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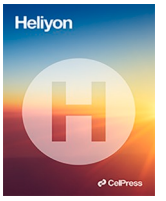

Research article

Anthocyanin profiling of genetically diverse pigmented potato (*Solanum tuberosum* L.) clonal accessions from north-eastern sub-Himalayan plateau of India

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ABSTRACT

White-fleshed potatoes have health concerns due to high glycemic index. Native and unexplored pigmented potato landraces may offer adequate and future smart alternatives with a balanced nutritional profile. Twenty-five pigmented potato clonal accessions across the eastern sub-Himalayan plateau of India were collected, purified and categorized into 'Badami' (UBAC) and 'Deshi' (UDAC) types. Evaluation of different nutritional attributes revealed that pigmented UBAC accessions are boosted with, high total dietary fibre, and total anthocyanin content and have remarkably low reducing sugar and glycemic index. Non-targeted LC-MS analysis identified caffeoyl and coumaroyl derivatives of delphinidin and petunidin glycosides, as major classes of anthocyanin compounds in pigmented potato accessions. HPLC-mediated quantification revealed high contents of delphinidin in the majority of accessions along with the selective presence of other anthocyanins. Selected accession was found to have polyphenolic compounds like gallic acid, vanillic acid, cinnamic acid and quercetin. The genetic cluster analysis of clonal accessions divided these genotypes into five major clusters. An ISSR repeat motif $(AGG)_6$ was tightly linked with the total anthocyanin content of the accessions in Single Marker Analysis. Altogether, these native pigmented potato accessions offer a nutritious and healthy alternative to the conventional white-fleshed potato genotypes.

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the predominant non-grain, starchy, readily available energy-rich tuber crops belonging to

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the family Solanaceae. Potatoes have a rich and intriguing history of acclimatization and cultivation around the world. Ancient to the outskirts of Peru and Bolivia near the Andes [1], potatoes are now grown in around 150 countries and contribute significantly to the world's economy [2]. Most of the cultivated potatoes are polyploid (2n = 4x = 48) with an average tuber weight of 70–150 g and exhibit tan to light brown skin colour with white flesh. The flesh is a rich reserve of nutrients and contains 20.6 % carbohydrates, 2.1 % protein, 0.3 % fat, 1.1 % crude fibre, 0.9 % ash and has a calorific value of 377 KJ/100 g [3]. Due to high carbohydrate content, potato tubers are classified as high glycemic index (GI) food and may result in insulin spikes upon consumption. Indian white potato cultivars are reported to have high GI values [4] making them an undesirable option for the health-conscious modern generation.

Eastern *sub-Himalayan* plains of India spanning over Sikkim, northern parts of West Bengal, Assam, and other north-eastern states, serve as a potential belt where potato is an automatic cash crop choice of farmers during rabi season [5]. Apart from the high-yielding varieties, few native potato landraces are traditionally grown in the North-Eastern and Terai agroecological regions of India. These potato accessions, popularly sobriquet as 'Badami aloo' and 'Deshi aloo' have smaller size of tubers than the released varieties, with pink to dark brown coloured skin and pigmented flesh [6]. These heirloom landraces have traditionally been cultivated and maintained by local farmers from ancient eras due to the preference among local consumers and premium market prices. The production scale of these pigmented landraces is small and regional to date and their predominant uses are in culinary applications due to their special taste and appearance. Pigmented small potatoes, also referred to as fingerling potatoes, have been reported from Southern Europe, South and North American countries, emphasizing the nutritional superiority of these genotypes over white-fleshed potatoes [7]. The red thumb fingerling potatoes are distinctive to normal-sized potatoes with yellow flesh in the shape, size and colour of the flesh. These potato accessions, originating from Peru, are small (5–7 cm), tubular or elliptical, with ruby red skin [8]. In the USA, two varieties of fingerling potatoes namely 'AmaRosa' and 'Purple Pelisse' were developed [9,10]. A few varieties of coloured potatoes including 'Hermanns Blaue', 'Highland' 'Burgundy Red', 'Shetland Black', and 'Vitelotte' have good market value [11]. Fingerling potatoes or pigmented potatoes are the natural sources of phytochemicals, especially acylated derivatives of anthocyanins and other important antioxidants [12]. Naturally occurring anthocyanin compounds like petunidin, pelargonidin, peonidin, malvidin, delphinidin and cyanidin [13] are found in pigmented potatoes that contribute towards their attractive colour. Several beneficial effects of the consumption of these potatoes on human health have been reported in recent times [14–16]. Anthocyanins have potential health benefits including enhancement of high antioxidant capacity, treatment of various blood circulation disorders, vaso-protective and anti-inflammatory properties, inhibition of platelet aggregation, maintenance of normal vascular permeability etc. [17].

