ± 0.01	1.1 ± 0.1	0.9 ± 0.1
W-UAE-10	6.6 ± 0.04	0.355 ± 0.001	1.47 ± 0.01	1.12 ± 0.01	1.2 ± 0.1	1 ± 0.1
W-UAE-20	6.3 ± 0.1	0.33 ± 0.01	1.51 ± 0.01	1.16 ± 0.1	1.2 ± 0.1	1 ± 0.1
W-UAE-30	6.8 ± 0.1	0.350 ± 0.004	1.54 ± 0.01	1.17 ± 0.01	1.1 ± 0.1	1 ± 0.1

E-CE-10; conventional extraction with ethanol for 10 min, E-CE-20; conventional extraction with ethanol for 20 min, E-CE-30; conventional extraction with ethanol for 30 min, E-UAE-10; ultrasonic-assisted extraction with ethanol for 10 min, E-UAE-20; ultrasonic-assisted extraction with ethanol for 20 min, E-UAE-30; ultrasonic-assisted extraction with ethanol for 30 min, W-CE-10; conventional extraction with water for 10 min, W-CE-20; conventional extraction with water for 20 min, W-CE-30; conventional extraction with water for 30 min, W-UAE-10; ultrasonic-assisted extraction with water for 10 min, W-UAE-20; ultrasonic-assisted extraction with water for 20 min, W-UAE-30; ultrasonic-assisted extraction with water for 30 min.

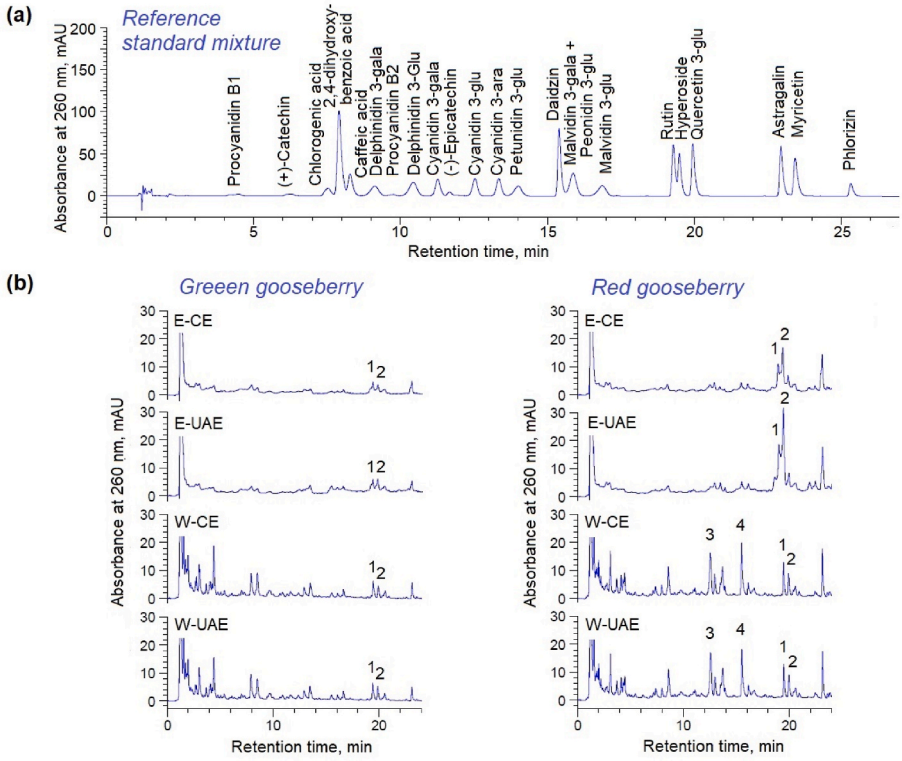

Fig. 1. (a). HPLC chromatograms of reference standard mixture at 260 nm. **(b).** HPLC chromatograms of green gooseberry (left) and red gooseberry (right) extracts at 260 nm. The identified anthocyanins and antioxidants are denoted as follows: 1 – rutin, 2 – quercetin 3-glucoside, 3 – cyanidin 3-glucoside, 4 – peonidin 3-glucoside. E-CE; conventional extraction with ethanol, E-UAE; ultrasonic-assisted extraction with ethanol, W-CE; conventional extraction with water, W-UAE; ultrasonic-assisted extraction with water. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

