Available Online

JSR Publications

J. Sci. Res. **14** (2), 569-581 (2022)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Ultrasonic-Assisted Extraction of Phytochemicals from Eggplant Peel: Physicochemical and Antibacterial Evaluation

S. Sharma, A. K. Ojha, S. Chakkaravarthi, B. Bhattacharya*

Department of Basic and Applied Sciences, National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sector - 56, HSIIDC Industrial Estate, Kundli, Sonipat 131028, Haryana, India

Received 15 September 2021, accepted in final revised form 16 January 2022

Abstract

The present study aimed to prepare and characterize eggplant peel extract (EPE) with suitability for application in the food industry. EPE was prepared *via* ultrasound-assisted extraction method employing ethanol as an extraction solvent, then reconstituted in water. The total phenolic content, anthocyanin content, and antioxidant activity of EPE in an organic and aqueous solvent were determined. In EPE, the major anthocyanin, delphinidin-3-rutinoside, was quantified *via* LC-MS/MS. The agar well diffusion assay and minimum inhibitory concentration (MIC) determination method were used to analyze the antibacterial activity of EPE against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. There was no significant difference ($p>0.05$) in the total phenolic content, anthocyanin content, and antioxidant activity of EPE when reconstituted in water. The inhibition zones of EPE (at 100 mg/mL concentration) ranged from 15.6 to 21.6 mm, whereas MIC ranged from 2.34 to 4.68 mg/mL against all tested bacteria. The lower values of MIC for *B. cereus* and *S. aureus* indicated that EPE was more effective for gram-positive bacterial strains. The water-reconstituted EPE with significant antioxidant and antibacterial activity would have potential food industrial applications, like food packaging, nutraceuticals, and functional foods.

Keywords: Eggplant peel extract; Anthocyanin; LC-MS/MS; Antioxidant activity; Antibacterial activity.

doi: http://dx.doi.org/10.3329/jsr.v14i2.55675 J. Sci. Res. **14** (2), 569-581 (2022)

1. Introduction

Eggplant (*Solanum melongena*) is one of the top ten antioxidant-rich vegetables. Eggplant fruit has perishable nature due to its high susceptibility to pests, nematodes, rot, and several plant diseases [1]. It is largely damaged by the infestation of Lepidopteron insects. Deterioration of eggplant reduces the market value of fruit and eventually leads to high economic loss [2]. The insect pest mostly eats its way toward the middle of the fruit and feeds on the pulp and seed of the eggplant. The eggplant peel is affected less than other parts of the fruit. The peel of the eggplant is a valuable by-product. The comparative

* *Corresponding author*: bhaswati.niftem@gmail.com

studies conducted on different parts of eggplant (peel, pulp, leaf, stem, and calyx) showed that the peel part had the highest anthocyanin and polyphenol contents, among other parts, and the second-highest antioxidant activity after calyx [3,4]. It has been reported that the main phenolic compounds in the flesh and peel of eggplant are chlorogenic acid and delphinidin, respectively [5]. Delphinidin-3-rutinoside has been reported as the major anthocyanin present in eggplant peel (ranging from 74 to 84 %) [6-8].

There are limited papers on the eggplant peel. In most papers, organic solvents such as acetone, ethanol, and methanol have been used to extract bioactive constituents from eggplant peels [6-9]. The papers available on the eggplant peel extract (EPE) provided the phytochemicals analysis (phenolic compounds, antioxidant activity, and antibacterial activity) of the organic solvent-based extract of eggplant peel. However, the analysis result would not be helpful if the future applicability of the extract is in the food system. The organic solvent limits the applicability of the extract into food due to the threatening effect of organic solvent on human health [10,11].

There was an attempt to prepare an organic solvent-free EPE using calcium-based extraction using the food-grade solution of calcium salt [12], but the reported phenolic content and antioxidant activity were significantly less than data reported for EPE prepared with organic solvent and water [3,6,13]. Also, the usage of water alone as an extraction solvent was not found as effective as the organic solvent (aqueous ethanol) in releasing the phytochemicals from the eggplant peel [3,4].

Therefore, preparation and chemical analysis of an organic solvent-free EPE abundant in bioactive compounds is the need of the hour, especially in the food industry. Hence, in the present study, aqueous ethanol was used as an extraction solvent for the efficient release of the phytochemicals, but the extraction solvent was removed successively, and EPE was reconstructed in water. The drying mode affects the phytochemicals and antioxidant activity of the plants [14]. In many studies, the freeze-drying process has been reported as an efficient method for the retention of phytochemicals and improving the antioxidant activity of plant extract [15-17]. In contrast, ultrasonication-assisted extraction is a 'green' and effective technology for efficiently releasing polyphenolic compounds [18]. Hence, the freeze-drying process (instead of oven or sun drying) for eggplant peel and the ultrasonication-assisted extraction was employed to ensure the efficient release of phytochemicals. The present study also provided a comparative analysis of phenolic content and antioxidant activity of ethanolic EPE and water-reconstituted EPE to check the effect of the reconstitution on these properties. Further, EPE was characterized *via* triple quadrupole liquid chromatography with a tandem mass spectrometry (LC-MS/MS) system for qualitative and quantitative analysis of delphinidin-3-rutinoside.

