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Research Article

Ultrasonic Extraction of Phenolic Compounds from Eggplant Peel and Formulation of Eggplant Peel Extract-Enriched Ice-Cream

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In this study, ultrasound-assisted extraction (UAE) was used for the extraction of bioactive compounds from eggplant peels (EPP) and conditions were optimized by using response surface methodology (RSM). Methanol concentrations (60%, 70%, and 80%), time (30 min, 45 min, and 60 min), and temperature (30°C, 45°C, and 60°C) were selected as independent extraction parameters, whereas total anthocyanin content (TAC), DPPH inhibition, and total phenolic content (TPC) were response variables. The optimal extraction was at 45°C for 45 min of extraction time with 70% solvent which corresponded to TAC, TPC, and DPPH inhibition values of 7.94 mg/g, 7.03 mg GAE/g, and 94.8%, respectively. The optimized EPP extract was chemically characterized by a Fourier-transform infrared spectrometer (FTIR), high-performance liquid chromatography (HPLC), and a gas chromatography mass spectrometer (GCMS). The optimized extract was further evaluated for antioxidant and anticancer potential. For antioxidant and anticancer potential, the corresponding IC_{50} values of the EPP extract were 243.2 μg/ml and 1.52–1.99 mg/ml, respectively. Furthermore, the EPP extract significantly inhibited the migratory ability of cancer cells in a dose-dependent manner. Major components identified in the EPP extract were chlorogenic acid (261.76 mg/kg), benzoic acid (184.41 mg/kg), syringic acid (78.48 mg/kg), p-coumaric acid (49.59 mg/kg), cinnamic acid (32.17 mg/kg), quercetin (5.24 mg/kg), sinapic acid (5.14 mg/kg), and gallic acid (4.34 mg/kg). The EPP extract-enriched ice-cream showed high antioxidant potential and consumer acceptability than the blank ice-cream formulation. The EPP extract, due to its antioxidant and anticancer potential, can be used in the formulation of functional food products.

1. Introduction

Eggplant (*Solanum melongena* L.) is a popular vegetable crop of the Solanaceae family and is believed to be originated from India. The eggplant fruit can be oval, elongated, or pear shaped with weight ranging from 20 to 400 g, and it is used as a cooking vegetable in various areas of the world [1]. Eggplant is low in fat content and contains fiber, carbohydrates, proteins, vitamins, minerals, and phenolic compounds particularly anthocyanins [2]. EPP exhibits high concentration of anthocyanins which imparts its dark purple color.

Plant secondary metabolites include phenolic compounds that exhibit the ability to improve human health [3]. The regular intake of polyphenol-enriched foods may reduce the chances of colon cancer, cardiovascular disease, obesity, liver disorders, and diabetes [4]. Eggplant fruit and its peels are a rich source of phenolic compounds particularly anthocyanins, which are associated with anti-inflammatory, anticancer, hepatoprotective, antioxidant, hypolipidemic, and antiallergic potential [5, 6]. Anthocyanins can be used as natural food colorants and can be extracted from various flower petals, leaves, fruits, and vegetables [7]. EPPs are generally discarded as waste by food processing units which

Table 1: Optimization of ultrasound-assisted extraction of eggplant peel.

Exp. no.	Independent variables			Response variables		
	Temperature (°C)	Time (min)	Solvent (%)	TAC (mg/g)	TPC (mg GAE/g)	DPPH inhibition (%)
1	45	30	60	4.568	4.605	79.63
2	60	45	60	4.52	4.48	81.21
3	30	60	70	5.6	5.41	84.7
4	45	45	70	5.34	5.755	85.6
5	45	45	70	7.42	7.13	94
6	30	45	60	3.68	3.475	72.51
7	60	60	70	5.44	4.89	83.07
8	45	45	70	6.9	7.13	89.98
9	45	45	70	7.52	6.7	88.41
10	60	30	70	4.98	4.06	81.5
11	30	45	80	3.76	3.505	73.03
12	60	45	80	4.2	3.52	80.6
13	45	45	70	7.94	7.03	94.8
14	45	60	60	3.52	3.62	79.12
15	45	30	80	4.14	3.13	60
16	30	30	70	5.62	5.665	84
17	45	60	80	4.84	4.48	83.4

TAC, total anthocyanin content; TPC, total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

may cause serious environmental hazards if not properly managed [8]. Recovery of bioactive compounds from EPP can provide polyphenols such as anthocyanins. Anthocyanins contain a wide spectrum of colors which is influenced by their pH and molecular structure. Due to their color diversity and antioxidant potential, anthocyanins can be used as natural food colorants and preservatives, replacing synthetic dyes [2]. The bioactive potential of eggplant is associated with the presence of various phenolic compounds such as quercetin, myricetin, p-coumaric acid, chlorogenic acid, cinnamic acid, and catechins [9].

Different techniques used for the extraction of plant materials are cold compression, solvent extraction (maceration, infusion, and digestion), distillation, and countercurrent extraction. Due to sensitivity of bioactive compounds, advanced extraction methods such as supercritical fluid extraction, microwave, and ultrasound-assisted extraction (UAE) technique have been extensively used [10]. UAE offers many advantages compared to conventional methods such as high extraction yield, being less time consuming, and requiring less amount of extraction solvent [7, 11].