Indian population is predominantly vegetarian and the nation depends heavily on potatoes to achieve nutritional security. The increase in lifestyle disorders like diabetes, obesity, cancer, cholesterol, etc. among the Indian population demands investigation for low GI, healthy potato alternatives. The present study aimed to identify novel potato accessions with low GI, and better nutritive value that offer health benefits. The current study presents evidence that the genetically diverse pigmented potato accessions from the eastern sub-Himalayan plains of India have low GI and high content of beneficial anthocyanins and phenolic compounds. Taken together, these accessions may serve as healthy potatoes in modern diets with abundant health benefits.

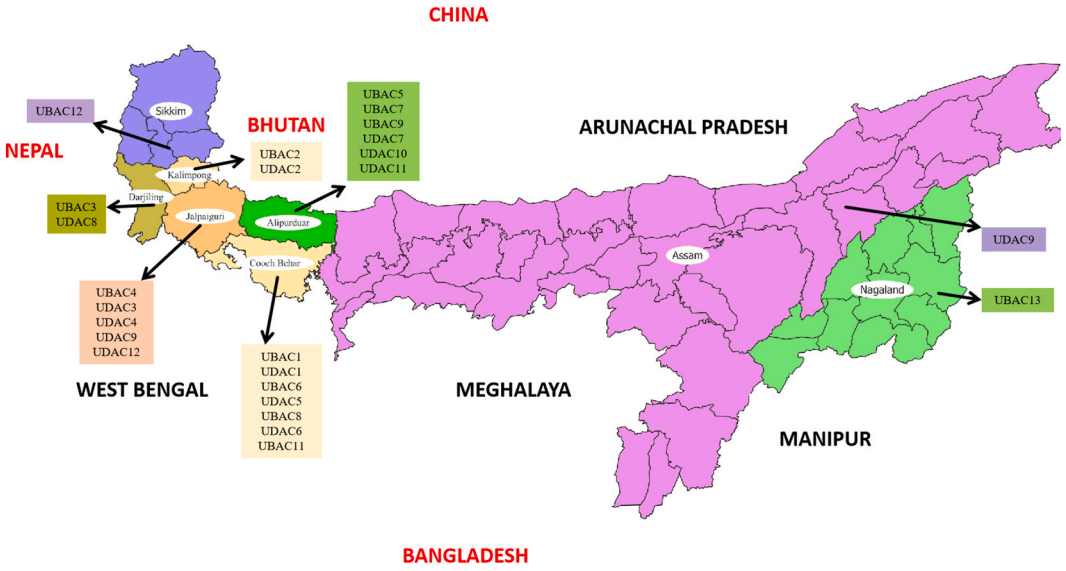

Fig. 1. The site of collection of the pigmented small potato accessions. The region is spanned over sub-Himalayan plain of the northeastern parts of India across four states. The map was generated using QGIS desktop 3.36.1. The districts of the collection site are highlighted on the map. The neighbouring Indian states are shown in black and neighbouring countries are shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2. Material and methods