To the best of the authors' knowledge, no previous publication has reported the minimum inhibitory concentration (MIC) of EPE against foodborne bacterial pathogens. Neither zone of inhibition was reported for water-reconstituted EPE. Therefore, the antibacterial activity of EPE was assessed *via* measurement of the zone of inhibition and

MIC against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium.

2. Materials and Methods

2.1. *Materials*

Long purple-colored eggplant (PUSA Shyamala) was obtained from the Indian Agricultural Research Institute (IARI), New Delhi, India. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, citric acid, and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt were purchased from Sigma-Aldrich, Merck, Maharashtra, India. Delphinidin-3-rutinoside chloride (≥95 % purity, Sigma-Aldrich, Maharashtra, India) was used as standard in LC-MS/MS. Other chemicals were of analytical grade and acquired from Hi-Media, Maharashtra, and SRL Pvt. Ltd., Haryana, India.

Four bacterial strains: two Gram-positive *Bacillus cereus* (NCDC240), *Staphylococcus aureus* (NCDC109), and two Gram-negative *Escherichia coli* (ATCC5922) and *Salmonella* Typhimurium (MTCC98), were used as a test organism for antibacterial activity study. Tryptone soya agar (TSA) and tryptone soya broth (TSB) were procured from Hi-media, Maharashtra, India.

2.1. *Preparation of eggplant peel extract (EPE)*

The extraction was carried out as per the method of Rabelo *et al*. [19] with some modifications. The eggplant was pre-washed with tap water to remove dirt, followed by surface sterilization with 200 ppm sodium hypochlorite, and finally washed with distilled water. After washing, eggplant was peeled using a sharp knife. The peel was freeze-dried (Mini Lyodel; Delvac Pumps, Chennai, India) with plate temperature at -42 °C for 48 h at 0.10 mbar pressure. The freeze-dried eggplant peel was ground using a mixer-grinder (Sujata Dynamix, Delhi, India). The eggplant peel powder (1 g) was added with 70 % aqueous ethanol solution containing 0.1 % citric acid. After 3 h stirring of the obtained mixture with a magnetic stirrer, the ultrasonication was performed at 45 °C for 2 h in an ultrasonic bath (Bransonic CPXH; Branson Ultrasonics, Connecticut, USA). The obtained mixture was centrifuged at 6000×*g* for 5 min at 4 °C. The collected supernatant was filtered through a syringe filter (0.45 µm) to obtain ethanolic EPE.

To prepare water-reconstituted EPE, the ethanolic EPE was placed in an oven, and the solvent was evaporated at 40 °C. After evaporating the solvent, the extract was suspended with water and placed in the oven at 40 °C for removal of solvent for one more time. After evaporation, the dried matter was weighed and again resuspended in water to prepare the desired concentration. Finally, both ethanolic EPE and water-reconstituted EPE were stored in glass vials at 4 °C and subjected to the analysis of total phenolic

content (TPC), total anthocyanin content (TAC), and antioxidant activity. The extraction yield percentage was calculated using the following Eq. (1).

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dry extract (g)}}{\text{weight of dry peel powder (g)}} \times 100 \quad (1)$$

2.3. *Total phenolics*

The TPC in the EPE was determined using Folin's colorimetric assay according to the method described by Kaur *et al*. [20] with slight modification. Individual sample extract (0.5 mL) was briefly mixed with Folin–Ciocalteu diluted reagent (0.25 mL). After 5 min, 20 % sodium carbonate solution (1.25 mL) was added and placed in a water bath for 10 min at 50 °C. After cooling down, the UV absorbance of the reaction mixtures was measured at 750 nm using a UV–Vis spectrophotometer (UV-2600; Shimadzu, Kyoto, Japan). The results were shown as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g) on a dry mass basis.

2.4. *Total anthocyanins*

The TAC in EPE was measured using the pH-differential method [8,21]. The TAC was calculated using Eq. (2) and expressed on a dry mass basis as mg of delphinidin-3-glucoside (Del-3-glc or D3G) equivalents per gram of sample extract (mg D3G/g).

$$\text{TAC (mg D3G/g)} = \frac{A \times M \times DF \times V \times 10^3}{\varepsilon \times l \times m} \quad (2)$$

where A is the absorbance value=(A_{520} - A_{700} at pH 1.0) − (A_{520} - A_{700} at pH 4.5), M is the molecular mass of Del-3-glc (465 g/mol), DF is the dilution factor, V is the volume of sample extract in a liter, 10^3 is the conversion factor from gram to the milligram, ε is the molar absorption coefficient of Del-3-glc (29,000 L/mol/cm), l is the path length of the cuvette (1 cm), and m is mass of sample in gram.