In this study, UAE was used to extract polyphenolic compounds from EPPs and evaluated them for their antioxidant and anticancer potential. The EPP extract was used as a functional ingredient to improve the antioxidant potential of ice-cream.

2. Materials and Methods

2.1. Sample Preparation. Fresh, elongated eggplants were purchased from the local market of Lahore district of Pakistan and washed with tap water to remove the surface dirt. The eggplant fruits were peeled, and peels were dried in an oven at 50°C for 48–72 h till constant weight, followed by mechanical grinding (HL7505, Philips Co., Ltd., China) and stored in Ziploc ® plastic bags covered with aluminum foil at 4°C till further use [5].

2.2. Ultrasound-Assisted Extraction. UAE of EPP was optimized by RSM using Design-Expert® software (Minneapolis, MN, USA) at a fixed frequency of 20 kHz. The three independent extraction variables were methanol concentration (60%, 70%, and 80%), time (30 min, 45 min, and 60 min), and temperature (30°C, 45°C, and 60°C). The variables were coded at three levels: –1, 0, and 1—using Box–Behnken design, and 17 experimental runs were used by using different combinations of independent extraction parameters (Table 1). The fixed sample-to-solvent ratio (1 : 20 w/v) was used for all extraction processes. The dried peels were added to a beaker containing the extraction solvent and subjected to an ultrasonic processor (LSP-500, Industrial Sonomechanics, USA). The extracts were immediately used after the extraction, and TAC, TPC, and DPPH were used as response variables.

2.3. Estimation of TAC, TPC, and Antioxidant Activity. TPC of EPP was determined using the Folin–Ciocalteau regent (Sigma-Aldrich, USA) by following the method of Sadiq et al. [12]. Gallic acid was used as the reference standard (20–100 μg/ml), and results were expressed as mg of gallic acid equivalent (GAE) per gram of the raw sample. The antioxidant activity of the EPP extract was determined by DPPH radical scavenging activity, following the method of Hiranrangsee et al. [5].

TAC of the EPP extract was estimated by the pH differential method [13]. The extract obtained by UAE (1 ml) was added to 9 ml of 0.025 M potassium chloride buffer (pH 1), and similarly, the extract (1 ml) was added to 9 ml of 0.4 M sodium acetate buffer (pH 4.5). The absorbance

of samples was read (within 20–50 min of preparation) by a UV-visible spectrophotometer (Aurius 2000 series, Cecil instruments, England) at 700 nm and 520 nm against a blank. TAC was expressed as mg of cyanidin-3-glucoside equivalent/g of the sample and measured by using the following equation:

$$\mathrm{TAC\,(mg/g)} = \frac{\mathrm{Abs} \times \mathrm{MW} \times \mathrm{DF} \times 1000}{\varepsilon \times \mathrm{L}}. \quad (1)$$

Absorbance (Abs) = $[(A_{520}-A_{700})_{\mathrm{pH\,1.0}}]$-$[(A_{520}-A_{700})_{\mathrm{pH4.5}}]$, ε (cyanidin-3-glucoside molar absorbance) = 26,900 $\mathrm{L{\cdot}mol^{-1}{\cdot}cm^{-1}}$, L = path length of the cell (1 cm), MW (molecular weight of anthocyanins) = 449.2 D, and DF = dilution factor.

2.4. Optimization of UAE Extraction. UAE extraction of EPP was optimized using RSM, and optimized experimental run was selected using 0.860 desirability. The optimized extraction conditions were 70% solvent, 47 min extraction time at 45°C. The optimized extract was lyophilized (Christ Alpha 1-2 LD plus, Germany) to obtain a powdered extract. The freeze-dried extracts were stored at 4°C until further use.

The optimized extract was evaluated for TAC, TPC, and DPPH inhibition assay. For DPPH assay, different concentrations (8000 μg/ml, 4000 μg/ml, 2000 μg/ml, 1000 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, and 31 μg/ml) of the EPP extract and vitamin C (positive control) were prepared in distilled water. IC_{50} value, representing the extract concentration required to scavenge 50% of the DPPH radical, was estimated by nonlinear regression using GraphPad Prism® version 7 (San Diego, US).

2.5. Anticancer Activity of the Eggplant Peel Extract

2.5.1. Cell Culture. Human breast cancer MDA-MB-231, colon cancer DLD-1, HCT-116, and prostate cancer PC3 cells were grown in a high glucose DMEM cell culture medium containing sodium pyruvate. The culture medium was supplemented with antibiotics penicillin/streptomycin and 10% fetal bovine serum (GIBCO; Thermo Fisher Scientific, USA). Cells were cultured in flasks with treated surface and vented caps and kept in a humidified incubator at 37°C with 5% CO_2. Cells were maintained below 80% confluence and passaged every two to three days using 0.1% trypsin EDTA solution and DPBS (GIBCO; Thermo Fisher Scientific, USA).