2.1. Planting material, field practices, soil and weather condition

A total of twenty-five pigmented small potato clonal accessions were collected from different areas of the north-eastern sub-Himalayan plateau region of India spanning the terai agroecological region of West Bengal, Lower Sikkim, Upper Assam and Nagaland (Fig. 1) in the year 2015. These accessions were acclimatized and purified for two cropping seasons and maintained as clonal accessions at the Horticultural Instructional Farm under the Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya (UBKV). The accessions were chosen for investigation based on the skin colour and flesh colour variation of the mature tubers as per the descriptors mentioned in IBPGR, [18]. The test cultivation was carried out in the rabi season of the consecutive three years (2017-20). The Purple Skin Purple Flesh (PSPF), Red Skin Purple Flesh (RSPF) and Red Skin White Flesh (RSWF), oval-shaped potato tubers are designated as UBKV Badami Aloo Collection (UBAC1-UBAC13), whereas the Red Skin Red flesh (RSRF), Red Skin White Flesh (RSWF), and Yellow Skin White Flesh (YSWF) round shaped tubers are designated as UBKV Deshi Aloo collection (UDAC1-UDAC12) (Fig. 2). The naming of the accessions was done based on their vernacular names. The experimental site was situated at 26° 19′ 86″ N latitude and 89° 23′ 53" E longitude and a height of 43 m above mean sea level. The plot size of 3.6 m × 3.0 m with 20 cm plant-to-plant and 30 cm row-to-row spacing was used for cultivation. Seed tuber was treated with mancozeb @ 2.5 g/L of water for 30 min and then dried under shade before planting. Farm Yard Manures (FYM) of 15 t/ha was applied five days before planting. Fertilizer was applied @ 150: 60: 120 kg/ha of Nitrogen (N): Phosphorus (P_2O_5): Potash (K_2O). Irrigation was done as and when required and stopped 15 days before harvesting. A regular spray of mancozeb 75 % WP @ 3 g/L of water were done at every 7-day interval as plant protection measures. The skin colour and flesh colour of the tubers were analyzed after harvest and after cooking for 20 min at boiling temperature. Soil samples were collected randomly from the whole experimental field and analyzed in the laboratory to know the chemical and physical properties of the soil (Table S1). The meteorological information for maximum and minimum temperature (°C), precipitation (mm) and relative humidity (%) were recorded during the cropping seasons (Fig. S1).

2.2. Chemicals and reagents

The chemicals used in the present investigation were of analytical grade. The source, product code, and purity percentage of the standards and other chemicals used in different experiments are listed in Table S2. Glycemic index kit (K-RSTAR) and TDF kit (K-TDFR-200A) were purchased from Megazyme, Bray, Ireland. Deionised water, Celite, CTAB, and PCR master mix were procured from

