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Original Research

Isolation, identification and chemical characterization of compounds from phenolic extracts of peels of Kufri Chipsona-3 and Kufri Jyoti potatoes having synergistic antioxidant interactions in combination

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Abstract: The study focused on the isolation, identification and characterization of the bioactive compounds that are responsible for synergistic antioxidant interactions in combination from the peel extract of Kufri Chipsona-3 and Kufri Jyoti potato varieties by Thin-Layer Chromatography (TLC) bioautography mediated isolation technique which was followed by isobologram analysis as well as characterization using different spectrometric methods. The results revealed that among the isolated seven antioxidant components of potato peel extracts (three components with Rf : 0.33, 0.52 and 0.57 from the peel extract of Kufri Chipsona-3 and four components with Rf : 0.14, 0.22, 0.36, 0.61 from the peel extract of Kufri Jyoti, only one component from Kufri Chipsona-3 (Rf : 0.57) and one component from Kufri Jyoti (Rf : 0.61) potato varieties in combination exhibited synergistic antioxidant interactions in combination. Spectrometric analysis (UV/Vis, FT-IR and HR-LCMS/MS) revealed that the active component with Rf: 0.57 from Kufri Chipsona-3 is prodelphinidin trimer and the other component with Rf : 0.61 from Kufri Jyoti is 5-hydroxy-3',4',7-trimethoxyflavone. In a root growth inhibition test (LC_{50} > 200 µg/ml), on Allium cepa, these two compounds showed no cytotoxicity. The results provide evidence that these two phenolic compounds, prodelphinidin trimer and 5-hydroxy-3',4',7-trimethoxyflavone in combination may serve as a stronger and efficacious novel natural antioxidant blend in pharmaceutical and food industries.

Keywords: Potato peel waste, Natural antioxidants, Synergistic interactions, TLC bioautography, Spectrometric analysis, Cytotoxicity study

Introduction

Lipid oxidation is considered to be one of the main problems with the preservation of fatty acids in food [1]. Since it has been established that omega-3 fatty acids exhibit significant health benefits, meals are now supplemented with them to boost the nutritional value of foods and lower the risk of acquiring chronic diseases. The two primary components of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are highly susceptible to autoxidation because of the high levels of unsaturation in their molecular structures [2]. This makes it challenging to produce stable functional foods fortified with omega-3 fatty acids. Synthetic antioxidants are frequently employed in the food sector to address these issues. However, there is a

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strong evidence to suggest that they can also be hazardous and in some cases nitrite and nitrate food preservatives may lead to stomach cancer [3]. Therefore, new, safer and effective natural antioxidants should continue to be sought as preservatives, especially those derived from plants, to protect foods from oxidative deterioration, particularly omega-3 fatty acid-fortified functional foods.

Due to their powerful antioxidant effectiveness, plant polyphenols may provide a possible source of such molecules [4, 5]. Given that these leftovers are one of the major sources of polyphenols, using fruit and vegetable waste for research has gained popularity recently. As a result, effective recycling and use of phenolic-rich fruit and vegetable waste could lead to financial gains for the food industry, contribute to addressing food nutritional issues, have a positive impact on health and reduce environmental pollution [6, 7]. Phenolic chemicals are abundant in potato peels. It has been discovered that potato peels have a higher phenol concentration than potato tubers. In the potato processing industry, large amounts of phenolic-rich potato peels are produced as a waste product. As a result, the industry in question faces a major challenge in terms of waste disposal [8]. Potato peels are another waste product that is produced in large quantities by families. However, due to their significant antioxidant activity, these potato peels with high phenolic content can be employed as a source of natural antioxidants for preserving foods [9, 10]. Researchers are now conducting a systematic search for these beneficial chemicals from used potato peels in response to the growing need to replace synthetic antioxidants with natural ones.

Only the peel extract of the potato varieties Kufri Chipsona-3 and Kufri Jyoti showed synergistic antioxidant interactions when combined, according to our recent investigation on the peel extracts of five different potato varieties [11]. Therefore, an attempt has been made in the current work to isolate, characterize and identify the bioactive compounds from peel extracts of these two potato varieties (Kufri Chipsona-3 and Kufri Jyoti). They were found to be responsible for synergistic antioxidant interactions when combined with each other using a TLC-bioautography led isolation technique, which was followed by isobologram analysis and spectrometric characterization.

Materials and methods

Collection and processing plant materials

These two potato varieties, Kufri Chipsona-3 and Kufri Jyoti, were collected from the vegetable farm of Bidhan Chandra Krishi Viswavidyalaya (BCKV), West Bengal, India, and were recognised by a botanist. The harvest period for them lasts for 90-100 days on average. They were used in the current investigation. These different kinds of potatoes were carefully rinsed in tap water before being manually peeled to a depth of around 1 mm. The peels were then air dried for 5-6 days until they had reached a constant weight and were then grounded into a coarse powder using a manual grinder.