2.5. *Antioxidant activity*

The antioxidant activity of EPE was evaluated by 3 assays, namely: DPPH, ABTS, and cupric reducing antioxidant capacity (CUPRAC) assays. Trolox was used as a reference standard for the standard curve for all three assays. The antioxidant activity of EPE was exhibited on a dry mass basis in µmol Trolox equivalents (TE) per gram of sample extract (µmol TE/g).

2.5.1. *DPPH assay*

The sample extract (0.1 mL) was mixed with 0.1 mM DPPH ethanolic solution (1 mL) [3]. After 30 min incubation, the absorbance was measured at 515 nm. The control contained 1 mL DPPH solution with respective solvents of extracts (aqueous ethanol and

water) instead of sample extract. Ethanol (1 mL) with 0.1 mL of respective solvents of extracts was taken as a reference.

2.5.2. *ABTS assay*

Briefly, the $ABTS^{\cdot+}$ working solution was prepared by diluting with respective solvent of extract to give an absorbance of 1-1.7 at 734 nm. 1 mL of $ABTS^{\cdot+}$ working solution was mixed with 0.1 mL of the sample extract. After 30 min incubation in the dark, the absorbance was measured at 734 nm. The control contained mixture of $ABTS^{\cdot+}$ solution (1 mL) and respective solvents of extracts (0.1 mL) [20].

2.5.3. *CUPRAC assay*

The method of Kaur *et al*. [20] was followed to perform the CUPRAC assay. The absorbance of the sample and reference was read at 450 nm (UV-2600; Shimadzu).

2.6. *LC-MS/MS analysis of eggplant peel extract*

LC-MS/MS system (API3000 Triple quadrupole mass spectrometer; ABSciex, California, USA) was used to identify and quantify delphinidin-3-rutinoside in water-reconstituted EPE [22,23]. The LC-MS/MS system was equipped with an electrospray ionization source (ESI) and a triple quadrupole-ion trap mass analyzer. The Analyst 1.6.2 software was used for data analysis. Nitrogen was used as curtain and collision gas. The standard solution and sample were injected 2 times. Detection was done in multiple reaction monitoring (MRM) mode. The amount of delphinidin-3-rutinoside compound in EPE was expressed on a dry mass basis as mg of delphinidin-3-rutinoside per 100 g of the sample (mg/100 g).

2.7. *Effect of pH variation on eggplant peel extract*

To assess the stability and color intensity of EPE at different pH conditions, the extracted sample was added to the solutions (0.1 M NaOH and 0.1 M HCl) in the pH ranges of 1–13 [24]. The color change of each sample was visually observed. The UV-Vis spectra of EPE in pH values of 1–12 were measured in the range of 400–800 nm.

2.8. *Antibacterial activity*

The agar well diffusion assay was used to evaluate the antibacterial activity of water-reconstituted EPE, whereas the broth microdilution technique was used to determine its MIC.

2.8.1. *Agar well diffusion assay*

Agar well diffusion method was used according to the method described by Basudan [25], with some modifications. Briefly, EPE was sterilized through a syringe filter (0.2 µm).

TSA plates were inoculated by spreading 100 µL of the actively grown bacteria cell suspension (adjusted to the turbidity of 0.5 McFarland standard). A sterile cork borer was used to make wells of 8 mm diameter, and 100 µL of the EPE at different concentrations (100, 75, 50, 25, 12.5 mg/mL) was introduced into the respective wells. The extract was allowed to diffuse for 1 h at room temperature. Standard antibiotic tetracycline (10 mg/mL) was used as a positive control. The inoculated agar plates were incubated (Forma 4111TS incubator; Thermo Fischer Scientific, Ohio, US) aerobically (37 °C, 24 h). The presence of inhibition zones was considered as an indication for antibacterial activity.

2.8.2. *Broth microdilution method*

The MIC of water-reconstituted EPE was determined by the broth microdilution technique following the Clinical and Laboratory Standards Institute (CLSI) [26,27]. Briefly, a series of ten dilutions of the extract ranging from 150 to 0.292 mg/mL were analyzed. The inoculums of test strains prepared from fresh overnight cultures were set to 0.5 McFarland standard, equivalent to $1\text{-}2\times10^8$ CFU/mL. The inoculums were diluted further in a 1:100 ratio using sterile TSB. A 50 µL of EPE (150 mg/mL) was serially two-fold diluted in wells of a microtiter plate (96-wells) containing 50 µL TSB. Wells were inoculated with 50 µL of the standard culture of bacteria. The inoculated plates were incubated at 37 °C in a moist condition. The well containing bacterial inoculums and broth served as a positive control, whereas the well-containing broth only served as a negative control. After 18 h, the optical density at 620 nm was measured with an ELISA microplate reader (Infinite F50; Tecan, Männedorf, Switzerland). MIC values were calculated as the minimum concentration (mg/mL) of EPE capable of inhibiting the bacteria growth.

2.9. *Statistical analysis*

Studies were performed in triplicate. Data were expressed as mean±standard deviation (SD) (N=3). The significance was determined with the student's t-test ($p<0.05$) using the Microsoft Excel software (2010), where a probability of $p<0.05$ was considered significant.