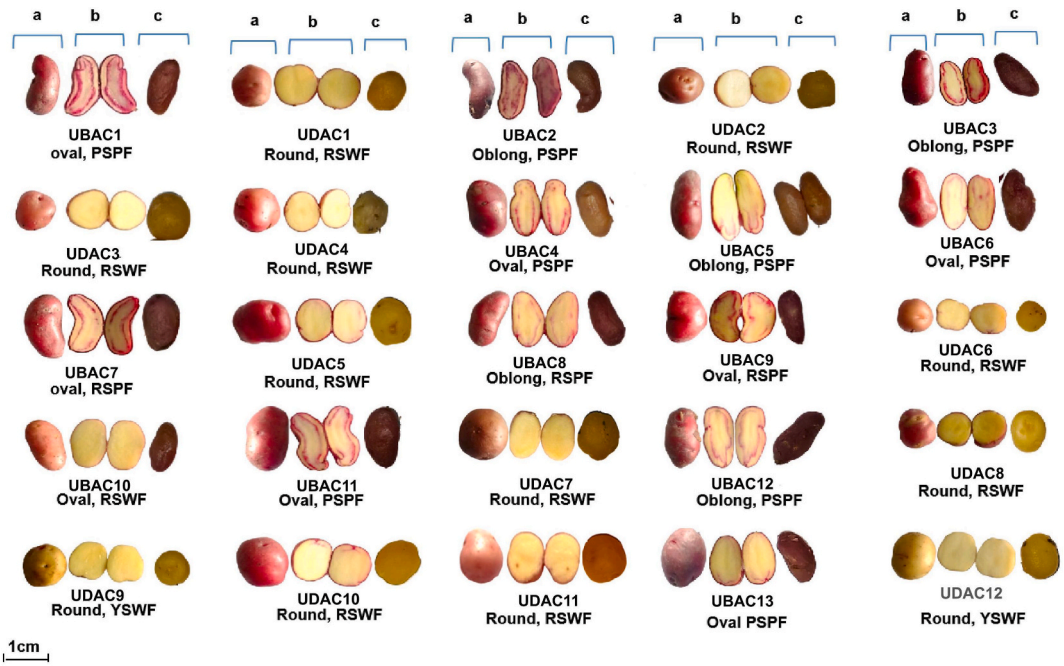

Fig. 2. Pictorial representation of the tubers of selected pigmented small potato accessions. The whole tuber (a), cut tubers (b), and boiled unskinned tubers (c) have been shown to address the variation in tubers colour before and after cooking. The Purple Skin Purple Flesh (PSPF), Red Skin Purple Flesh (RSPF) and Red Skin White Flesh (RSWF), oval-shaped potato tubers are designated as UBKV Badami Aloo Collection (UBAC), whereas the Red Skin Red flesh (RSRF), and Red Skin White Flesh (RSWF), round shaped tubers are designated as UBKV Deshi Aloo collection (UDAC). The naming of the genotypes was based on their local names i. e. 'Badami aloo' and 'Deshi aloo', following the international potato descriptor rules. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Promega. USA. The primers were custom synthesised from GCC Biotech Pvt. Ltd., India.

2.3. Nutritional parameters

Freshly harvested and cleaned tubers of the potato accessions were used to investigate the following nutritional parameters at the Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya.

2.3.1. Carbohydrate content

Total Starch content (STA) and Total Soluble Sugar (TSS) content for the accessions were estimated by the anthrone method [19]. Fresh potato tubers (200 mg) were homogenized with 80 % ethanol and centrifuged at 11200×*g* for 20 min. The precipitate was resuspended in 5 mL of distilled water and 6.5 mL of 52 % perchloric acid. After centrifugation, the final volume was made up to 100 mL with distilled water. Finally, 4 mL of anthrone reagent was added to 100 μL supernatant for green colour development. Absorbance was measured at 630 nm and STA was calculated in percentage on a fresh weight basis. For estimation of TSS content, 100 mg of fresh sample was hydrolysed for 3 h in 5 mL of 2.5 N HCl in a pre-heated water bath. The extract was then cooled and final volume was made up to 50 mL with distilled water and then centrifuged at 11200×*g* for 20 min. Afterwards, 100 μL of supernatant was diluted to 1 mL with distilled water and finally, 4 mL of anthrone reagent was added. The absorbance was measured at 630 nm. TSS is expressed in mg per 100 g of fresh weight using the standard reference plot of glucose (10–100 μg). The Reducing and Non-Reducing sugars (RES and NRS) were estimated by following the dinitrosalicylic acid (DNS) method [20]. Fresh potato tuber weighing 200 mg sample was homogenized in 80 % ethanol for extraction of sugars. After centrifugation at 11200×*g* for 20 min the supernatant was subjected to evaporation in a hot water bath at 80 °C. To estimate RES content, DNS reagent added to evaporated samples and boiled. Subsequently, 40 % potassium sodium tartrate (Rochelle salt) was added and absorbance was measured at 510 nm. For NRS estimation, 100 μL of the evaporated solution was taken and 1 mL of 1 N H_2SO_4 was added and hydrolysed by heating for 30 min at 50 °C. The absorbance was measured at 510 nm. The concentration of RES and NRS is expressed in mg per 100 g of fresh weight of sample using a standard reference plot of glucose (20–100 μg). The Amylose content (AMY %) was estimated by following the iodine-colorimetric method described by Morrison and Laignel., (1983) [21]. For AMY (%) estimation, 500 g of fresh tuber were homogenized in 1 mL of 100 % ethanol and mixed in 10 mL of 1 N NaOH. This solution was neutralized against 0.1 N HCl solution dropwise, until the colour of phenolphthalein disappeared, 1 mL of iodine solution was added and the final volume was made up to 50 mL with distilled water. The absorbance was taken at 590 nm. The concentration of AMY (%) was calculated from a standard reference plot of pure amylose from potato (200–1000 μg). Amylopectin (AMYL) content (%) was determined by subtracting the AMY content (%) from the STA (%) content. In every case, three or more replicates were analyzed and absorbance was measured in a UV–VIS spectrophotometer (Model UV-1800, Shimadzu, Japan). The calibration equation for different parameters used in the study is presented in Table S3.

2.3.2. Protein content

The protein content from the pigmented potato tuber was measured by the Bradford method [22]. The samples were prepared in 5 mL PBS solution using 500 mg of fresh sample. The homogenized samples were centrifuged at 112000×*g* for 20 min. 0.2 mL of supernatant was added in 5 mL of Bradford solution after volume made up to 1 mL and mixed well. The absorbance was recorded at 595 nm after 20 min of incubation. The protein content (%) was calculated on a fresh weight basis from a reference plot of Bovine Serum Albumin (20–100 μg, Table S3).