Extraction of plant materials

The phenolic extract of the potato peels of Kufri Chipsona-3 and Kufri Jyoti was made respectively, according to the procedure described by Abu-Reidah et al. [12]. 25 g of coarsely ground potato peels were macerated at 25 ± 1º C in the dark for 24 hours with 250 ml of a solution of water and ethanol at a ratio of 20:80 v/v ratio, while being occasionally agitated. The mixture was then filtered using Whatman filter paper No. 1. Then the filtrates were blended after the second and third extraction of the residue. 100 ml of the pooled filtrate and 4 ml of Carrez reagent A were mixed and vortexed for two minutes before being left for one minute. The mixture was then combined with 4 ml of Carrez reagent B, then vortexed for 20 seconds. These two chemicals were utilized to separate the proteins and polysaccharides, respectively. The mixture was then centrifuged at 2000 g for 20 minutes. Next, the supernatant was removed, centrifuged at 2000 g for a further 10 minutes and then filtered. Anhydrous sodium sulphate was used as a dehydrating agent, and the filtrate was subsequently dried by evaporation in a rotary evaporator at a bath temperature of 40°C. Most of the dried potato peel extracts were stored at 20 °C until needed [yield: Kufri Chipsona-3: 3.92%; and Kufri Jyoti: 3.72%].

Isolation, identification and chemical characterization of bioactive compounds

The phenolic extract from the peels of Kufri Chipsona-3 and Kufri Jyoti were chemically analyzed by TLC-bioautography guided detection and isolation, followed by isobologram analysis and spectrometric characterization as follows. This was done for isolating, identifying, and chemically characterizing the bioactive compounds responsible for antioxidant efficacy.

TLC bioautography guided detection and isolation of antioxidant components from potato peel extract

Analytical TLC

In accordance with the methodology by Gu et al. (2009) [13], the Rf values of separated components of active extracts from the peels of the potato varieties Kufri Chipsona-3 and Kufri Jyoti (which showed synergistic antioxidant and antimicrobial interactions in combination) were determined using analytical TLC separation technique. The analytical TLC plates (5 × 10 cm, 0.25 mm thickness, Silica gel G 60 F254, Merck, Darmstadt, Germany) were baked in aluminium. These plates were preconditioned by heating for an hour at 120 °C. 5µl (10 mg/ml) of the active extracts from the peels of Kufri Chipsona-3 and Kufri Jyoti potato varieties were applied

using a capillary pipette. The plate was then developed for 3-4 hours at room temperature using a solvent solution made up of butanol, ethyl acetate, and formic acid [5:4:1 (v/v)]. After developing the TLC plate, the absorbent layer was air dried to eliminate all traces of the solvent mixture. For visualisation, the p-anisaldehyde-sulphuric acid reagent was sprayed on the sample and heated at 110°C for 5 minutes. The spots on the plate that contained separated active components of peel extract were measured for their Rf values using the equation below [14].

$$R_f = \frac{Distance\ traveled\ by\ solute}{Distance\ traveled\ by\ solvent}$$

In order to prevent potential interference with the visualisation reagent, which could affect the antioxidant activity of test samples in bioautograms, TLC plates for the detection of antioxidant compounds in TLC bioautography were prepared simultaneously using the same procedure as described above without spraying the visualisation reagent [15].

TLC bioautography guided detection of antioxidant components

To confirm the presence of antioxidant components, the developed air-dried plate from analytical TLC that had been prepared for the detection of antioxidant compounds was then placed in a sterile Petri dish and sprayed with a 0.02% w/v DPPH solution in methanol. This was then heated at 110°C for 5 minutes. The Rf values of the antioxidant spots on the analytical TLC plate were calculated from the corresponding Rf values of the component's spots.

Preparative TLC for isolation of antioxidant components

To separate the antioxidant components of the active extracts of Kufri chipsona-3 and Kufri Jyoti potato varieties, a streak of active test peel extracts was manually applied to a preparative TLC glass plate (20 × 20 cm, 1 mm thickness) (Sigma-Aldrich, USA) and air dried. After air drying, the plate was developed in a pre-saturated glass chamber with the same solvent mixture used for analytical TLC. On the produced bioautograms, a 0.02% (w/v) DPPH solution in methanol was sprayed on the entire plate. Antioxidant spots with Rf values that showed antioxidant activity in the TLC bioautography were scraped out of the processing. The bands with antioxidant activity were carefully removed from the silica gel and dissolved in 80% methanol. Then the methanol-based antioxidant components were centrifuged at around 10,000 g for an additional 15 minutes. The supernatants and silica gel were collected. The supernatants were then dried in vacuum after being filtered through a 0.22 m membrane filter. The extracted antioxidants, which had been dried at various Rf values, were then stored in a refrigerator for later use.

Following the isobologram analysis, the extracted antioxidant components from the peel extracts of Kufri Chipsona-3 and Kufri Jyoti potato varieties were submitted to a combined antioxidant efficacy research.

Determination of antioxidant activity of active components alone and in combination by DPPH radical scavenging method

According to Wang et al. (1998) [16], the ability of antioxidant elements from the potato peel extracts of Kufri Chipsona-3 and Kufri Jyoti to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was investigated both separately and in combination. In brief, test tubes were added with 100 µl of antioxidant compounds alone and in combination (1:1) at different concentrations (3.12 µg/ml to 100 µg/ml), and then 3.9 ml of a 0.1 mM solution of DPPH in methanol was added. The test tubes were then shaken vigorously. The tubes were then left at room temperature in the dark for 30 minutes. The test substances described above were used to establish the control. The zero value was changed by using methanol. The absorbance of the samples was measured at 517 nm. The percentage radical scavenging activity of antioxidant components alone and in combination was evaluated as follows:

$$(\%)\ Free\ radical\ scavenging = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

Where, A_{sample} is the absorbance of DPPH solution after reacting with a given concentration of antioxidant component and A_{blank} is the absorbance of DPPH solution with methanol blank instead of antioxidant component. The IC_{50} values (the concentration required to scavenge 50% of the DPPH radical) of antioxidant components alone and in combination were determined from dose-response curve.