3. Results and Discussion

3.1. *Extraction yield, TPC, and TAC*

Table 1 summarizes data on the determination of TPC and TAC of EPE before and after reconstitution with water.

Table 1. Total phenolic content (TPC) and total anthocyanin content (TAC) in eggplant peel extracts.

Sample	TPC (mg GAE/g)	TAC (mg D3G/g)
Ethanolic EPE	$(39.02\pm0.08)^{a}$	$(11.55\pm0.02)^{b}$
Water-reconstituted EPE	$(38.89\pm0.02)^{a}$	$(11.54\pm0.01)^{b}$

Mean value±SD (*N*=3). Same letter in the same column represents no significant difference ($p>0.05$). EPE=Eggplant peel extract, GAE=gallic acid equivalent, D3G=delphinidin-3-glucoside.

The TPC and TAC of EPE before and after reconstitution with water didn't show any significant difference ($p>0.05$). TPC value of ethanolic EPE and water-reconstituted EPE was found to be 39.02±0.08 and 38.89±0.02 mg GAE/g, respectively. TPC value of EPE in the current study was found to be substantially higher than the TPC content of 13.64 mg GAE/g reported by Condurache *et al.* [6] for a similar kind of extract (ethanolic extract of eggplant peel), 23.01-29.01 mg GAE/g by Ferarsa *et al.* [28] for acidified water extract of eggplant peel; and 13.64 and 14.72 mg GAE/g, respectively reported for the ethanolic and methanolic extract of eggplant peel by Horincar *et al.* [13]. Rochín-Medina *et al.* [12] prepared EPE by solvent-free calcium-based extraction and reported TPC of 0.48-1.24 mg GAE/g, which is significantly very meager in comparison to the current study. On the other hand, Jung *et al.* [3] reported quite high TPC for aqueous and ethanolic extracts of EPE resuspended in dimethyl sulfoxide, *i.e.*, 54.94 and 55.19 mg GAE/g, respectively. This could be due to the nature of the solvent, DMSO being a polar aprotic solvent that probably acts as a better resuspension solvent for solubilizing the bioactive compounds than the latter, which is a polar protic solvent [29].

The TAC of EPE before and after reconstitution with water was found to be 11.55±0.02 and 11.54±0.01 mg D3G/g, respectively. There are limited studies in which Del-3-glc was used as a reference for calculating TAC in eggplant peel. The total anthocyanin content in the present study was determined to be higher than 0.45 mg D3G/g reported by Sadilova *et al.* [8] for aqueous acetone-based EPE; 0.58 mg D3G/g reported by Condurache *et al.* [6] for ethanolic extract of eggplant peel; and 0.58 mg D3G/g and 0.74 mg D3G/g reported for ethanol and methanol extract of eggplant peel, respectively by Horincar *et al.* [13].

The extraction yield for EPE was found to be 41.19±0.94 %. The value of extraction yield and TPC are coherent with few available studies on eggplant peel. The extraction yield of EPE in the present work surpassed the extraction yield reported by Jung *et al.* [3] for water and ethanolic extract of eggplant peel, i.e., 36.1 % and 33.5 %, respectively. The results indicated that ultrasonication helped increase the extraction yield; this substantiated that ultrasonication-assisted extraction is effective for better release of polyphenolic compounds [18,28].

The variations in the results of extraction yield, TPC, and TAC might be due to conditions such as drying method for eggplant peel, i.e., oven, sun, or freeze-drying, extraction time, extraction temperature, i.e., ultrasonic water bath temperature; extraction solvent, and eggplant variety. In all the above-mentioned researches, the eggplant was purchased from the local market.

3.2. *Determination of antioxidant activity*

Table 2 depicts the results of the antioxidant activity of EPE addressed by 3 different methods. The use of multiple antioxidant assays is important to get a better estimate of antioxidant activity rather than a single assay. A similar trend of TPC and TAC was followed as ethanolic EPE, and water-reconstituted EPE did not show any significant difference ($p>0.05$) in their antioxidant activity. The antioxidant activity of ethanol-based EPE assayed by DPPH, ABTS, and CUPRAC assays were found to be 1.82±0.01, 2.11±0.01, and 7.17±0.02 µmol TE/g, respectively. Similarly, the antioxidant activity of water-reconstituted EPE assayed by DPPH, ABTS, and CUPRAC assays was found to be 1.81±0.01, 2.09±0.00, and 7.16±0.01 µmol TE/g, respectively.

Table 2. Antioxidant activity of eggplant peel extracts as measured by DPPH, ABTS, and CUPRAC assays.

Sample	DPPH	ABTS	CUPRAC
Ethanolic EPE	$(1.82\pm0.01)^{a}$	$(2.11\pm0.01)^{b}$	$7.17\pm0.02)^{c}$
Water-reconstituted EPE	$(1.81\pm0.01)^{a}$	$(2.09\pm0.00)^{b}$	$7.16\pm0.01)^{c}$

Mean value±SD (*N*=3). Same letter in the same column represents no significant difference ($p>0.05$). EPE=Eggplant peel extract, TE=Trolox equivalent. The unit of antioxidant activity is µmol TE/g.