2.5.2. MTT Cytotoxicity Assay. Survival/viability of human cancer cell lines in the presence of the eggplant peel extract was quantified using MTT (methylthiazolyldiphenyl-tetrazolium bromide) cytotoxicity assay as described by Jawad et al. [14]. The water soluble EPP extract was dried using a vacuum concentrator, and the dried sample was used to make 20 mg/ml solution in serum-free DMEM. The EPP extract stock solution was filtered through a 0.22-micron syringe filter to sterilize and stored at −20°C. For cytotoxicity assay, 4×10^3 MDA-MB-231 and PC3 cells and 8×10^3 DLD-1 and HCT-116 cells per well were seeded in 96-well culture plates with complete DMEM. Cells were grown overnight before addition of the sterile eggplant peel extract at varying concentrations (10 μg/ml to 5 mg/ml). Doxorubicin was used as a positive control cytotoxic substance (10 ng/ml to 5 μg/ml). Four replicate wells per concentration of the test substance were tested, and the experiment was repeated three times on separate days. Cells were grown in the presence of the test substance for 72 h before MTT assay. MTT stock solution of 5 mg/ml was prepared in PBS and filter sterilized. 10 μl MTT solution was added to 100 μl of cell culture media in each well of the cell culture plate. Cells were further incubated for 4 h at 37°C. 100 μl of acidified isopropanol (0.04 N HCL) was added per well to dissolve formazan crystals. Plates were read at 492 nm, and raw data were normalized to control wells without cells. Cell survival at any particular substance concentration was expressed as percentage survival of control cells. IC_{50} values were obtained by plotting cell survival against concentration of the EPP extract using GraphPad Prism software.

2.5.3. Cell Migration-Wound Healing Assay. PC3 and MDA-MB-231 cells were cultured in 6-well plates at 1.5×10^5 cells per well. Cells were grown for 24 h to form confluent monolayers which were scratched using sterile 200 μl tips. Detached cells and cellular debris were removed by washing with serum-free DMEM media. Cells were allowed to grow in the DMEM medium with 2.5% FBS (reduced serum to minimise cell proliferation) along with varying concentrations of the eggplant peel extract. Wound gaps were photographed at 0 h, 12 h, and 24 h to record the migration of cells into the wounded area, using an inverted phase contrast microscope [14]. Results from three independent experiments were used to quantify cell migration using Image J software (open source). Image J software was used to assess wound closure in an unbiased manner. Wound edges were traced automatically by thresholding (using a histogram to identify an intensity cutoff that separates the wound from cells), and areas were calculated.

2.6. FTIR Analysis. The EPP extract was analyzed by an FTIR spectrometer (Cary 630, Agilent technologies, Santa Clara, CA, USA) in the range of 4000–650 $\mathrm{cm^{-1}}$ with a resolution of 4 $\mathrm{cm^{-1}}$. The sample (5 mg) was placed in FTIR, and 50 sample scans were averaged.

2.7. Gas Chromatography Mass Spectrometry (GCMS) Analysis. The EPP extract was analyzed by the GC-MS system (GC-7890A/MS-5975C, Agilent Technologies, Santa Clara, CA, USA) with an HP-5 MS capillary column. Helium was used as the mobile phase (1.0 ml/min), and the temperature for sample injection was maintained at 200°C. Data were acquired in the range 50–600 amu, and identification was made using the NIST 05 spectral library (Gaithersburg, MD, USA).

2.8. High-Performance Liquid Chromatography (HPLC) Analysis. Gradient HPLC (LC-10A, SHIMADZU, JAPAN)

was used for the separation of extract components by using shim pack CLC-ODS (C-18), 25 cm × 4.6 mm, 5 μm column. The chromatographic separation was carried out using the following mobile phase gradients: A (H2O: acetic acid-94 : 6, pH = 2.27) and B (acetonitrile 100%). The gradient used was 15% solvent B for 0–15 min, 45% solvent B for 15–30 min, and 100% solvent B for 30–45 min, with a 1 ml/min flow rate. The UV-visible detector was monitored at 280 nm. Phenolic compounds were identified by comparing retention time and UV-visible spectra with standards (chlorogenic acid, benzoic acid, syringic acid, p-coumaric acid, cinnamic acid, quercetin, sinapic acid, and gallic acid), and quantification was performed by peak area comparison with standards [15].

2.9. Formulation of Eggplant Peel Extract-Enriched Ice-Cream. The EPP extract-enriched ice-cream was made by using 75.76% milk, 4.45% cream, 0.74% stabilizer, 0.74% emulsifier, and 18.36% sugar (w/w). Initially, all ingredients were mixed and pasteurized at 80°C for 10 min followed by cooling and addition of different concentrations (0.5%, 1%, 2%, and 5% w/w) of the extract. After blending, the ice-cream mixtures were processed by an ice-cream processor (La Gelatiera, Termozeta, Italy) at −18°C for 30–45 min [16]. The ice-cream without the extract was used as a control. After production, the ice-cream was stored at −18°C for 24 h before the analysis of functional characteristics.

For the determination of functional characteristics of EPP-enriched ice-cream, the sample (1 ml of ice-cream) and 9 ml of methanol were mixed and centrifuged for 10 min at 1300 × g to obtain the supernatant which was used for the determination of TAC, TPC, and DPPH inhibition [16].

2.10. Sensory Analysis of Ice-Cream. Before conducting sensory evaluation, ice-cream was stored for 24 h at −18°C and then kept for 5 min at 20°C [17]. A 20 g serving of the EPP extract (5%)-enriched ice-cream was assessed by 16 participants with food science background using the 9-point hedonic scale (1 = highly disliked to 9 = highly liked). Color, texture, aroma, hardness, melting, taste, appearance, and overall acceptance were evaluated. Ice-cream without the extract was used as a blank, and commercially available ice-cream was used as a control. Samples were assigned codes of three digits and presented to the panelist in random order.