Isobologram analysis for determination of combination index (CI) based on IC_{50} values

The antioxidant combination index (CI) of combined isolated antioxidant components was evaluated using isobologram analysis based on IC_{50} values. The data were analyzed using the traditional calculation by isobologram combination index (CI) [17].

$$CI = \frac{D1}{Dx1} + \frac{D2}{Dx2}$$

where (D_1) and (D_2) are the doses (IC_{50} values) of two different test antioxidant components in combination; (D_{x1}) and (D_{x2}) are the doses (IC_{50} values) of two individual test antioxidant components that are combined [17].

Determination of type of antioxidant interactions based on combination index (CI)

Based on the CI values, the type of antioxidant

interactions in combination were interpreted as follows: CI < 1: synergistic; CI = 1: additive; CI > 1: antagonistic [18].

Spectrometric analysis

In order to characterize the chemical composition of the antioxidant compounds, the spectrometric (UV/Vis, FTIR, HR-LCMS/MS) analyses of the peel extract of the potato varieties Kufri Chipsona-3 and Kufri Jyoti were performed on the antioxidant components, which demonstrated synergistic antioxidant interactions in combination in isobologram analysis.

Following spectrometric investigation for chemical identification, only one component from peel extract of Kufri Chipsona-3 (Rf: 0.57) and Kufri Jyoti (Rf: 0.61) in combination was found to show synergistic antioxidant interactions.

UV/Vis absorption spectra

A Perkin Elmer Lambda 950 UV-Vis spectrometer was used to record the UV/Vis absorption spectra of antioxidant components from peel extract of these two selected potato varieties, which together demonstrated synergistic antioxidant interactions. The spectral range was 200-2500 nm. The calibration curve was prepared. Samples were subjected to UV/Vis absorption spectral analysis.

FT-IR analysis

The FT-IR spectral analysis of test antioxidant components from Kufri Chipsona-3 and Kufri Jyoti was conducted by using the Fourier-transform infrared instrument (make and model:3000 Hyperion Microscope with Vertex 80 FTIR System, Burker, Germany) in the scan range 450-4000 cm^{-1}.

HR-LCMS/MS analysis

The HR-LCMS/MS spectrometric analysis of test antioxidant compounds was done by using HR-LCMS instrument (make and model : Agilent Technologies, USA, 1290 Infinity UHPLC System,1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs) in the mass range 50-32200 amu and resolution 40000 FWHM. The instrument was equipped with (1) UHPLC & Nano HPLC for small molecules as well as for large molecules; (2) Direct Infusion Mass with ESI & APCI (Positive & Negative mode ionization; (3) UHPLC PDA Detector -Mass spectrometer and (4) Nano HPLC with Chipcube (Microfluidic column) -Mass spectrometer. Agilent software was used for data analysis.

Evaluation of cytotoxic potential of antioxidant compounds that showed synergistic antioxidant interactions in combination

Allium cepa root growth inhibition assay

When evaluating the cytotoxic potential of antioxidant compounds in the peel extract of the potato varieties Kufri Chipsona-3 and Kufri Jyoti, which exhibited synergistic antioxidant interactions in combination, Fiskesjo's method from 1985 was used [19]. Briefly, we purchased 20 to 25 g of robust common onion bulbs from a neighboring market in Bura Bazar, Kolkata, India. Before the experiment, the bulbs were allowed to dry and the outer scales were removed, which were made simpler by stopping the destruction of the root primordial. The remainders of the pesticides and herbicides were then carefully removed from the bulbs by washing them under running water. After that, bulbs were kept in tap water at room temperature for 3-4 days without light to germinate, at this time the new roots were 3-4 cm long. Nine groups of bulbs, with six for each group, were collected after the development of new roots. Each glass tube contained 10 ml of tap water. The antioxidant compounds of Kufri Chipsona-3 and Kufri Jyoti were then added into tubes containing seven groups of onion bulbs at seven different concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml), which demonstrated synergistic antioxidant interactions in combination in the DPPH method and isobologram analysis. Positive control group received sodium azide (10 µg/ml), while the negative control group did not receive any medication. Following that, both control and antioxidant component-treated groups were stored at room temperature in the dark for 96 hours. The top 10 roots of Allium cepa bulbs in each group were measured at the conclusion of the exposure period (96h), and the mean root length (cm) was calculated. Onion bulbs in the negative control group were considered as a standard, and measurements of the average root length in the treatment groups that received antioxidant chemicals and the positive control group were made in comparison. At the conclusion of the exposure period, whether the roots have any bulges, hooks, or twists, as well as other clear morphological transformations, including root consistency and color changes, were also carefully studied.

Statistical analysis

The statistical analysis of data was performed using the SPSS software: version 18.0. A one-way ANOVA (analysis of variance) followed by a Tukey range test was used to analyse the data with the significance level set at $P < 0.05$.

Results

Separation and detection of antioxidant components by analytical TLC and TLC bioautography analysis

Figure 1(a) shows the Rf values of separated components of peel extract of Kufri Chipsona-3 obtained by analytical TLC analysis, and Figure 1(b) shows the Rf values of antioxidant components of peel extract of Kufri Chipsona-3 obtained by TLC- bioautography. The analytical TLC separation of peel extract of Kufri Chipsona-3 (Figure 1(a)) revealed the presence of four

components (Rf: 0.33, 0.47, 0.52 and 0.57) in Kufri Chipsona-3 potato peel extract. Of these four separated components, only three components with Rf : 0.33, 0.52 and 0.57 were found to have antioxidant activity in TLC bioautography guided detection technique as yellow spots against a purple background (Figure 1(b)).