There are limited studies in which the antioxidant activity of eggplant peel is reported as a Trolox equivalent/g sample. Rochín-Medina *et al.* [12] reported the antioxidant activity value of 0.033 mmol TE/g (DPPH assay) and 0.056 mmol TE/g (ABTS assay) for EPE prepared with calcium-based solution, which is significantly low. On the contrary, Condurache *et al.* [6] estimated the antioxidant activity of 157.8 mmol TE/g (DPPH assay) for ethanolic EPE, whereas Horincar *et al.* [13] reported antioxidant activity of 20.3 and 27.2 mmol TE/g (DPPH assay), respectively for the ethanolic and methanolic extract of eggplant peel. The antioxidant activity of EPE reported by Horincar *et al.* [13] and Condurache *et al.* [6] was considerably lower than the present findings. This could be further supported if there is available data on antioxidant activity based on other assays like ABTS or CUPRAC for the two studies cited above.

In this work, TPC and antioxidant activity of the eggplant peel were found to be very high in comparison to that reported by Kaur *et al.* [20] for the whole eggplant of the same eggplant variety (PUSA Shyamala), *i.e.*, TPC (0.41±0.01 mg GAE/g), and antioxidant activity (DPPH, ABTS, and CUPRAC scavenging activity were 1.45±1.3, 1.66±0.5, and 0.98±0.5 µmol TE/g, respectively)**.** This finding further substantiates the fact that the eggplant peel has higher phenolic and antioxidant activity than whole eggplant fruit.

3.3. *Analysis by LC-MS/MS*

A quantitative run of water-reconstituted EPE with the authentic delphinidin-3-rutinoside standard was performed with the LC-MS/MS system (Table 3). The LC-MS/MS analysis of EPE revealed the presence of a delphinidin-3-rutinoside compound at the amount of

180.18±8.36 mg/100 g of sample. Identification and peak assignment of delphinidin-3-rutinoside in EPE were primarily based on comparison of their retention times (RT) and mass spectrometric data, *i.e*., molecular cation $[M]^+$ (m/z) with those of delphinidin-3-rutinoside standard (Fig. S1)**.** Delphinidin-3-rutinoside was detected at $[M]^+$ (611 m/z, RT 3.11). The quantification of delphinidin-3-rutinoside in EPE was achieved by using the peak areas of the EPE sample and delphinidin-3-rutinoside standard. The $[M]^+$ (611 m/z) of delphinidin-3-rutinoside was in agreement with the literature [8,22]. Sadilova *et al.* [8] also reported delphinidin-3-rutinoside **(**using HPLC-DAD-MS) as a major anthocyanin but at a lower concentration of 37.8 mg/100 g of EPE. Whereas, Condurache *et al.* [7] indicated a higher amount of delphinidin-3-rutinoside (157-562 mg/100 g) in ethanolic EPE (using HPLC). The variations in the results might be due to different measurement systems, operating conditions, extraction solvent, and eggplant variety.

Table 3. LC-MS/MS analysis of water-reconstituted eggplant peel extract.

Sample	RT/min	m/z $[M]^+$	Peak area (counts)
Delphinidin-3-rutinoside standard	3.12	611	67750±2192
Eggplant peel extract	3.11	611	20000±282

Mean value±SD (*N*=3). RT=retention time

3.4. *Effect of pH*

Although a report on color studies of eggplant anthocyanins with pH variation [8], the observation was limited only to three discreet pH values, pH 1, pH 3.5, and pH 6.0. Therefore, to obtain the detailed changes with continuous pH variation, the following study was carried out where Fig. 1a shows the variation in the color of EPE with different pH values (1-13). The color of EPE changed from pink-red to faded pink, purple, violet, blue, yellow-green, and yellow when the pH value varied from 1-13. The EPE became pink-red when the pH was no more than 3, faded pink at pH 4-5, purple at pH 6, violet at pH 7, blue at pH 8, greenish-yellow at pH 9, and yellow at pH 10-13. Thus, the EPE is sensitive to the acidity and alkalinity of the environment, which may be beneficial for certain other applications as sensing.

In order to get a better insight behind the color variation of the eggplant anthocyanins with pH variation, absorption spectra were recorded. Fig. 1b represents the UV-Vis spectra of EPE at all the different pH values (1-12). The maximum absorption peak appeared at around 525 nm at pH 1. The intensity of the absorbance band significantly reduced with the increase of pH, and there was a gradual bathochromic shift of the absorption maxima. Such changes in the absorption band are similar to other plant anthocyanins, where the color variation due to pH is observed due to the different equilibrium forms of anthocyanins [30-32].