923.75 mg/100 g in E-UAE extract 270.42 to 332.29 mg/100 g in W-CE extract and 263.13 to 289.58 mg/100 g in W-UAE extract. On the other hand, TPC in green gooseberries were ranged from 304.96 to 391.90 mg/100 g in E-CE extract, 207.44 to 268.13 mg/100 g in E-UAE extract, 161.88 to 198.42 mg/100 g in W-CE extract and 204.05 to 265.03 mg/100 g in W-UAE extract.

Intake of gooseberries can offer possibly higher antioxidant availability that may have potential health benefits. Assessment of TPC is an accepted method to reveal the quantity of accessible antioxidants a sample possibly contain. However, limited research work has been carried out on antioxidants in gooseberries as compared to other berry fruits (Chiang et al., 2013; Duda-Chodak and Tarko, 2007). According to an earlier study by Kähkönen et al. (1999), TPC analysis in crowberry and aronia exhibited 70% more antioxidant content as compared to gooseberries. Another study by (Da Silva Pinto et al., 2010), reported that TPC of gooseberry was lesser than that of black currant. An assessment of our findings with an analysis of phenols and antioxidant activity in various berries shows that berries, such as black currant, chokeberry and bilberry are richer in polyphenols than in gooseberry (Kähkönen et al., 2001). Moreover, in a similar study Chiang et al. (2013) on Tixia gooseberry, a considerably elevated antioxidant activity than that of Invicta gooseberry was reported. These two gooseberry species are of similar shapes, but different in colour. Tixia is of red colour while Invicta is of green colour.

3.4.2. DPPH assay

The health beneficial activity of berries is because of its antioxidant activity which have ability to scavenge free radical produced from oxidation-reduction reaction during the process of metabolism (Sharma et al., 2022). The DPPH assay is routinely employed as a rapid and reliable method that offers an initial indication of radical scavenging potential of a sample extract. The findings of our study showed green and red gooseberry extracts (obtained from UAE and conventional extraction techniques) have radical inhibition activity and can serve as major antioxidants (see Table 4). In berries, including gooseberry fruits, significant amounts of antioxidants are present. Majority of these bioactive antioxidants are polyphenolic compounds and these include flavonols, quercetin, kaempferol, myricetin, coumaric, caffeic, ellagic and hydroxybenzoic acids (Borkowska, 2003; Moyer et al., 2002).

Gooseberry extracts obtained from conventional and UAE techniques with ethanol showed significantly ($P < 0.05$) higher percent inhibition of radical scavenging activity than that of UAE and conventional extract with water. This study also revealed that red gooseberry extract to have significantly ($P < 0.05$) higher percent inhibition of radical scavenging activity when compared with green gooseberry extracts. The red gooseberry extract obtained from conventional extraction technique with ethanol ranged from 90.36 to 91.23%, extract obtained from UAE with ethanol ranged between 89.52 and 90.52%, extract obtained from conventional extraction with water ranged between 86.85 and 90.31%, whereas, extract obtained from UAE with water ranged from 88.47 to 89.43%. On the other hand, green gooseberry extract obtained from conventional extraction technique with ethanol showed a range from 85.86 to 86.26%, extract obtained from UAE with ethanol showed a range between 82.56 and 84.47%, extract obtained from conventional extraction with water ranged between 73.35 and 74.41% and extract obtained from UAE with water showed a range from 74.64 to 77.23%. The results of this study clearly indicate significantly ($P < 0.05$) higher potential in scavenging DPPH radicals to occur in the conventional and UAE technique with ethanol when compared with water extracts.

To support our results, there are some relevant reports available. In a study by Jordheim et al. (2007) 14 different cultivars of European gooseberries were evaluated and accordingly various proportions of 3-rutinoside, 3-glucoside, the 3-xyloside, and 3-glucoside of peonidin were identified. Babbar et al. (2011) screened the extract of kinnow

Table 4
TPC and DPPH estimation of red and green gooseberries extracts obtained from conventional and green extraction methods.