2.3.3. Glycemic index (GI) content

The Glycemic Index (GI) of pigmented potato tuber was estimated using *in vitro* method following Lal et al. (2021) [4] using K-RSTAR Kit (Megazyme). To mimic the human small intestine, a dialysis tube was used (width- 24.26 mm, diameter- 14.3 mm, Himedia, India) to calculate GI. A total of three digestive enzymes were used in this experiment namely, pepsin, amyloglucosidase and α -amylase for digesting the potato starch. Potato tubers were boiled cooled and oven dried before grinding. Potato powder (200 mg) was boiled in a water bath for 2 min, and mixed with 5 mL of 0.1 M phosphate buffer (pH 6.9). After mixing, the pH was reduced to 2.5 with 10 % orthophosphoric acid. Afterwards, 200 μL of pepsin (250 mg/mL) was added to the mixture and incubated at 37 °C in a low pH condition (pH 2.5) with shaking. Subsequently, the pH was increased to 6.9 and 200 μL of α -amylase (125 mg/mL) was added, placed in a beaker containing 40 mL of phosphate buffer (0.1 M, pH 6.9) and incubated at 37 °C with shaking. To the aliquots, 1.5 mL of 0.4 M sodium acetate buffer (pH 4.75) and 30 μL of amyloglucosidase enzyme (3300 U/mL) were added and incubated at 50 °C for 30 min on a hot plate magnetic stirrer (Tarsons, USA). The solutions were diluted using 10 mL of distilled water, and 0.3 mL of each solution was incubated with GOPOD (glucose oxidase-peroxidase) reagent at 50 °C for 20 min. The absorbance was measured at 510 nm. Maltose (200 mg) was used as a reference carbohydrate. Average values were used to plot curves by computing the area under the curve (AUC). The Hydrolysis index (HI) for each potato accession was calculated by dividing the AUC of the sample by that of maltose and expressed in percentage. The predicted Glycemic Index was calculated using the potato-specific following formula (PGI) = 39.71 + (0.803 × HI).

2.3.4. Total dietary fiber (TDF) content

The TDF of pigmented potato tuber was estimated using *in vitro* method of Megazyme kit (K-TDFR-200A) following manufacturers' instructions. The raw potato tubers were dried before grinding. Initially, 100 mg of potato powder was taken. The final weight of the sample was taken after treatment with different enzymes (α-amylase, protease, and amyloglucosidase). TDF content was calculated using the formulae % TDF = 100 × Corrected sample residue (CSR)/mg sample, where CSR = Uncorrected average sample residue -

Sample protein residue- Sample ash residue -Corrected blank. TDF is expressed in % on a dry weight basis.

2.3.5. Total anthocyanin content (TAC) content

The TAC content of pigmented potato tuber was determined by the method described by Johnson et al. (2020) [23]. Fresh potato tuber (1 g) was homogenized with 10 mL of acidified methanol (1 % HCl in methanol) followed by centrifugation at 11200×*g* for 10 min. The supernatant was collected and the extraction process was repeated with the pellet. The color intensity was measured at 525 nm, against the acidified methanol blank. The concentration of Anthocyanin was calculated from a reference plot of standard Cyanidin-3-glucoside (10–100 μg, Table S3). TAC is expressed as mg/100 g on fresh weight basis.

2.3.6. Ascorbic acid (vitamin C) content

The vitamin C content from the pigmented potato tuber was analyzed the by 2, 4 Dinitrophenyl Hydrazine (DNPH)-colorimetric method [24]. The absorbance was measured at 540 nm wavelength. The concentration of vitamin C was calculated from a reference plot of standard ascorbic acid (10–100 μg).

2.3.7. Carotenoid (vitamin A) content

The vitamin A content from the pigmented potato tuber was analyzed by the trichloroacetic acid (TCA)-colorimetric method [25]. The final absorbance was measured at 620 nm wavelength. The concentration of vitamin A was calculated from a reference plot of standard vitamin A palmitate (1.5–7.5 μg).