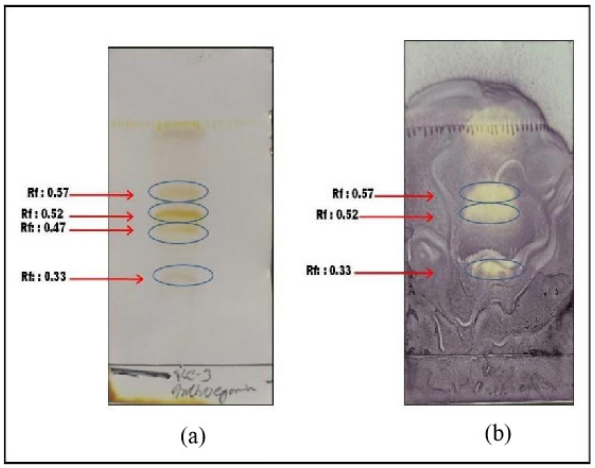


Figure 1. (a). Rf values of separated components of Kufri Chipsona-3 by analytical TLC; (b). Rf values of antioxidant components of Kufri Chipsona-3 by TLC-bioautography

Figure 2(a) and Figure 2(b) respectively represent the chromatograms of analytical TLC and TLC bioautography analyses of peel extract of Kufri Jyoti potato variety. It was observed from Figure 2 (a) that the peel extract of Kufri Jyoti potato variety contains five components (Rf : 0.14, 0.22, 0.36, 0.61, and 0.73) and of them only four components (Rf : 0.14, 0.22, 0.36, and 0.61) demonstrated antioxidant activity in TLC bioautography analysis (Figure 2 (b)).

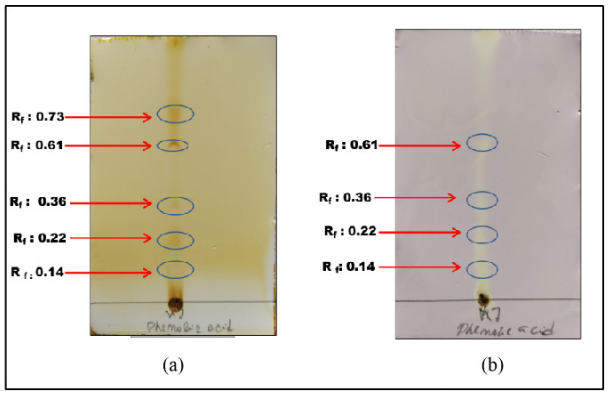


Figure 2. (a) Rf values of separated components of Kufri Jyoti by analytical TLC; (b) Rf values of antioxidant components of KufriJyoti by TLC-bioautography

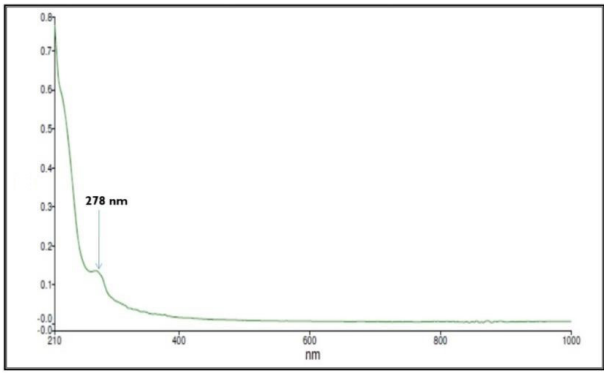
Results of isobologram analysis for determination of combination index (CI) based on IC_{50} values

Table 1 shows the Rf values of seven antioxidant components of peel extracts of Kufri Chipsona-3 (Rf: 0.33, 0.52, 0.57) and Kufri Jyoti (Rf: 0.14, 0.22, 0.36, 0.61) potato varieties obtained by TLC bioautography analysis and their antioxidant potential (IC_{50}) alone and in combination determined by DPPH radical scavenging method as well as their combination index (CI) values based on IC_{50} values using isobologram analysis. It was observed from Table 1 that among the possible combinations tested, only one combination between one component (Rf : 0.57) of Kufri Chipsona- 3 and one component (Rf : 0.61) of Kufri Jyoti peel extract exhibited synergistic antioxidant interactions (CI < 1), whereas other possible tested combinations showed additive antioxidant effect (CI=1).

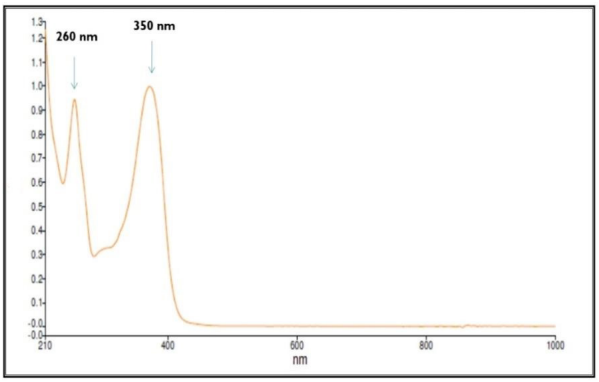
Results of spectrometric analysis

UV/Vis absorption spectra results

Figure 3(a) represents the UV/Vis spectra of Kufri Chipsona -3 peel extract component (Rf: 0.57). It was observed that the maximum absorbance (UVλmax) of Kufri Chipsona-3 peel extract component (Rf: 0.57) was 278 nm, which corresponds with the absorbance maxima of proanthocyanidin compounds [20].