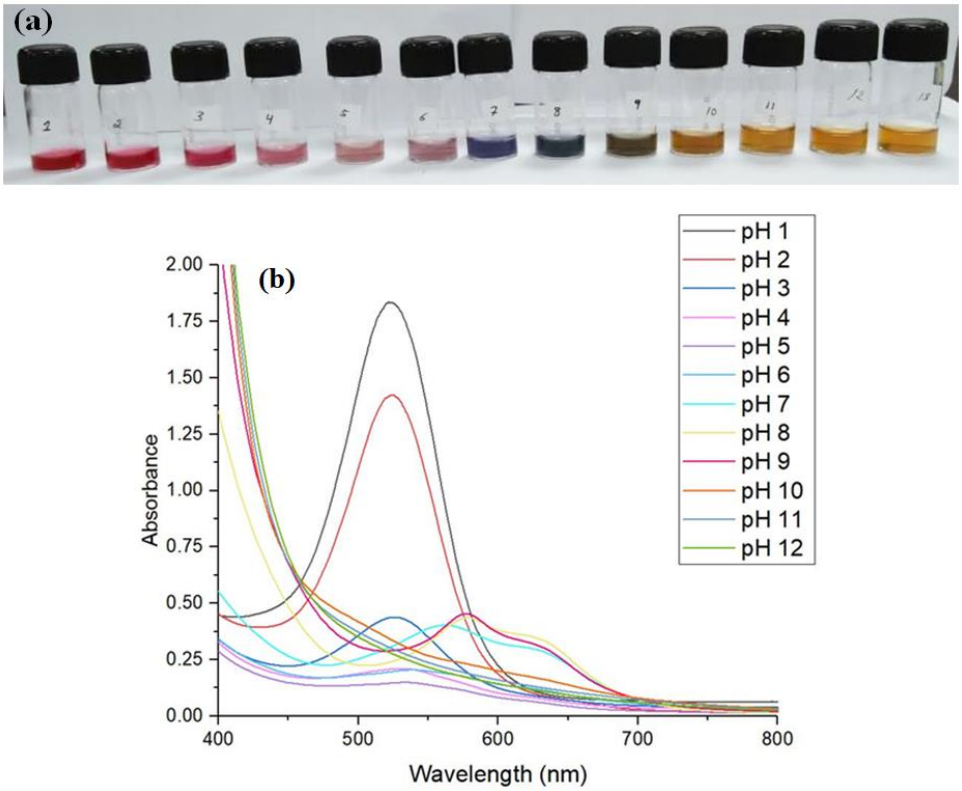

Fig. 1. Effect of pH variation on eggplant peel extract: a) Color of eggplant peel extract at different pH (1-13) and b) UV-Vis spectra of water-reconstituted eggplant peel extract at pH 1-12.

3.5. *Antibacterial activity of EPE*

3.5.1. *Agar well diffusion assay*

The water-reconstituted EPE exhibited an antibacterial effect against Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella* Typhimurium) (Fig. S2). The zone of inhibition of EPE at different concentrations against all the tested bacteria is tabulated in Table 4. The EPE had a significant inhibitory effect on all tested bacteria at a concentration of 50, 75, and 100 mg/mL. The results showed that the inhibitory effect of EPE at the concentration of 100 mg/mL on *B. cereus* and *E. coli* were maximum, and the inhibition zone diameter was 19±0.00 and 21.6±0.57 mm, respectively. The diameter of the inhibition zone increased with the increasing concentration of EPE in the range of 50-100 (mg/mL) for all the tested bacteria. The larger zone of inhibition represents the higher antibacterial activity of the extract. However, no zone of inhibition was detected at an EPE concentration below 50 mg/mL against all tested bacteria.

Table 4. Zone of inhibition of water-reconstituted eggplant peel extract against various bacteria.

Eggplant peel extract (mg/mL)	Zone of inhibition (mm)			
	S. aureus	*B. cereus*	*E. coli*	*S.* Typhimurium
12.5	ND.	ND.	ND.	ND.
25	ND.	ND.	ND.	ND.
50	12±0.00	12.6±0.57	16±0.00	13±0.00
75	13.6±0.57	16±1.73	19±0.00	15±0.00
100	15.6±1.15	19±0.00	21.6±0.57	18.6±1.52
Tetracycline (10 mg/mL)	34.6±0.57	40±0.00	35.6±0.57	35±0.00

Mean value±SD (*N*=3). ND.=not detected.

Basudan [25] reported the zone of inhibition of a methanolic extract of eggplant peel against *S. aureus* (18-24 mm) and *E. coli* (18-26 mm). On the other hand, Rochín-Medina *et al.* [12] reported the antibacterial effect of EPE obtained by extraction with a calcium-based solution against *S.* Typhimurium (8-13 mm) using the disk diffusion method. However, these two studies did not mention the concentration of the EPE; hence the results could not be compared with the current work.

AL-Janabi and AL-Rubeey [33] reported the zone of inhibition of 15 mm for aqueous extract (50 mg/mL) of whole eggplant fruit against *S. aureus* and *E. coli.* In the present study, the zones of inhibition of EPE (at a concentration of 50 mg/mL) against *S. aureus* and *E. coli* were 12 mm and 16 mm, respectively. The result of the inhibition zone for EPE and whole eggplant fruit extract did not show any significant difference, which may be attributed to the content of phytochemicals in EPE and the w{hole fruit, due to the difference in eggplant variety and geographical location of eggplant.