2.4. Non targeted identification of anthocyanin by LC-MS

2.4.1. Sample preparation

For LC-MS analysis, 100 mg dried potato sample was mixed thoroughly with 5 mL extraction buffer comprising of methanol: water: 1 N HCl (50: 45: 5) in the dark. This mixture was then vortexed and transferred to a water bath at 40 °C for 3 min. In the next step, centrifugation of the mixture was done at 1792×*g* for 10 min followed by collection of the supernatant. This supernatant was further filtered with a 0.45 μm nylon syringe filter and stored in amber vials at 4 °C for further analysis.

2.4.2. LC-MS analysis

LC-MS operation was performed with Agilent LC-MS (version: LC/MSD iQ 1260 Infinity II), pump module type G7104C with a total run time of 7 min. The analysis was carried out with a C_{18} column (Infinity Lab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 μm). The samples were run using the instrumentation method as follows, injection volume is kept at 5 μL, flow rate (1.2 mL/min), pressure limit (0–800 bar), and maximum flow ramp Up and Down both maintained at 100 mL/min^2. The gradient solvent system was maintained by supplying 100 % water through channel A (80 %), and 100 % acetonitrile through channel B (20 %). The auxiliary draw speed was 200 μL/min and the eject speed was 400 μL/min and a wait time of 1.2 s was fixed. Detection was done via DAD method with UV lamp (bandwidth 4 nm) at 254 nm wavelength. The spectrum range is kept at 190–800 nm. MS was done using an SQ mass spectrometer (Model type: LC/MSD iQ), ion source ESI. Time segment conditions were kept at start mass 100 m/z, end mass 800 m/z and scan time 475 ms. Source parameters were kept as Gas temperature 350 °C, gas flow 10 L/min, nebulizer 35 psi, and capillary voltage 4000-2500 V.

2.5. Quantification of anthocyanin by HPLC

2.5.1. Sample preparation

Dried powder of potato tubers (200 mg) was macerated with 20 mL of HPLC grade methanol (0.05 % HCl) and incubated for 24 h with continuous shaking maintaining pH 2.823. The extracts were filtered through a syringe filter (0.22 μm). An extract volume of 20 μl of 1000 ppm concentration was used for the HPLC analysis to determine the different anthocyanin content.

2.5.2. HPLC analysis

The high-performance liquid chromatography (HPLC) system (Waters, USA) with UV–Vis detector and Empower software was used for anthocyanin estimation [26]. The separation was achieved by Waters C_{18} Spherisorb reversed-phase ODS2 column (4.6 mm × 250 mm; 5 μm). The mobile phase contained Water, acetronitrile and TFA in a ratio of 53:46:1 (Solvent A) and 0.1 % of TFA in HPLC grade Water (Solvent B), the flow rate was adjusted to 0.60 mL/min, the column was thermostatically controlled at 25 °C and the injection volume was kept at 20 μl. The total analysis time per sample was 15 min. A gradient solvent system was used with Solvent A (20:60:20:20) and Solvent B (80:40:80:80) at 0–7 min, 7–11 min, 11–16 min and 16–20 min, respectively, to achieve maximum resolution. HPLC Chromatogram was obtained using a UV–Vis detector at 517 nm. Standards curves for delphinidin, cyanidin, petunidin and pelargonidin were constructed using HPLC grade standards of delphinidin 3, 5 di-glucosides, cyanidin 3, 5 diglucoside, petunidin chloride salt and pelargonidin chloride salt (10–100 μg/mL). Each compound was identified by its retention time and by spiking with standards under the same conditions. Results are presented as micrograms per gram (μg/g) of sample.

2.6. Quantification of phenolics by HPLC

2.6.1. Sample preparation

Fresh potato tubers weighing 200 mg of the UBAC12 accession were macerated with 20 mL of HPLC-grade acetonitrile for 72 h with continuous shaking. The extracts were filtered through a syringe filter (pore size 0.22 μm). Extract volume of 1 μl of 1000 ppm concentration was used for the HPLC analysis to determine phenolic contents.