(a)



(b)

Figure 3. (a) UV-Vis absorption spectra of Kufri Chipsona-3 peel extract component (Rf : 0.57); (b) UV/Vis absorption spectra of Kufri Jyoti peel extract component (Rf : 0.61)

Figure 3(b) represents the UV/Vis absorption spectra of Kufri Jyoti peel extract component (Rf: 0.61). It was observed that the maximum absorbance (UVλmax) of Kufri Jyoti peel extract component (Rf: 0.61) was at 350 nm and 260 nm, which corresponds with the absorbance maxima of flavones [21] .

Table 1. Results of combined antioxidant efficacy (CI) of active components from peel extracts of Kufri Chipsona-3 and Kufri Jyoti potato varieties based on IC_{50} values of antioxidant components alone and in combination

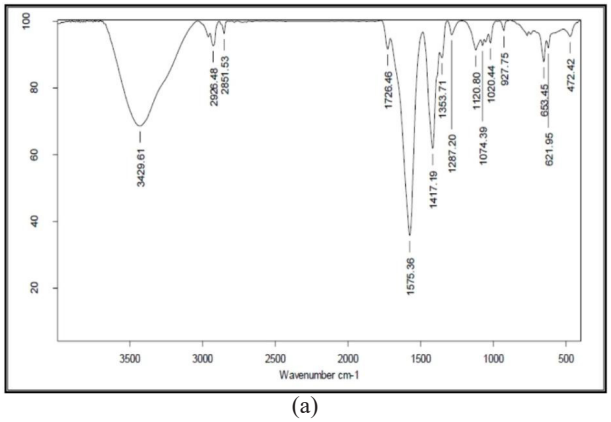
Test potato peel	Rf value of active components	IC_{50} (µg/ml)	$CI_1=(D)_1/(DX)_1$	$CI_2=(D)_2/(DX)_2$	$CI=CI_1+CI_2$	Remarks
KufriChipsona-3	0.33	71.67±1.23	-	-	-	-
KufriChipsona-3	0.52	64.34±1.05	-	-	-	-
KufriChipsona-3	0.57	34.56±1.02	-	-	-	-
KufriJyoti	0.14	75.78±2.01	-	-	-	-
KufriJyoti	0.22	59.43±1.34	-	-	-	-
KufriJyoti	0.36	66.37±1.54	-	-	-	-
KufriJyoti	0.61	42.64±2.23	-	-	-	-
KufriChipsona-3 + KufriJyoti	0.33+0.14	37.54±1.43	0.52	0.49	1.01≈1	Additive
KufriChipsona-3 + KufriJyoti	0.33+0.22	33.32±1.11	0.46	0.56	1.02≈1	Additive
KufriChipsona-3 + KufriJyoti	0.33+0.36	41.35±1.21	0.57	0.62	1.19	Antagonistic
KufriChipsona-3 + KufriJyoti	0.33+0.61	26.98±2.13	0.37	0.63	1	Additive
KufriChipsona-3 + KufriJyoti	0.52+0.14	35.65±1.57	0.55	0.47	1.02	Additive
KufriChipsona-3 + KufriJyoti	0.52+0.22	42.18±2.07	0.65	0.70	1.35	Antagonistic
KufriChipsona-3 + KufriJyoti	0.52+0.36	32.98±1.78	0.51	0.49	1	Additive
KufriChipsona-3 + KufriJyoti	0.52+0.61	25.91±1.28	0.40	0.60	1	Additive
KufriChipsona-3 + KufriJyoti	0.57+0.14	29.63±1.11	0.85	0.39	1.23	Antagonistic
KufriChipsona-3 + KufriJyoti	0.57+0.22	22.12±2.43	0.64	0.37	1.01≈1	Additive
KufriChipsona-3 + KufriJyoti	0.57+0.36	24.67±1.09	0.71	0.37	1.08 ≈1	Additive
KufriChipsona-3 + KufriJyoti	0.57+0.61	16.94±1.15	0.49	0.39	0.88	Synergistic

FT-IR analysis results

Figure 4(a) represents the FT-IR spectrum of Kufri Chipsona -3 peel extract component (Rf: 0.57). From FT-IR curves (Figure 4(a)) the major peaks of peel extract component (Rf : 0.57) of Kufri Chipsona-3 were found at 3429.61 (stretching vibrations of O-H groups); 2906.48 and 2851.53 (stretching vibration of C-H (CH_2, CH_3); 1575.36 (aromatic ring vibrations); 1417.19 (related to CH_2, CH_3, flavonoids and aromatic rings where the vibrations would be bending (δ) vibrations of C-H and stretching vibration of aromatics); 1120.80-1020.44 (stretching vibration of C-O-C). These bands represent the structure of proanthocyanidins [20].

Figure 4(b) represents the FT-IR spectrum of Kufri Jyoti peel extract component (Rf : 0.61). Major peaks at FT-IR spectra of Kufri Jyoti peel extract component (Rf: 0.61) were found at 3442.28 (stretching vibration of O-H group); 2927.83-2821.82 (stretching vibration of C-H); 1594.57 (aromatic ring vibration); 1353.23 (stretching vibration of aromatic C=C); 1075.60 (C-O

stretching). These bands represent the molecular structure of flavonoids [22].