3.5.2. *Determination of minimum inhibitory concentration*

Table 5 shows that the EPE had a similar MIC of 2.34 mg/mL (against *B. cereus* and *S. aureus*) and *a* similar MIC of 4.68 mg/mL (against *E. coli* and *S.* Typhimurium). *B. cereus* and *S. aureus* exhibited more susceptibility to the extract than *E. coli* and *S.* Typhimurium. The result showed that EPE had better antibacterial potential against Gram-positive bacteria (*B. cereus* and *S. aureus*) than Gram-negative bacteria (*E. coli* and *S.* Typhimurium).

Table 5. Minimum inhibitory concentration (MIC) of water-reconstituted eggplant peel extract against different bacterial strains tested.

Test organism	MIC (mg/mL)
S.aureus	2.34
B. cereus	2.34
E. coli	4.68
S. Typhimurium	4.68

AL-Janabi and AL-Rubeey [33] reported the MIC (48 mg/mL) of aqueous extract of whole eggplant fruit against *S. aureus* and *E. coli.* On the contrary to the zone of

inhibition result reported in the same study (discussed in the previous section), the significant difference in MIC results depicts that the eggplant peel has better antibacterial activity than whole eggplant fruit.

4. Conclusion

The results indicated promising perspectives for using eggplant peel by-product, an under-used product, and may contribute to the efficient use of eggplant peel. A significant level of total phenol and total anthocyanin content and a considerable level of antioxidant activity was exhibited by water-reconstituted EPE, which established the fact that there was no loss of phytochemicals of EPE in the process of reconstitution of EPE in water. Delphinidin-3-rutinoside was determined significantly in LC-MS/MS analysis of EPE. The zone of inhibition displayed by EPE against the bacterial strains explains that eggplant peel is a good source of biologically active antibacterial agents. Findings from the current MIC assay showed that the growth of Gram-positive bacteria (*B. cereus* and *S. aureus*) was inhibited at a lower concentration of EPE than Gram-negative bacteria (*E. coli* and *S.* Typhimurium), which implied that EPE had more antibacterial activity against *B. cereus* and *S. aureus.* Thus, EPE could be an efficient and natural source of an antioxidant and antibacterial agent with future applicability in the food industry as an additive. The sharp and distinct color changes of EPE towards variation in pH may extend its applicability in the food packaging sector.

Acknowledgments

The authors would also like to thank Indian Agricultural Research Institute (IARI), New Delhi, for providing the eggplant variety. The authors also extend their gratitude to Sigma test and Research center, Delhi, for their LC-MS/MS facility. The authors are also grateful to the Ministry of Food Processing Industries, Government of India, for a research project grant (Q-11/23/2018-R&D).

References

1. I. C. Okwulehie and E. U. Okon, Int. J. Agric. Innov. Res. **3**, 2319 (2015).
2. L. Kitinoja and A. A. Kader. Measuring Postharvest Losses of Fresh Fruits and Vegetables in Developing Countries (The Postharvest Education Foundation, 2015) pp.1–26.
3. E. Jung, M. Bae, E. Jo, Y. Jo, and S. J. Lee, Med. Plants. Res. **5**, 4610 (2011).
4. L. Salerno, M. N. Modica, V. Pittalà, G. Romeo, M. A. Siracusa, C. Di Giacomo, V. Sorrenti, and R. Acquaviva, Sci. World. J. **2014**, 1 (2014). https://doi.org/10.1155/2014/719486
5. G. Niño-Medina, V. Urías-Orona, M. D. Muy-Rangel, and J. B. Heredia, S. Afr. J. Bot. **111**, 161 (2017). https://doi.org/10.1016/j.sajb.2017.03.016
6. N. N. Condurache, I. Aprodu, O. Crăciunescu, R. Tatia, G. Horincar, V. Barbu, E. Enachi, G. Râpeanu, G. E. Bahrim, A. Oancea, and N. Stănciuc, Food Bioprocess Technol. **12**, 1316 (2019). https://doi.org/10.1007/s11947-019-02302-1
7. N. N. Condurache, C. Croitoru, E. Enachi, G. E. Bahrim, N. Stănciuc, and G. Râpeanu, Plants **10**, 577 (2021). https://doi.org/10.3390/plants10030577