2.6.2. HPLC for phenolic compounds

The quantification of phenolic compounds was performed using HPLC system (Waters Alliance e2695, Waters, USA) equipped with 2489 UV/Vis-detector and Empower3 software using a previously published method [27]. The samples were separated using a reverse-phase Nova-pack C_{18} column with a dimension of 3.9 mm × 150 mm. The mobile phase consisted of two solvents: solvent A (0.1 % trifluoroacetic acid in 5 % aqueous acetonitrile) and solvent B (0.1 % trifluoroacetic acid in acetonitrile). Gradient run of mobile phase was maintained as 0–5 min (A: 100 %), 5–10 min (A: 30 %, B: 70 %), 10–15 min (A: 100 %) and 15–25 min (A: 100 %) respectively. The flow rate was 0.45 mL/min, and the column temperature was maintained at 25 °C. Each sample injection volume was 1 μL and detection was carried out at a wavelength 280 nm with a pressure range from 600 to 1200 psi. Calibration curves for gallic acid, vanillic acid, caffeic acid, cinnamic acid, and quercetin were constructed using concentrations ranging from 5 to 500 ppm to ensure linearity. To identify compounds, retention times were compared with standards. Quantification was performed by injecting 1 μL of each sample (1000 ppm concentration) into the HPLC system and interpolating peak areas from the standard curves. Results were expressed as μg/g of sample. For all the above analyses through HPLC, the signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ was maintained uniformly.

2.7. Genetic diversity analysis and marker association with biochemical traits

2.7.1. DNA isolation and PCR

DNA from the pigmented accessions was isolated using the CTAB method from germinating buds of potatoes. PCR was run using a 20 μL reaction mixture with 50 ng DNA, 10 pmole primers, 2× buffer which contains $MgCl_2$ and Taq polymerase (2 unit) in a thermal cycler (Veritii, Applied Biosystems, USA) [28,29]. The PCR product was analyzed in a 2–3 % Agarose gel. The resultant bands in the agarose gel were scored, where the presence of the band was represented as one and the absence of the band was scored as zero.

2.7.2. Bioinformatic analysis

The efficiency of the markers was analyzed using iMEC server (https://irscope.shinyapps.io/iMEC/) for PIC, Resolution power (R) Discriminating power (D) and heterozygosity (H). The population structure of the pigmented potato genotypes was performed using STRUCTURE 2.3.4 platform. The genetic diversity of the accessions was analyzed using DARWIN version 6 utilizing dissimilarity matrix and UPGMA clustering. To evaluate the association of the markers with biochemical traits, the Single Marker Association test (SMA) was performed using the TASSEL program. The results were presented in a Manhattan plot [30].

2.8. Statistical analysis

All the data of different nutritional and biochemical parameters are provided as Mean ± SD from three or more independent replications of each sample. Statistical analyses were performed using Completely Randomized Designing (CRD). The calculated F values were compared with table F value for testing of the significance. The critical differences (CD) were calculated to find out the superiority of one accession over the other. Dancun's test was performed to compare between the means in R-studio software (https://www.R-project.org/). Spearman's rank correlation coefficient was performed using the OriginPro-2024 (OriginLab Corp., Northampton, MA, USA). The level of significance is set at 5 % achieving α value (p-value) at 0.05. A p-value more than 0.05 is determined to be significant and suggests there is a significant correlation between the two parameters.

3. Results and discussion

3.1. Phenotypic characterization of the pigmented potato tubers

The pigmented potato accessions collected from sub-Himalayan plateau can be divided into two classes i.e. UBAC and UDAC, depending on their shape, skin and flesh colour described previously in detail in para 2.1. The accessions vary significantly in phenotypic characters namely plant height, number of tubers per plant, length and diameter of tubers, stolon length, weight of individual tubers etc. The average yield of the accessions varied between 11.06 t/ha to 36.04 t/ha [31]. Nine accessions (UBAC1-UBAC6; UBAC11-UBAC13) bear tubers with purple to dark brown skin colour and considerable purple pigmentation inside the tubers. Their signature pigmentations are present beneath the periderm and around the vascular ring. Upon boiling, the pigmentation diffuses and the whole tuber turns dark red or purple. These accessions are categorized as Purple-Skin Purple Flesh (PSPF) (Fig. 2). Three accessions (UBAC7, UBAC8 and UBAC 9) have tubers with dark red skin colour, after boiling the tuber turned to purple colour and these accessions are termed Red-Skin Purple Flesh (RSPF). Eleven accessions (UBAC10; UDAC1-UDAC8; UDAC10 and UDAC11) bear tubers having light red to pink skin colour, with negligible pigmentation. When boiled, the skin colour diffuses into the flesh and turns the boiled tuber light pink. These accessions are referred to as Red-Skin White Flesh (RSWF). Two accessions (UDAC9 and UDAC12) have

light yellow skin with no pigmentation and are therefore referred to as Yellow-Skin White Flesh (YSWF). YSWF tubers turn light yellow to off-white when boiled (Fig. 2).