Extract	Green Gooseberry		Red Gooseberry	
	TPC (mg GAE/100 g)	DPPH (% inhibition)	TPC (mg GAE/100 g)	DPPH (% inhibition)
E-CE-10	337.29 ± 1.59^{g}	86.17 ± 0.08^{g}	922.5 ± 8.27^{b}	90.36 ± 0.19^{b}
E-CE-20	304.96 ± 3.44^{h}	85.86 ± 0.17^{h}	771.88 ± 2.17^{c}	90.94 ± 0.31^{a}
E-CE-30	391.90 ± 0.44^{f}	86.26 ± 0.07^{g}	987.29 ± 8.25^{a}	91.23 ± 0.09^{a}
E-UAE-10	260.23 ± 1.9^{m}	82.56 ± 0.07^{k}	608.13 ± 8.68^{d}	90.32 ± 0.09^{b}
E-UAE-20	207.44 ± 0.07^{o}	83.89 ± 0.04^{j}	559.79 ± 2.00^{e}	90.52 ± 0.20^{b}
E-UAE-30	268.13 ± 1.16^{kl}	84.47 ± 0.21^{i}	923.75 ± 4.37^{b}	89.52 ± 0.39^{c}
W-CE-10	161.88 ± 0.75^{r}	74.08 ± 0.15^{o}	332.29 ± 6.88^{g}	86.85 ± 0.04^{f}
W-CE-20	185.08 ± 2.39^{q}	74.41 ± 0.16^{n}	270.42 ± 6.62^{jk}	88.99 ± 0.16^{d}
W-CE-30	198.42 ± 3.25^{p}	73.35 ± 0.07^{p}	331.88 ± 1.87^{g}	90.31 ± 0.06^{b}
W-UAE-10	204.05 ± 0.59^{op}	77.23 ± 0.39^{l}	263.13 ± 3.34^{lm}	89.43 ± 0.55^{c}
W-UAE-20	245.07 ± 1.62^{n}	76.68 ± 0.08^{m}	289.58 ± 2.52^{i}	89.03 ± 0.42^{d}
W-UAE-30	265.03 ± 1.63^{klm}	74.64 ± 0.18^{n}	275.42 ± 2.81^{j}	88.47 ± 0.19^{e}

E-CE-10; conventional extraction with ethanol for 10 min, E-CE-20; conventional extraction with ethanol for 20 min, E-CE-30; conventional extraction with ethanol for 30 min, E-UAE-10; ultrasonic-assisted extraction with ethanol for 10 min, E-UAE-20; ultrasonic-assisted extraction with ethanol for 20 min, E-UAE-30; ultrasonic-assisted extraction with ethanol for 30 min, W-CE-10; conventional extraction with water for 10 min, W-CE-20; conventional extraction with water for 20 min, W-CE-30; conventional extraction with water for 30 min, W-UAE-10; ultrasonic-assisted extraction with water for 10 min, W-UAE-20; ultrasonic-assisted extraction with water for 20 min, W-UAE-30; ultrasonic-assisted extraction with water for 30 min.

(Tangerine) peel, litchi pericarp and banana peel for total phenolic content (TPC) and DPPH· free radical scavenging activities. In this study, methanolic extracts obtained from kinnow peel contained 17.5 mg GAE/g of TPC with 51.7 mg TE/g of antioxidant activity. Similarly, litchi peel extract showed 24.6 mg GAE/g of TPC and 36.42 mg TE/g of antioxidant activity, while banana peel contained 3.8 mg GAE/g of TPC and 5.67 mg TE/g of antioxidant activity (all on d.w. basis). In a comparable study on sea buckthorn pomace extract, Sharma et al. (2022) reported the TPC values to range from 341.02 to 405.58 mg gallic acid equivalent (GAE)/g of extract with DPPH free radical inhibition ranging from 27.50 to 94.16 %. In another study, Sharma and Bhat (2021) studied the carotenoid content in the extracts of pumpkin peels and reported TPC to range from 535.58 to 588.68 mg gallic acid equivalent (GAE)/g of extract and with DPPH free radical assay ranging between 91.35 and 93.53 % inhibition.