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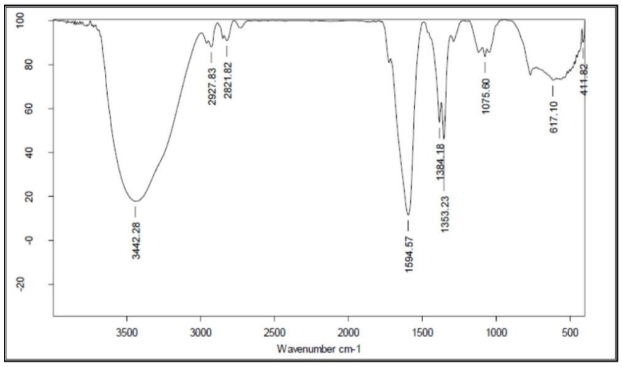


(b)

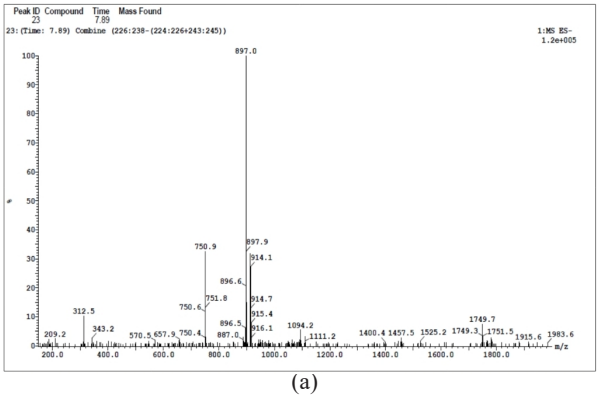
Figure 4. (a) FTIR spectra of Kufri Chipsona-3 peel extract component (Rf : 0.57); (b) FTIR spectra of Kufri Jyoti peel extract component (Rf : 0.61)

Figure 4(b) represents the FT-IR spectrum of Kufri Jyoti peel extract component (Rf : 0.61). Major peaks at FT-IR spectra of Kufri Jyoti peel extract component (Rf: 0.61) were found at 3442.28 (stretching vibration of O-H group); 2927.83-2821.82 (stretching vibration of C-H); 1594.57 (aromatic ring vibration); 1353.23 (stretching vibration of aromatic C=C); 1075.60 (C-O stretching). These bands represent the molecular structure of flavonoids [22].

HR-LCMS/MS analysis results

Figure 5(a) represents the HR-LCMS/MS analysis of Kufri Chipsona-3 peel extract component (Rf : 0.57). The retention time of this component was found to be 7.89 min and its molecular mass (m/z) was found to be 897.0.

Figure 5(b) represents the HR-LCMS/MS analysis of Kufri Jyoti peel extract component (Rf: 0.61). From HR-LCMS/MS analysis curves, the retention time of Kufri Jyoti peel extract component (Rf : 0.61) was found to be 10.04 min and its molecular mass (m/z) was 327.5.



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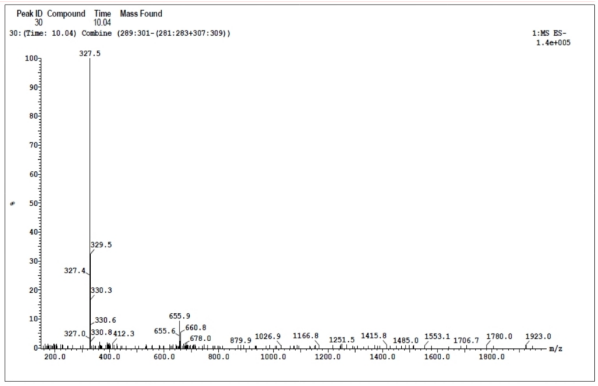


(b)

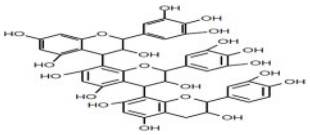
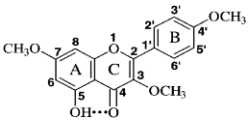
Figure 5. (a) HR-LCMS/MS spectra of Kufri Chipsona-3 peel extract component (Rf : 0.57); (b) HR-LCMS/MS spectra of Kufri Jyoti peel extract component (Rf : 0.61)

Table 2 shows the spectrometric analyses data (UV/Vis, FT-IR, HR-LCMS/MS) of two antioxidant components, one from peel extract of Kufri Chipsona-3 (Rf : 0.57) and the other from peel extract of Kufri Jyoti (Rf : 0.61) that showed synergistic antioxidant interactions in combination. Comparing the obtained data with MS library data as well as available literature data [23, 24], the antioxidant component (Rf : 0.57) obtained from peel extract of Kufri Chipsona-3 was identified as prodelphinidin trimer and the antioxidant component (Rf : 0.61) obtained from peel extract of Kufri Jyoti was identified as 5-Hydroxy-3',4',7-trimethoxyflavone.