8. E. Sadilova, F. C. Stintzing, and R. Carle, Z. Naturforsch. C. J. Biosci. **61**, 527 (2006). https://doi.org/10.1515/znc-2006-7-810
9. L. Boulekbache-Makhlouf, L. Medouni, S. Medouni-Adrar, L. Arkoub, and K. Madani, Ind. Crops. Prod. **49**, 668 (2013). https://doi.org/10.1016/j.indcrop.2013.06.009
10. D. R. Joshi and N. Adhikari, J. Pharm. Res. Int. **28**, 1 (2019). https://doi.org/10.9734/jpri/2019/v28i330203
11. S. Kumar and D. Subhashini, Rev. J. Chem. **4**, 90 (2015).
12. J. J. Rochín-Medina, J. A. Sotelo-Castro, N. Y. Salazar-Salas, J. A. López-Valenzuela, and K. Ramírez, CyTA - J. Food **17**, 873 (2019). https://doi.org/10.1080/19476337.2019.1675762
13. G. Horincar, E. Enachi, N. Stănciuc, and G. Râpeanu, Ann. Univ. Dunarea. Jos. Galati Fascicle VI: Food Technol. **43**, 40 (2019). https://doi.org/10.35219/foodtechnology.2019.1.03
14. M. Shafiq, S. Firdous, Q. Irfan, S. J. Khan, and A. Qadir, J. Sci. Res. **11**, 365 (2019). https://doi.org/10.3329/jsr.v11i3.40493
15. M. Saifullah, R. McCullum, A. McCluskey, and Q. Vuong, Heliyon **5**, 1 (2019). https://doi.org/10.1016/j.heliyon.2019.e03044
16. J. Vuthijumnok, IOSR J. Pharm. Biol. Sci. **8**, 42 (2013). https://doi.org/10.9790/3008-0814248
17. G. Horincar, E. Enachi, V. Barbu, D. G. Andronoiu, G. Râpeanu, N. Stănciuc, and I. Aprodu, Antioxidants **9**, 351 (2020). https://doi.org/10.3390/antiox9040351
18. N. Medina-Torres, T. Ayora-Talavera, H. Espinosa-Andrews, A. Sánchez-Contreras, and N. Pacheco, Agron. J. **7**, 47 (2017). https://doi.org/10.3390/agronomy7030047
19. C. A. S. Rabelo, N. Taarji, N. Khalid, I. Kobayashi, M. Nakajima, and M. A. Neves, Food. Res. Int. **106**, 542 (2018). https://doi.org/10.1016/j.foodres.2018.01.017
20. C. Kaur, S. Nagal, J. Nishad, R. Kumar, and Sarika, Food Res. Int. **60**, 205 (2014). https://doi.org/10.1016/j.foodres.2013.09.049
21. P. Nisha, P. Abdul Nazar, and P. Jayamurthy, Food Chem. Toxicol. **47**, 2640 (2009). https://doi.org/10.1016/j.fct.2009.07.026
22. A. Todaro, F. Cimino, P. Rapisarda, A. E. Catalano, R. N. Barbagallo, and G. Spagna, Food Chem. **114**, 434 (2009). https://doi.org/10.1016/j.foodchem.2008.09.102
23. A. Oniszczuk, M. Olech, T. Oniszczuk, K. Wojtunik-Kulesza, and A. Wójtowicz, Arab. J. Chem. **12**, 4719 (2019). https://doi.org/10.1016/j.arabjc.2016.09.003
24. C. Ma, L. Yang, F. Yang, W. Wang, C. Zhao, and Y. Zu, Int. J. Mol. Sci. **13**, 14294 (2012). https://doi.org/10.3390/ijms131114294
25. N. Basudan, Int. J. Chem.Tech. Res. **11**, 161 (2018). https://doi.org/10.20902/ijctr.2018.110817
26. V. Tyagi, C. Saravanan, Y. Wang, and B. Bhattacharya, Food Technol. Biotechnol. **59**, 31 (2021). https://doi.org/10.17113/ftb.59.01.21.6878
27. CLSI M07-A9, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard — 9th Edition, 32 (Wayne, PA, USA: Clinical and Laboratory Standards Institute (CLSI), 2012).
28. S. Ferarsa, W. Zhang, N. Moulai-Mostefa, L. Ding, M. Y. Jaffrin, and N. Grimi, Food Bioprod. Process **109**, 19 (2018). https://doi.org/10.1016/j.fbp.2018.02.006
29. M. Jabbari, N. Khosravi, M. Feizabadi, and D. Ajloo, RSC Adv. **7**, 14776 (2017). https://doi.org/10.1039/c7ra00038c
30. B. Tang, Y. He, J. Liu, J. Zhang, J. Li, J. Zhou, Y. Ye, J. Wang, and X. Wang, Dyes Pigm. **170**, 1 (2019). https://doi.org/10.1016/j.dyepig.2019.107643
31. S. Wahyuningsih, L. Wulandari, M. W. Wartono, H. Munawaroh, and A. H. Ramelan, IOP Conf. Ser. Mater. Sci. Eng. **193**, 1 (2017). https://doi.org/10.1088/1757-899x/193/1/012047
32. J. Zhang, X. Zou, X. Zhai, X. W. Huang, C. Jiang, and M. Holmes, Food Chem. **272**, 306 (2019). https://doi.org/10.1016/j.foodchem.2018.08.041
33. A. A. H. S. AL-Janabi and S. A. H. AL-Rubeey, Pharmacogn. J. **2**, 35 (2010). https://doi.org/10.1016/s0975-3575(10)80076-8