Previously, Chiang et al. (2013) reported stronger scavenging DPPH· radicals in Tixia (red gooseberries) than that of Invicta (green gooseberries). Their findings are comparable with our results. In addition, it is worth to note that differences in the DPPH assay results can depend on the difference in cultivar, extraction technique, solvent used and extraction time.

3.5. Colour analysis of green and red gooseberry extracts

Colour is a major contributor of visual appeal that can enhance the market value and demand of any food products (Sharma et al., 2022). Green and red gooseberries are rich in natural pigments; hence, the extraction of anthocyanins in water and ethanol significantly ($P < 0.05$) increased the colour in extracts. As shown in Table 5, there was a high value of L^* in W-UAE, extracts (28.08–36.76) followed by W-CE extract (28.25–31.09). Whereas, lowest values of lightness (L*) was recorded in case of E-CE extracts (13.20–17.09) followed by E-UAE extract (17.68–24.09). This can be justified by reflecting the higher yield of chlorophyll and anthocyanin content in E-CE and E-UAE extracts and poor yield in W-CE and W-UAE extracts.

4. Conclusions

Green extraction methods (UAE and conventional extraction techniques) combined with green extraction solvents (water and ethanol) were successfully employed for the extraction of natural pigments from European green and red gooseberries. Data obtained from HPLC analysis showed that conventional extraction and UAE with water to exhibit higher yield of targeted anthocyanins. Results of this study promote utilization of green extraction techniques and green solvents for extraction of natural pigments (anthocyanins), thus playing a significant part in contributing regarding a cleaner environment. Consequently, the established procedure is an innovative one with an environment friendly

Table 5
Colour attributes of red and green gooseberries extracts obtained from conventional and green extraction methods.

Extract	Colour attributes					
	Green gooseberries			Red gooseberries		
	L*	a*	b*	L*	a*	b*
E-CE-10	14.39 ± 0.79^{efg}	6.62 ± 0.57^{hi}	25.24 ± 0.87^{bcd}	6.75 ± 0.19^{kl}	25.78 ± 1.11^{d}	11.05 ± 0.7^{fg}
E-CE-20	17.09 ± 1.25^{def}	6.88 ± 0.67^{ghi}	26.47 ± 0.89^{bcd}	4.03 ± 0.13^{lm}	18.26 ± 0.67^{e}	6.47 ± 0.32^{gh}
E-CE-30	13.20 ± 0.84^{ghi}	5.85 ± 0.44^{i}	20.81 ± 0.75^{de}	1.56 ± 0.05^{m}	7.73 ± 0.31^{fghi}	2.46 ± 0.19^{h}
E-UAE-10	24.09 ± 1.21^{c}	9.41 ± 0.83^{fghi}	35.36 ± 1.08^{a}	13.55 ± 0.34^{fgh}	34.19 ± 1.02^{bc}	22.79 ± 0.43^{cd}
E-UAE-20	18.08 ± 1.08^{d}	7.86 ± 0.51^{fghi}	27.21 ± 1.01^{bc}	8.50 ± 0.32^{k}	27.17 ± 0.85^{d}	14.02 ± 0.29^{f}
E-UAE-30	17.68 ± 1.23^{de}	8.98 ± 0.39^{fghi}	27.90 ± 0.94^{bc}	10.07 ± 0.43^{hijk}	27.26 ± 0.87^{d}	16.70 ± 0.54^{ef}
W-CE-10	31.09 ± 0.51^{b}	10.63 ± 0.6^{f}	28.65 ± 0.72^{bc}	9.46 ± 0.72^{jk}	31.56 ± 0.83^{c}	15.92 ± 0.16^{ef}
W-CE-20	30.41 ± 1.11^{b}	10.18 ± 0.57^{fgh}	29.84 ± 0.67^{ab}	10.01 ± 0.63^{hijk}	31.58 ± 1.09^{c}	16.44 ± 0.22^{ef}
W-CE-30	28.25 ± 0.46^{b}	9.36 ± 0.19^{Fghi}	27.56 ± 0.95^{bc}	9.72 ± 0.47^{ijk}	31.96 ± 1.21^{c}	16.38 ± 0.47^{ef}
W-UAE-10	36.76 ± 0.82^{a}	10.23 ± 0.41^{fg}	31.06 ± 1.13^{ab}	15.91 ± 0.76^{defg}	38.33 ± 1.45^{a}	26.93 ± 0.93^{bc}
W-UAE-20	30.10 ± 1.18^{b}	8.83 ± 0.45^{fghi}	27.42 ± 0.81^{bc}	15.09 ± 0.66^{defg}	38.04 ± 1.61^{a}	25.67 ± 0.67^{bcd}
W-UAE-30	28.08 ± 1.67^{b}	8.79 ± 0.61^{fghi}	26.95 ± 0.59^{bc}	13.05 ± 0.58^{ghij}	35.91 ± 1.36^{ab}	23.09 ± 0.82^{cd}