Allium cepa root growth inhibition assay results

The results of the inhibition assay on Allium cepa root growth were used to determine the cytotoxic potential of the bioactive compounds prodelphinidin trimer and 5-Hydroxy-3',4',7- trimethoxyflavone, which were isolated respectively from active peel extracts of the potato varieties Kufri Chipsona-3 and Kufri Jyoti. Cell viability of the bioactive compounds tested in combination did not differ significantly from the negative control up to a concentration of 200 µg/ml, but it did differ significantly ($p < 0.05$) from the positive control (Sodium azide), and the IC_{50} of the tested bioactive compounds in combination was discovered to be greater than 200 µg/ml (Figure 6).

Table 2. Spectrometric analyses data of active compound from peel extract of Kufri Chipsona-3 (Rf : 0.57) and Kufri Jyoti (Rf : 0.61) potato varieties that showed synergistic antioxidant interactions

Spectrometric parameters	Active components from peel extract of Kufri Chipsona-3 potato (Rf : 0.57)	Active components from peel extract of Kufri Jyoti potato (Rf : 0.61)
UVλmax(MeOH)	Only one band at 278nm	Two bands. Band A at 350 nm and B at 260 nm
HR-LCMS/MS(m/z) [M-H]-	897(calcd.molecular formula:C45H38O20;898.8)	327.5 (calcd.molecular formula:C18H16O6;328.3)
Retention Time(min)	7.89	10.04
FT-IRKBr(cm-1)	Major peaks are at 3429.61 (stretching vibrations of OH groups); 2906.48 and 2851.53 (stretching vibration of C-H (CH_2,CH_3); 1575.36 (could be related to aromatic ring vibrations); 1417.19 (related to CH_2, CH_3, flavonoids and aromatic rings where the vibrations would be bending(δ) vibrations of C-Hand stretching vibration of aromatics); 1120.80-1020.44 (stretching vibration of C-O-C).	Majorpeaksareat3442.28 (stretching vibration of O-H group); 2927.83-2821.82 (stretching vibration of C-H); 1594.57 (aromatic ring vibration); 1353.23 (stretching vibration of aromatic C=C);1075.60(C-O stretching).
Proposed compound	Prodelphinidin trimer	5-Hydroxy-3',4',7-trimethoxy flavone
Molecular structure		
References	NIST MS search v.2.3	NIST MS search v.2.3

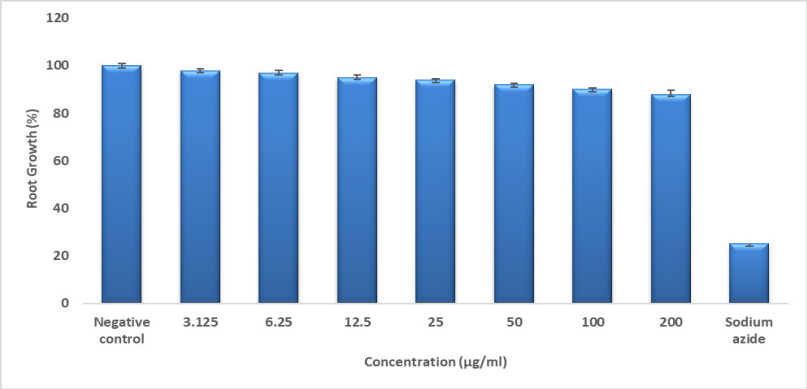


Figure 6. Results of cytotoxic potential of bioactive compounds in combination isolated from potato varieties Kufri Chipsona-3 (Rf : 0.57) and Kufri Jyoti (Rf : 0.61) in Allium cepa root growth inhibition assay. *No significant ($P > 0.05$) difference in root growth up to 200µg/ml concentration when compared with negative control but significantly ($P < 0.05$) different when compared with positive control Sodium azide

Discussion

It has been reported by several researchers that potato peels are rich in compounds, such as anthocyanins, glycoalkaloids, phenolic compounds and flavonoids [25]. These bioactive compounds are associated with good health because they protect the body's cells from oxidative processes and have antioxidative, anti-inflammatory, antitumor and anticancer properties. Due to their extensive application potential and beneficial properties, potato peels are well worth the attention of researchers and industry. Hence, new methods to discover these compounds and

their applications to food matrices are being explored [26, 27]. Take this into consideration, in our present investigation, an attempt has been made to isolate, identify and chemically characterize the bioactive compounds responsible for synergistic antioxidant interactions in combination from potato peel extracts of potato varieties Kufri Chipsona-3 and Kufri Jyoti with a aim to develop novel natural antioxidants for food and pharmaceutical industries.

To achieve the goal, at first, TLC bioautography guided detection and isolation of antioxidant components from peels extracts of potato varieties Kufri Chipsona-3 and Kufri Jyoti were carried out. TLC bioautography guided detection and isolation technique were used in the present investigation because (1) this assay results in the direct and quick localization of bioactive compounds obtained from plant extracts; (2) it is a quick, low-cost and straightforward method for isolating compounds from complex plant extracts; and (3) it is discovered to be able to accurately identify chemical compounds in plant extracts; (4) It is crucial to avoid time consumption for inactive compound isolation, and this assay makes it easier to combine chromatographic separation with an in-situ activity measurement, which enables to locate and identify bioactive compounds and this technique in recent years has become an important tool for detection and isolation of bioactive compounds [15].