2.11. Statistical Analysis. All the experiments were conducted in triplicates, and one-way analysis of variance (ANOVA) and Tukey's HSD tests were carried out to determine significant differences ($p < 0.05$) among mean observations by using the SPSS statistical software package (SPSS, version 22.0, USA).

3. Results and Discussion

3.1. Optimization of UAE of Eggplant Peel. UAE extraction of EPPs was optimized by using the Box–Behnken design (Table 1). The results indicated that the optimal extraction was at 45°C for 45 min extraction time with 70% solvent which corresponded to TAC, TPC, and DPPH inhibition values of 7.94 mg/g, 7.03 mg GAE/g, and 94.8%, respectively.

The quadratic model was used to evaluate the effect of extraction parameters on TAC, TPC, and DPPH inhibition. The model was well fitted to the experimental data which was evident from R^2 (coefficient of determination) values of 0.8446, 0.9157, and 0.8257 for TAC, TPC, and antioxidant activity, respectively. Moreover, the lack of fit value was insignificant which indicated that the model was well fitted to experimental data. TAC, TPC, and DPPH inhibition values of the EPP extract were in the ranges of 3.52–7.94 mg/g, 3.13–7.13 mg GAE/g, and 60–94.8%, respectively. All the extraction parameters influence the response variables; however, the nature of influence was not significant. The fruit peels have been reported to exhibit high TPC and antioxidant activities and can be used as an ingredient in the formulation of food, pharmaceutical, and cosmetic products [18]. UAE was reported to be a better extraction technique due to its short extraction time, high extraction yield, and requirement of a lower amount of extraction solvent [11]. Khan et al. [19] reported that 48 h of conventional extraction of almond hull resulted in TPC of 2.56 mg GAE/g and 64.34% DPPH inhibition, whereas UAE for 20 min resulted in TPC of 11.31 mg GAE/g and 86% DPPH inhibition.

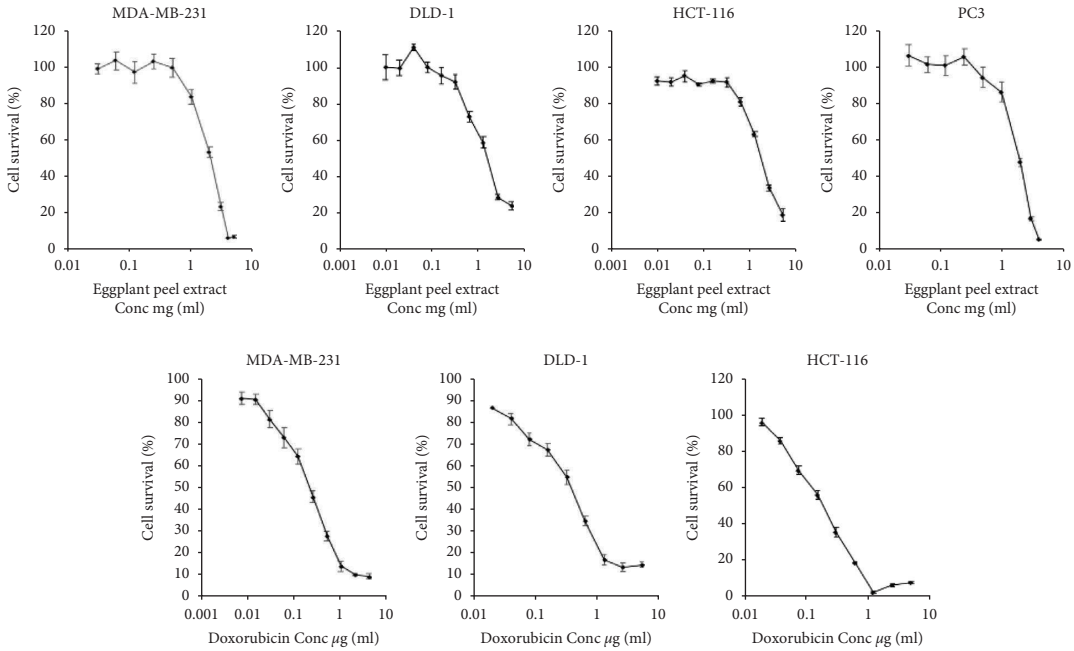
UAE extraction conditions were optimized by using the optimization function of the design expert and optimized extraction conditions (70% solvent, 47 min extraction time at 45°C) with a desirability of 0.860 were used for extraction. At optimized extraction conditions, TAC, TPC, and DPPH inhibition values were 7.33 ± 0.43, 6.91 ± 0.31 mg/g, and 91.5 ± 2.7%, respectively.

Table 2: % DPPH inhibition of the optimized eggplant peel extract.