E-CE-10; conventional extraction with ethanol for 10 min, E-CE-20; conventional extraction with ethanol for 20 min, E-CE-30; conventional extraction with ethanol for 30 min, E-UAE-10; ultrasonic-assisted extraction with ethanol for 10 min, E-UAE-20; ultrasonic-assisted extraction with ethanol for 20 min, E-UAE-30; ultrasonic-assisted extraction with ethanol for 30 min, W-CE-10; conventional extraction with water for 10 min, W-CE-20; conventional extraction with water for 20 min, W-CE-30; conventional extraction with water for 30 min, W-UAE-10; ultrasonic-assisted extraction with water for 10 min, W-UAE-20; ultrasonic-assisted extraction with water for 20 min, W-UAE-30; ultrasonic-assisted extraction with water for 30 min.

approach that can be implemented for extracting natural pigments (bioactive) not only from gooseberries, but also from other raw materials, such as those of agri-food industrial processing wastes and by-products. Our results support consumers demand and opens up the avenue to explore pigments as natural colourants in food and cosmetics applications.

CRediT authorship contribution statement

Shehzad Hussain: Investigations, Methodology, Data collection, Writing. **Minaxi Sharma:** Investigations and Data collection. **Tatsiana Jarg:** Investigations and Data collection. **Riina Aav:** Methodology, Supervision. **Rajeev Bhat:** Conceptualization, Visualization, Reviewing, Editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rajeev Bhat reports financial support was provided by Estonian University of Life Sciences. Riina Aav reports financial support was provided by Tallinn University of Technology.

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 810630 (VALORTECH). In addition, funding was received from Mobilitas Pluss ERA-Chair support [Grant No. MOBEC006 ERA Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences]. Author (RB) acknowledges base funding of the Estonian University of Life Sciences (No. P210162VLTQ) provided to support research and development activities. The authors (from Tallinn University of Technology) thank Estonian Research Council Grant PRG399 and European Regional Development Fund (CoE 2014-2020.4.01.15-0013).

References

Amchova, P., Kotolova, H., Ruda-Kucerova, J., 2015. Health safety issues of synthetic food colorants. Regul. Toxicol. Pharmacol. 73, 914–922. https://doi.org/10.1016/j.yrtph.2015.09.026.

Anastasiadi, M., Mwangi, P.M., Ordaz-Ortiz, J.J., Redfern, S.P., Berry, M., Simmonds, M. S., Terry, L.A., 2016. Tissue biochemical diversity of 20 gooseberry cultivars and the effect of ethylene supplementation on postharvest life. Postharvest Biol. Technol. 117, 141–151. https://doi.org/10.1016/j.postharvbio.2016.02.008.

Babbar, N., Oberoi, H.S., Uppal, D.S., Patil, R.T., 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int. 44, 391–396. https://doi.org/10.1016/j.foodres.2010.10.001.

Bachalla, N., 2016. Identification of synthetic food colors adulteration by paper chromatography and spectrophotometric methods. Int. Arch. Integ. Med. 3, 182–191.