TLC bioautography analysis showed the presence of three antioxidant components in peel extract of Kufri Chipsona-3 that have Rf values 0.33, 0.52 and 0.57, respectively (Figure 1(b)) and four antioxidant components with Rf : 0.14, 0.22, 0.36 and 0.61 in peel extract of potato varieties Kufri Jyoti (Figure 2 (b)). These seven antioxidant components of different Rf values from peel extracts of two selected potato varieties were subjected to combined antioxidant efficacy study in DPPH radical scavenging method followed by isobologram analysis to observe their possible antioxidant interactions (synergistic, additive or antagonistic). Results obtained from combined antioxidant efficacy study revealed that among the possible combinations tested, only one combination between components with Rf : 0.57 of Kufri Chipsona-3 peel extract and Rf : 0.61 of Kufri Jyoti peel extract exhibited synergistic antioxidant interactions (CI < 1). Besides, most of the possible combinations showed an additive antioxidant effect (CI =1), whereas a few combinations showed antagonistic antioxidant efficacy (C>1) (Table 1).The possible reason behind the antagonistic antioxidant interactions between a few tested combinations observed in the present investigation is not clear right now. According to Olszowy et al., it may be due to (1) antioxidant adduct formation that is competitive, (2) antioxidant regeneration by an antioxidant that is more effective and (3) antioxidant oxidation by the radicals of a less effective antioxidant; and antioxidant microenvironment modification by another antioxidant [28]. In our previous study on antioxidant activity of potato peel extracts, we have used BHT as reference standard antioxidant [29]. But, in the present investigation , our main objective is to identify and chemically characterize the bioactive components from active extracts of potato peels responsible for synergistic antioxidant interactions. That is why, in the present investigation we have not used any reference standard antioxidant like BHT.

Now, these two bioactive components from peel extract of potato varieties Kufri Chipsona-3 (Rf : 0.57) and Kufri Jyoti (Rf : 0.61) were subjected to spectrometric (UV/Vis, FT-IR, HR-LC MS/MS) analyses for chemical characterization of bioactive compounds responsible for synergistic antioxidant interactions. Collective spectrometric analyses data of these two antioxidant components of peel extracts of potato varieties Kufri Chipsona-3 and Kufri Jyoti are shown in Table 2. These data were compared with MS library data as well as relevant references [23, 24] to chemically characterize the bioactive compounds. Comparing the obtained spectral data of bioactive components from peel extracts of potato varieties Kufri Chipsona-3 and Kufri Jyoti (Table 2) with MS library data as well as relevant literature data, the antioxidant component from peel extract of Kufri Chipsona-3 with Rf : 0.57 and m/z: 327.5 was found to be prodelphinidin trimer and the antioxidant component from peel extract of Kufri Jyoti (Rf : 0.61; m/z 897) was found to be 5-Hydroxy-3',4',7-trimethoxyflavone.

Cytotoxicity studies of plant extracts or isolated bioactive compounds are essential because to be minimal nontoxicity is a crucial process that results in the successful development of a pharmaceutical or cosmetic product. In the present investigation, we therefore, evaluated the cytotoxic potential of two phenolic compounds prodelphinidin trimer and 5-hydroxy-3',4',7-trimethoxyflavone isolated respectively from potato peel extracts of Kufri Chipsona-3 and Kufri Jyoti that showed synergistic antioxidant interactions in combination using Allium cepa root growth inhibition assay [30, 32]. The Allium cepa test was chosen because it is straightforward with a lower relative cost than other tests and is versatile, and only requires a minimal amount of laboratory equipment to be used [31]. Due to the fact that root development inhibition can be seen as a manifestation of cell division arrest, it is considered to be the most sensitive measure [32, 33]. The IC_{50} of the tested bioactive components in combination in Allium cepa root growth inhibition assay was found to be >200µg/ml in this investigation. Furthermore, no bulges, hooks, or twists in the roots, as well as any discernible morphological alterations in root consistency and colour were seen and therefore the test compounds in combination can generally be considered as safe [34, 35].

Conclusion

Thus, phenolic compounds prodelphinidine trimer and 5-hydroxy-3',4',7-trimethoxyflavone isolated respectively from peel extracts of Kufri Chipsona-3 and and Kufri Jyoti

were found to be responsible for synergistic antioxidant interactions in combination and can generally be considered as safe. In our previous study, peel extracts of Kufri Chipsona-3 and Kufr Jyoti showed synergistic antioxidant interactions both in various in-vitro models as well as against oxidation of omega-3 fatty acids enriched food supplements. Therefore, these two phenolic compounds isolated from Kufri Chipsona-3 and Kufr Jyoti potato peel extracts may serve as a potent antioxidant applied both in food and pharmaceutical industries. The findings may also help to convert waste potato peels from zero value waste to value added products. To the best of our knowledge this is the first report of synergistic antioxidant interactions of phenolic compounds isolated from potato peel extracts.

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Ethical approval

This article does not contain any experiment on human participants or animals performed by any of the authors.

Consent to participate

Informed consent was obtained from all individual authors included in the study.

Consent to publish

All authors consent for the publication of identifiable details, which can include data, tables, figures and details within the text to be published in the Journal of Food, Nutrition and Diet Science.

Authors' contribution

The study conception and design were done by Dr. Rabi Ranjan Chattopadhyay. Material preparation, data collection and analysis were performed by Dr. Abhishek Bhattacharya. The first draft of the manuscript was written by Dr. Rabi Ranjan Chattopadhyay and all authors commented on previous version of the manuscript. All authors read and approved the final version of this manuscript.

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Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials

All data are included within the manuscript.

Declaration of generative AI and AI assisted technologies in the writing process

No AI or AI assisted technologies were used in writing process.

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