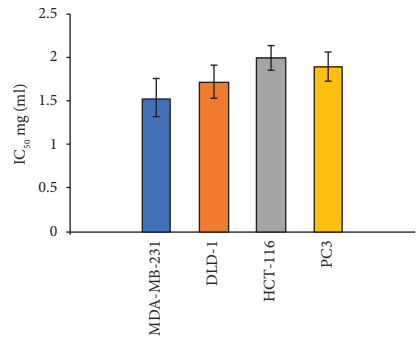
Quantity (μg/ml)	Vitamin C	Eggplant peels extract
8000	96.77 ± 0.37^{a}	95.23 ± 2.39^{a}
4000	95.31 ± 1.31^{a}	86.63 ± 3.00^{b}
2000	89.97 ± 1.42^{b}	71.74 ± 0.99^{c}
1000	88.20 ± 0.40^{b}	55.75 ± 2.08^{d}
500	59.92 ± 0.98^{c}	48.82 ± 1.84^{de}
250	41.05 ± 3.11^{d}	44.46 ± 2.06^{ef}
125	24.50 ± 0.85^{e}	40.57 ± 1.47^{fg}
62.5	18.00 ± 1.00^{f}	37.64 ± 2.94^{fg}
31.25	15.87 ± 1.02^{f}	35.87 ± 4.04^{g}

Different superscript small letters (a–g) indicate means which are significantly ($p < 0.05$) different.

3.2. Antioxidant Activity of the Optimized Eggplant Peel Extract. DPPH inhibition (%) of the EPP extract and vitamin C (positive control) are summarized in Table 2. With the increase in concentration, DPPH inhibition of the EPP peel extract was also increased, and at highest test concentration (8000 μg/ml), 95.23 ± 2.39% DPPH inhibition was observed which was not significantly different from vitamin C (96.77 ± 0.37%). The corresponding IC_{50} values of the EPP extract (243.2 μg/ml) and vitamin C (322.5 μg/ml) were estimated by nonlinear regression. IC_{50} value represents the minimum concentration required for 50% DPPH inhibition, and a lower IC_{50} is associated with high antioxidant

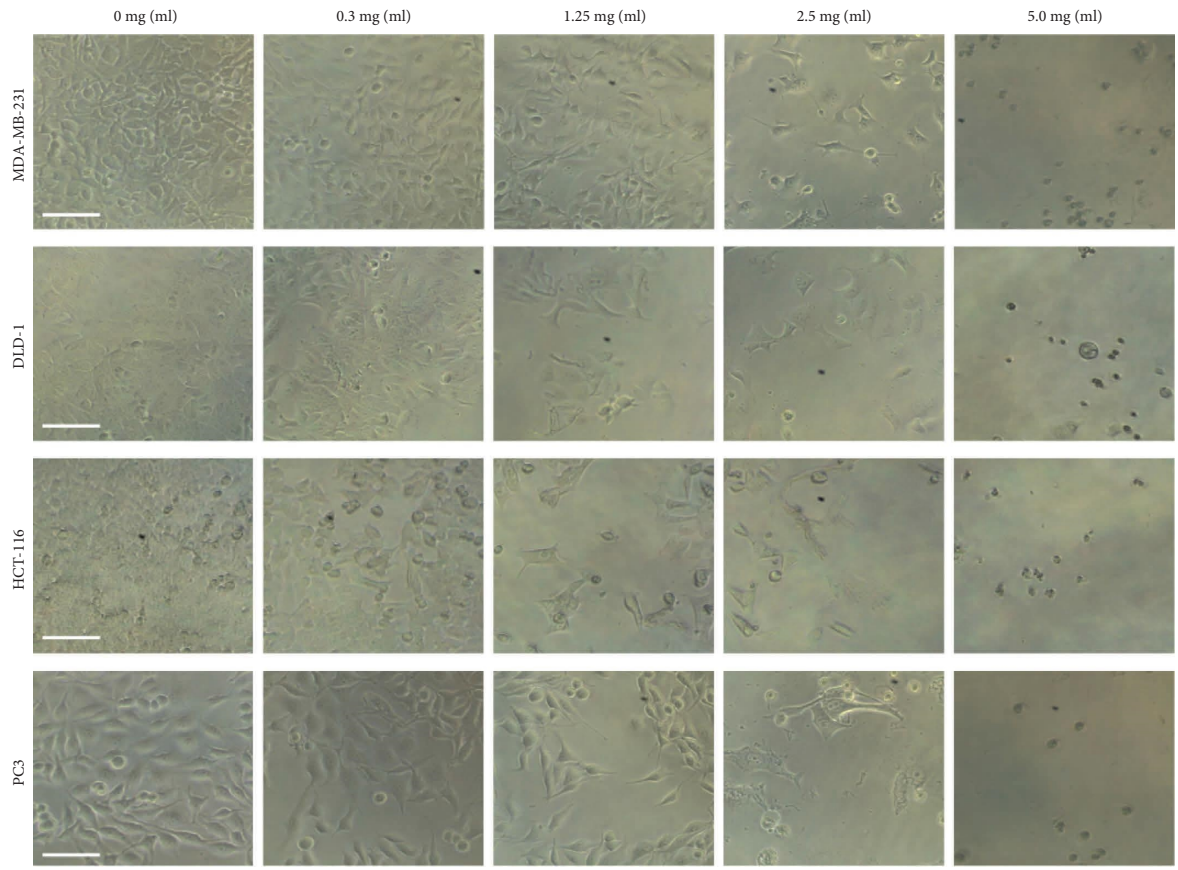


(a)



(b)

Figure 1: Continued.