Belwal, T., Ezzat, S.M., Rastrelli, L.I., Bhatt, D., Daglia, M., Baldi, A., Devkota, H.P., Orhan, I.E., Patra, J.K., Das, G., Anandharamakrishnan, C., 2018. A critical analysis of extraction techniques used for botanicals: trends, priorities, industrial uses and optimization strategies. TrAC Trends Anal. Chem. 100, 82–102. https://doi.org/10.1016/j.trac.2017.12.018.

Borkowska, J., 2003. Fruits and vegetables as source of natural antioxidant. Przem. Ferm. Ow. Warz. 5 (11.12).

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A.S., Strube, J., Uhlenbrock, L., Gunjevic, V., Cravotto, G., 2019. Green extraction of natural products. Origins, current status, and future challenges. TrAC Trends Anal. Chem. 118, 248–263. https://doi.org/10.1016/j.trac.2019.05.037.

Chiang, C.J., Kadouh, H., Zhou, K., 2013. Phenolic compounds and antioxidant properties of gooseberry as affected by in vitro digestion. LWT-Food Sci. Technol. 1 (51) https://doi.org/10.1016/j.lwt.2012.11.014, 417-322.

Da Silva Pinto, M., Kwon, Y.I., Apostolidis, E., Lajolo, F.M., Genovese, M.I., Shetty, K., 2010. Evaluation of red currants (*Ribes rubrum L.),* black currants (*Ribes nigrum* l.), red and green gooseberries (*Ribes uva-crispa*) for potential management of type 2 diabetes and hypertension using *in vitro* models. J. Food Biochem. 34, 639–660. https://doi.org/10.1111/j.1745-4514.2009.00305.x.

Dafallah, A.A., Abdellah, A.M., Abdel-Rahim, E.A., Ahmed, S.H., 2015. Physiological effects of some artificial and natural food coloring on young male albino rats. J. Food Technol. Res. 2, 21–32. https://doi.org/10.18488/journal.58/2015.2.2/58.2.21.32.

Duda-Chodak, A., Tarko, T., 2007. Antioxidant properties of different fruit seeds and peels. Acta Sci. Pol. Technol. Aliment. 6, 29–36.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Mérillon, J.M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57 (5), 1768–1774.

Fang, J., 2015. Classification of fruits based on anthocyanin types and relevance to their health effects. Nutrition 31 (11–12), 1301–1306. https://doi.org/10.1016/j.nut.2015.04.015.

Gengatharan, A., Dykes, G.A., Choo, W.S., 2015. Betalains: Natural plant pigments with potential application in functional foods. LWT-Food Sci. Technol. 64 (2), 645–649.

Gentili, R., Fenu, G., Mattana, E., Citterio, S., De Mattia, Bacchetta, G., 2015. Conservation genetics of two island endemic Ribes spp.(Grossulariaceae) of Sardinia: survival or extinction? Plant Biol. 17 (5), 1085–1094.

Giusti, M.M., Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. Curr. Protoc. Food Anal. Chem. 1, F1–F2. https://doi.org/10.1002/0471142913.faf0102s00.

Goti, D., Dasgupta, S., 2023. A comprehensive review of conventional and non-conventional solvent extraction techniques. J. Pharmacogn. Phytochem. 12, 202–211. https://doi.org/10.22271/phyto.2023.v12.i3c.14682.

Häkkinen, S.H., Kärenlampi, S.O., Heinonen, I.M., Mykkänen, H.M., Törrönen, A.R., 1999b. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J. Agric. Food Chem. 47, 2274–2279. https://doi.org/10.1021/jf9811065.

Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., Törrönen, R., 1999a. Screening of selected flavonoids and phenolic acids in 19 berries. Food Res. Int. 32, 345–353. https://doi.org/10.1016/S0963-9969(99)00095-2.

He, J., Giusti, M., 2010. Anthocyanins: natural colorants with health-promoting properties. Annu. Rev. Food Sci. Technol. 1, 163–187. https://doi.org/10.1146/annurev.food.080708.100754.

Heinonen, I.M., Lehtonen, P.J., Hopia, A.I., 1998. Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem. 46, 25–31. https://doi.org/10.1021/jf970489o.

Jeana, G., 1983. Chlorophyll and carotenoid pigments in Ribes fruits. Sci. Hortic. 18, 131–136. https://doi.org/10.1016/0304-4238(82)90127-3.

Jordheim, M., Måge, F., Andersen, Ø.M., 2007. Anthocyanins in berries of Ribes including gooseberry cultivars with a high content of acylated pigments. J. Agric. Food Chem. 55, 5529–5535. https://doi.org/10.1021/jf0709000.

Kähkönen, M.P., Hopia, A.I., Heinonen, M., 2001. Berry phenolics and their antioxidant activity. J. Agric. Food Chem. 49, 4076–4082. https://doi.org/10.1021/jf010152t.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47, 3954–3962. https://doi.org/10.1021/jf990146l.

Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I., Kontominas, M.G., 2013. Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses. Food Res. Int. 54, 1950–1958. https://doi.org/10.1016/j.foodres.2013.09.023.

Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M., 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr. Res. 61, 1361779 https://doi.org/10.1080/16546628.2017.1361779.

Määttä-Riihinen, K.R., Kamal-Eldin, A., Mattila, P.H., González-Paramás, A.M., Törrönen, A.R., 2004. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. J. Agric. Food Chem. 52, 4477–4486. https://doi.org/10.1021/jf049595y.

Mahdavi, S.A., Jafari, S.M., Assadpour, E., Ghorbani, M., 2016. Storage stability of encapsulated barberry's anthocyanin and its application in jelly formulation. J. Food Eng. 181, 59–66. https://doi.org/10.1016/j.jfoodeng.2016.03.003.

McDougall, G.J., Fyffe, S., Dobson, P., Stewart, D., 2005. Anthocyanins from red wine e their stability under simulated gastrointestinal digestion. Photochemistry 66, 2540–2548. https://doi.org/10.1016/j.phytochem.2005.09.003.

Minguez-Mosquera, M.I., Rejano, L., Gandul, B., Sanchez, A.H., Garrido, J., 1991. Color-pigments correlation in virgin olive oil. J. Am. Oil Chem. Soc. 68, 322–337. https://doi.org/10.1007/BF02657688.

Miranda, P.H., dos Santos, A.C., de Freitas, B.C., de Souza-Martins, G.A., Boas, E.V., Damiani, C.A., 2021. A scientific approach to extraction methods and stability of pigments from Amazonian fruits. Trends Food Sci. Technol. 113, 335–345. https://doi.org/10.1016/j.tifs.2021.04.047.

Mishra, V.K., Bacheti, R., Husen, A., Le, H., Salcedo, A.E., 2011. Medicinal uses of chlorophyll: a critical overview. In: Le, H., Salcedo, E. (Eds.), Chlorophyll: Structure, Function and Medicinal Uses. Hua Le and Elisa Salcedo. Nova Science Publishers, Inc., Hauppauge, NY, pp. 177–196, 11788 (ISBN 978-1- 62100-015-0).

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., Wrolstad, R.E., 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, Rubus, and Ribes. J. Agric. Food Chem. 50, 519–525. https://doi.org/10.1021/jf011062r.

Ngamwonglumlert, L., Devahastin, S., Chiewchan, N., 2017. Natural colorants: pigment stability and extraction yield enhancement via utilization of appropriate

pretreatment and extraction methods. Crit. Rev. Food Sci. Nutr. 57, 3243–3259. https://doi.org/10.1080/10408398.2015.1109498.

Novais, C., Molina, A.K., Abreu, R.M., Santo-Buelga, C., Ferreira, I.C., Pereira, C., Barros, L., 2022. Natural food colorants and preservatives: a review, a demand, and a challenge. J. Agric. Food Chem. 70, 2789–2805. https://doi.org/10.1021/acs.jafc.1c07533.