(c)

Figure 1: Quantification of cytotoxic effects of the eggplant peel extract on human cancer cell lines in vitro. (a) Results of MTT cytotoxicity assay showing reduction in survival of MDA-MB-231, DLD-1, HCT-116, and PC3 cells. Cells were treated with different concentrations of the eggplant peel extract for 72 hours, and data shown were normalized to zero drug controls. Doxorubicin was used as a positive-control cytotoxic substance. Error bars show the standard error of the mean of four wells, and results shown are representative of three independent repeats. (b) IC_{50} of eggplant peel extract treatment in cancer cell lines. Data shown were obtained by nonlinear regression curve fitting of normalized cell survival, and error bars represent 95% confidence interval. (c) Assessment of morphological changes in different cancer cells after 72 hours of treatment with increasing concentration of the eggplant peel extract. The scale bar represents 100 micrometers, and all images were captured at the same magnification.

potential. The antioxidant activity of the EPP extract was associated with the presence of phenolic compounds. Kazemi et al. [8] reported an increase in the antioxidant activity of the EPP extract, and at a concentration of 10 mg/ml, 94% DPPH inhibition was reported.

3.3. Anticancer Activity of the Optimized Eggplant Peel Extract

3.3.1. MTT Cytotoxicity Assay. To determine the cytotoxic effect of eggplant peel extract on human cancer cell lines, one breast cancer cell line, MDA-MB-231, two colon cancer cell lines, DLD-1 and HCT-116, and one prostate cancer cell line, PC3, were cultured in the presence of different concentrations of the EPP extract (10 μg/ml to 5 mg/ml). MTT assay was performed after 72 h of exposure, and the percentage of cell survival was calculated. We observed that EPP extract treatment reduced cell survival in a dose-dependent manner (Figure 1(a)). All cancer cell lines showed significant reduction in survival, and the IC_{50} values were 1.52 mg/ml (MDA-MB-231), 1.71 mg/ml (DLD-1), 1.99 mg/ml (HCT-116), and 1.88 mg/ml (PC3) (Figure 1(b)). Doxorubicin, a well-known chemotherapeutic agent, was used as a positive control, and the IC_{50} values obtained after MTT assay were 0.34 μg/ml (MDA-MB-231), 0.55 μg/ml (DLD-1), and 0.29 μg/ml (HCT-116). Assessment of morphological changes in different cancer cells after 72 h of treatment with increasing concentration of the EPP extract is summarized in Figure 1(c).

3.3.2. Cell Migration-Wound Healing Assay. Cancer cell migration is a critical process in cancer metastasis. To study the functional impact of the EPP extract on cancer cell migration, we performed in vitro scratch wound healing assay using prostate cancer PC3 and breast cancer MDA-MB-231 cells. As shown in Figure 2, the treatment of the EPP extract significantly inhibited the migratory ability of cancer cells in a dose-dependent manner. The inhibitory effect was more pronounced for PC3 cells where 24 h of treatment with