Panić, M., Gunjević, V., Cravotto, G., Radojčić Redovniković, I., 2019. Enabling technologies for the extraction of grape-pomace anthocyanins using natural deep eutectic solvents in up-to-half-litre batches extraction of grape-pomace anthocyanins using NADES. Food Chem. 300, 125185 https://doi.org/10.1016/j.foodchem.2019.125185.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis, G.R., 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem. 1023, 777–783. https://doi.org/10.1016/j.foodchem.2006.06.021.

Pauli, H., 1976. Proposed extension of the CIE recommendation on uniform colour spaces, colour difference equations, and metric colour terms. J. Opt. Soc. Am. 66 8, 866–867. https://doi.org/10.1364/JOSA.66.000866.

Pérez, B., Endara, A., Garrido, J., Ramírez-Cárdenas, L., 2021. Extraction of anthocyanins from mortiño (*Vaccinium floribundum*) and determination of their antioxidant capacity. Rev. Fac. Nac. Agron. Medellín 74, 9453–9460. https://doi.org/10.15446/rfnam.v74n1.89089.

Roriz, C.L., Barros, L., Prieto, M.A., Morales, P., Ferreira, I.C., 2017. Floral parts of Gomphrena globosa L. as a novel alternative source of betacyanins: optimization of the extraction using response surface methodology. Food Chem. 15, 223–234. https://doi.org/10.1016/j.foodchem.2017.02.073.

Sadar, P., Dande, P., Kulkarni, N., Pachori, R., 2017. Evaluation of toxicity of synthetic food colors on human normal flora and yeast. Int. J. Health Sci. Res. 7, 110–114.

Sharma, M., Bhat, R., 2021. Extraction of carotenoids from pumpkin peel and pulp: comparison between innovative green extraction technologies (ultrasonic and microwave-assisted extractions using corn oil). Foods 10, 787. https://doi.org/10.3390/foods10040787.

Sharma, M., Hussain, S., Shalima, T., Aav, R., Bhat, R., 2022. Valorization of seabuckthorn pomace to obtain bioactive carotenoids: an innovative approach of using green extraction techniques (ultrasonic and microwave-assisted extractions) synergized with green solvents (edible oils). Ind. Crops Prod. 175, 114257 https://doi.org/10.1016/j.indcrop.2021.114257.

Sharmila, G., Muthukumaran, C., Suriya, E., Muppidathi Keerthana, R., Kamatchi, M., Kumar, N.M., Anbarasan, T., Jeyanthi, J., 2019. Ultrasound aided extraction of yellow pigment from Tecoma castanifolia floral petals: optimization by response surface method and evaluation of the antioxidant activity. Ind. Crops Prod. 130, 467–477. https://doi.org/10.1016/j.indcrop.2019.01.008.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J Enol. Vitic. 16 (3), 144–158.

Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S., Xue, H., 2022. Extraction and purification of anthocyanins: a review. J. Agric. Food Res. 8, 100306 https://doi.org/10.1016/j.jafr.2022.100306.

Turkiewicz, I.P., Wojdyło, A., Tkacz, K., Nowicka, P., 2020. Carotenoids, chlorophylls, vitamin E and amino acid profile in fruits of nineteen Chaenomeles cultivars. J. Food Compos. Anal. 93, 103608 https://doi.org/10.1016/j.jfca.2020.103608.

Velázquez, E., Tournier, H., de Buschiazzo, P.M., Saavedra, G., Schinella, G., 2003. Antioxidant activity of Paraguayan plant extracts. Fitoterapia 74, 91–97. https://doi.org/10.1016/S0367-326X(02)00293-9.

Wani, F.A., Rashid, R., Jabeen, A., Brochier, B., Yadav, S., Aijaz, T., Makroo, H.A., Dar, B. N., 2021. Valorisation of food wastes to produce natural pigments using non-thermal novel extraction methods: a review. Int. J. Food Sci. Technol. 56, 4823–4833. https://doi.org/10.1111/ijfs.15267.

Zhang, A., Han, Y., Zhou, Z., 2023. Characterization of citric acid cross-linked chitosan/gelatin composite film with enterocin CHQS and red cabbage pigment. Food Hydrocolloids 135, 108144. https://doi.org/10.1016/j.foodhyd.2022.108144.