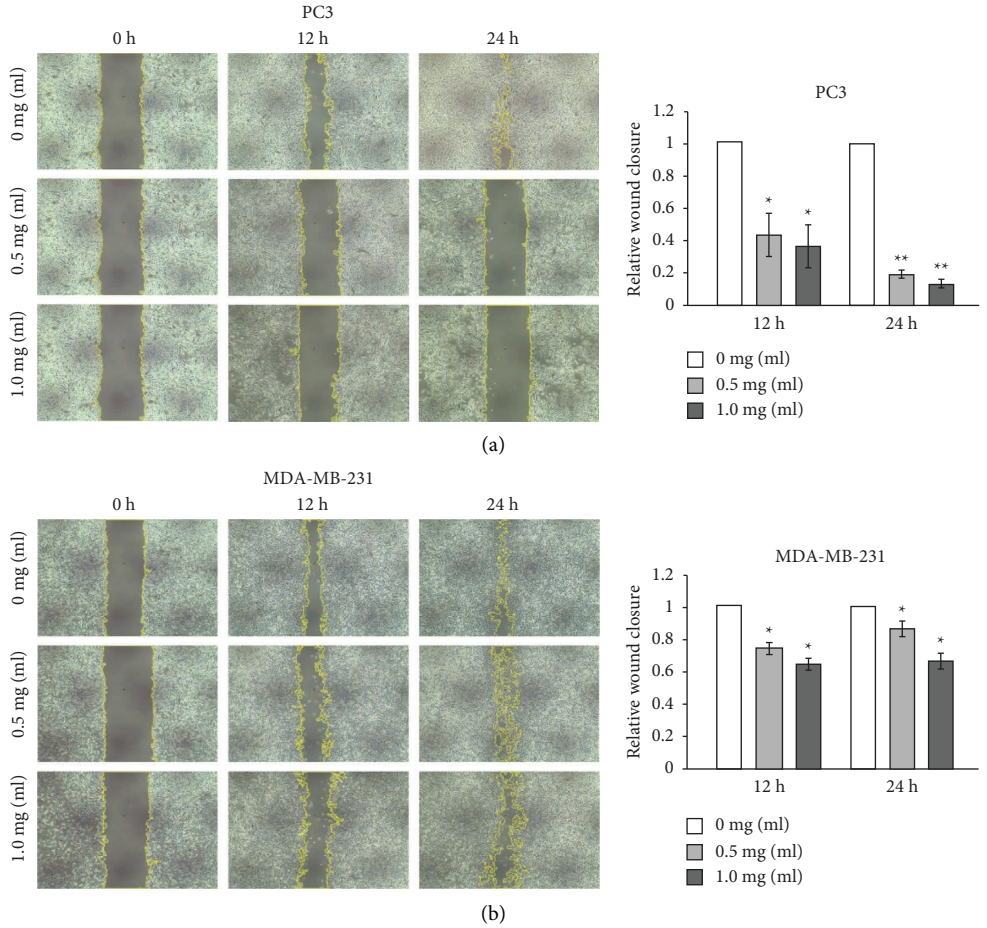


Figure 2: Treatment of the eggplant peel extract reduces the migratory capacity of PC3 (a) and MDA-MB-231 (b) cells. Images on the left represent scratch wound healing assay; cell monolayers were scratched and allowed to heal in the presence of different concentrations of the eggplant peel extract. Scratched areas were photographed at 0 h, 12 h, and 24 h, and yellow lines represent scratched areas/after healing areas were calculated using ImageJ software. The right panels show quantitative analysis of wound closure. Relative wound closure was calculated as a fold change relative to the wound area of control images with no eggplant peel extract. Error bars show the standard error of the mean, $^{*}p < 0.05$, $^{**}p < 0.001$ compared with no eggplant peel extract controls.

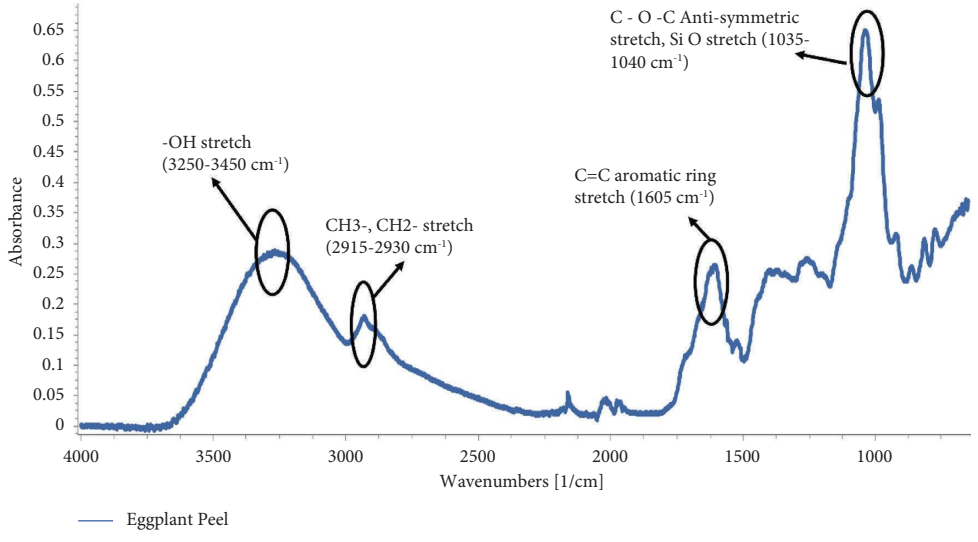


Figure 3: FTIR spectrum of the optimized eggplant peel extract.

Table 3: GC-MS analysis of the eggplant peel extract.

Serial #	Retention time	Molecular weight	Compounds	Molecular formula
1	6.564	151	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$
2	6.788	86	Butyrolactone	$C_4H_6O_2$
3	10.534	144	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$
4	20.376	256	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
5	22.068	282	Oleic acid	$C_{18}H_{34}O_2$
6	22.243	372	Octadecanoic acid	$C_{22}H_{44}O_4$
7	26.762	388	22, 23-Bisnor-5-cholenic acid, 3β-hydroxy, acetate	$C_{24}H_{36}O_4$

Table 4: HPLC analysis of the optimized eggplant peel extract.

Phenolic compound	Retention time (min)	Area (%)	Concentration (mg/kg)
Quercetin	2.753	0.2	5.24
Gallic acid	4.827	0.2	4.34
Benzoic acid	14.587	3.6	184.41
Chlorogenic acid	15.100	6.9	261.76
Syringic acid	16.427	6.4	78.48
p-Coumaric acid	17.700	7.8	49.59
Cinnamic acid	25.247	1.9	32.17
Sinapic acid	26.000	0.9	5.14

Table 5: TAC, TPC, and DPPH inhibition of different ice-cream formulations.

Different ice-cream formulations with the eggplant peel extract (%)	TAC (mg/100 g)	TPC (mg GAE/100 g)	DPPH inhibition (%)
0.5	1.09 ± 0.04^{c}	3.30 ± 0.06^{d}	71.36 ± 0.81^{b}
1	1.31 ± 0.03^{c}	4.14 ± 0.22^{c}	72.33 ± 1.94^{b}
2	1.75 ± 0.22^{b}	5 ± 0.20^{b}	74.06 ± 2.00^{b}
5	3.08 ± 0.20^{a}	7.52 ± 0.35^{a}	83.00 ± 2.64^{a}

Different superscript small letters (a–d) indicate means which are significantly ($p < 0.05$) different.

Table 6: Sensory evaluation of eggplant peel extract (5%)-enriched ice-cream.

Characteristics	Blank (ice cream without the extract)	Control (commercial ice-cream)	Sample (ice-cream with 5% extract)
Texture	6.125 ± 1.204^{b}	7.312 ± 1.25^{a}	7.437 ± 1.41^{a}
Aroma	6.187 ± 1.37^{b}	7.375 ± 1.14^{a}	6.875 ± 1.31^{ab}
Hardness	5.937 ± 1.91^{a}	6.625 ± 1.58^{a}	7.187 ± 1.22^{a}
Melting	6.75 ± 1.52^{a}	6.312 ± 1.99^{a}	7.56 ± 1.75^{a}
Color	7.25 ± 1.65^{a}	7.625 ± 1.5^{a}	6.437 ± 2.03^{a}
Appearance	6.3125 ± 1.99^{a}	7.5 ± 1.154^{a}	7.062 ± 1.48^{a}
Taste	5.93 ± 1.43^{b}	7.5 ± 1.414^{a}	7.25 ± 1.57^{a}
Overall acceptance	6.125 ± 1.5^{b}	7.312 ± 1.45^{a}	7.812 ± 1.16^{a}

Different superscript small letters (a-b) within a row indicate means which are significantly ($p < 0.05$) different.

0.5 and 1.0 mg/ml EPP extract resulted in reduction in their wound closure ability to $19 \pm 2\%$ and $13.2 \pm 2.5\%$ of untreated control cells, respectively.

Eggplant exhibits anticancer activity due to the presence of a diverse group of phenolic acids, and previous studies reported the therapeutic potential of eggplant bioactive compounds in the treatment of gastric and lung cancer [20, 21]. Chlorogenic acid and P-coumaric acid were two major phenolic compounds detected in the EPP extract and previously reported for remarkable antioxidant and anticancer potential [22, 23].

3.4. FTIR Analysis. The FTIR spectrum was used to identify the functional groups corresponding to bioactive compounds present in the EPP extract. The FTIR spectrum of the EPP extract and assignment of functional groups to characteristics peaks are presented in Figure 3.

The prominent peak in the range of 3450–3250 cm^{-1} was attributed to -OH in phenols and alcohols (O-H stretch) [24]. The peak in the range of 1618–1498 cm^{-1} was attributed to C=C aromatic ring stretch. The intense peak in the range of 1040–1030 cm^{-1} was attributed to C-O-C in aliphatic ether asymmetrical stretch and silicates Si-O [12]. In

polyphenolic compounds, the presence of hydroxyl groups on the aromatic ring enables them to donate a proton to a radical and act as an antioxidant [25].

3.5. GCMS Analysis. The major phytoconstituents identified in the EPP extract were oxime-, methoxy-phenyl-, butyrolactone, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, n-hexadecanoic acid, oleic acid, octadecanoic acid, 22, 23-bisnor-5-cholenic acid, and 3β-hydroxy, acetate (Table 3). The eggplant was reported to contain terpenoids, flavonoids, and fatty acids which contribute to the antioxidant and nutritional potential of eggplant [26].

3.6. HPLC Analysis. HPLC was used for the identification and quantification of phenolic compounds in the EPP extract (Table 4). The major components identified in the EPP extract were chlorogenic acid (261.76 mg/kg), benzoic acid (184.41 mg/kg), syringic acid (78.48 mg/kg), P-coumaric acid (49.59 mg/kg), cinnamic acid (32.17 mg/kg), quercetin (5.24 mg/kg), sinapic acid (5.14 mg/kg), and gallic acid (4.34 mg/kg). Eggplant is recognized for its high phenolic content and antioxidant activity. Phenolic compounds in the EPPs can serve as an alternative to synthetic additives for food fortification and improvement of shelf life [27]. Chlorogenic acid was reported to be the most abundant phenolic compound in eggplant [9].

3.7. Eggplant Extract-Enriched Ice-Cream. Ice-cream was made by incorporating the optimized EPP extract. Ice-cream with 5% EPP extract showed significantly higher TPC (7.52 ± 0.35 mg GAE/g), TAC (3.08 ± 0.20 mg/100 g), and DPPH inhibition ($83.00 \pm 2.64\%$) (Table 5). The EPP extract was previously used to develop functional beer with enhanced TPC, TAC, and antioxidant activity [28]. After the incorporation of the EPP extract in ice-cream, there was reduction in antioxidant activity and TPC, which was due to the degradation of antioxidants during ice-cream processing and storage [16].

Sensory evaluation of eggplant peel extract-enriched ice-cream was performed using the 9-point hedonic scale. Ice-cream with the eggplant peel extract showed high scores for texture (7.437 ± 1.41), aroma (6.875 ± 1.31), hardness (7.187 ± 1.22), melting (7.56 ± 1.75), appearance (7.062 ± 1.48), and taste (7.25 ± 1.57) as compared to the blank ice-cream. However, the score for color was lower than the blank ice-cream. The overall acceptance score of EPP extract-enriched ice-cream (7.812 ± 1.16) was higher than control (7.312 ± 1.45) and blank (6.12 ± 1.5) samples (Table 6). Sensory analysis of EPP extract-enriched ice-cream was based on 16 panelists which indicated the overall product's liking by selective participants. However, for commercial applications, sensory evaluation by a large number of consumers can provide comprehensive sensory perception. Ice-cream is a highly acceptable product among consumers and can be modified by the incorporation of bioactive formulations such as the EPP extract without compromising its sensory attributes [16, 29].

4. Conclusion

Under optimized ultrasonic-assisted extraction conditions, high concentrations of phenolics and anthocyanins were obtained from eggplant peel. The eggplant peel extract was found to be a good source of phenolic compounds with demonstrated antioxidant and anticancer activity. Due to its high antioxidant activity and anticancer potential against human cancer cell lines, the EPP extract can be used in the formulation of functional food and feed products.

Data Availability

The data supporting the findings of the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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