3.2. Nutritional characterization of the pigmented potato tubers

3.2.1. Estimation of STA and its components from pigmented potato tubers

The total starch content (STA) of potatoes impacts the dry matter content, specific gravity, cooking quality and texture of the tubers [32]. The STA content of the pigmented potato tubers varied significantly from 16.79 % to 23.58 % on a fresh weight basis with an average content of 20.58 % (Table 1). The STA content of the commercial white-fleshed potato cultivars ranges from 18 to 20 %. The pigmented potatoes showed comparable total starch content with their commercial white-fleshed counterparts. Starch granules consist of amylose (AMY) and amylopectin (AMYL) polysaccharides. The AMYL to AMY ratio determines the stickiness of the potato tubers. White fleshed Potato tubers usually contain 75–80 % AMYL and 20–25 % AMY and maintain a ratio of 3:1 approximately [33]. The AMYL (mean value = 63.78 %) to AMY (mean value = 36.23 %) ratio was found to be widely variable, in pigmented accessions ranging from 0.36 to 12.00 (Table 1). PSPF accessions exhibited typical characteristics of mealy potatoes (mean AMY = 30.69 %; mean AMYL = 69.32 %). Thirteen accessions were found to contain AMYL to AMY ratio lesser than the normal 3:1 ratio including six accessions viz. UDAC2, UDAC5, UBAC11, UBAC12, UDAC8, UDAC10 which exhibited remarkably low ratio (<1) indicating high amylose content. AMYL content of eleven accessions was found with ratio 3:1 or higher explaining its sticky texture. Accessions like UDAC9 and UDAC11 exhibited a ratio of 10.03–12 which is comparable to waxy potatoes [34] indicating richness in amylopectin. Amylopectin rich potatoes are reported to have good industrial applications for frozen foods as food additives, food binders, and alcohol production [35].

3.2.2. Determination of TSS, RES, NRS and PRO from pigmented potato tubers

The TSS content of the native pigmented potato accessions varied significantly from 326.09 to 416.09 mg per 100 g fresh weight. The highest TSS content was found in accession UBAC7 and UBAC3 and the lowest was found in the UDAC12 and UDAC3 (Table 1). Earlier reports suggested that the white-fleshed potato tubers can have TSS content of 750 mg/100 g fresh weight, which can increase after storage [36]. Pigmented potatoes in the present study show lower TSS content than white potatoes. Reducing (RES) and Non-reducing (NRS) sugar content play crucial roles in blood glucose spikes. The RES value of these pigmented tubers varied significantly 89.5 mg–122.16 mg per 100 g fresh weight (Table 1). NRS values ranged between 223.81 mg and 303.43 mg per 100 g fresh weight. RES and NRS content in potato tuber may be influenced by genotype, maturity, season, production site, storage, cultural and environmental conditions [37]. Freshly harvested Kufri Jyoti tubers grown in the districts of West Bengal have very high RES containing 300 mg/100 g to 585 mg/100 g [38]. Native pigmented potatoes in the present study were found to be significantly low in RES and TSS content, which may prevent them from non-enzymatic browning through maillard reaction during cooking and promote long-term storage in ambient conditions. The protein content of the pigmented potato accessions varied from 1.37 % in UBAC13 to 4.75 % in UBAC6 (mean value 2.75 %). Most of these pigmented accessions have protein content higher than the white-fleshed genotypes [39].

3.2.3. Estimation of GI and TDF of pigmented potato tubers

The glycemic index (GI) indicates the rate at which the carbohydrate in the food is broken down into glucose and released into the bloodstream. Low GI of a food indicates lesser glucose released into the blood after consumption. As per the Food Safety & Standards Authority of India (FSSAI), food with a GI value below 55 is considered a low GI food [40]. The boiled tubers of pigmented potato accessions viz. UBAC1, UBAC3, UBAC5, UBAC7, UDAC5, UBAC6, and UBAC11 can be considered low GI potatoes having GI values of 54.21, 52.46, 54.76, 55.25, 50.16 and 52.22 respectively. The mean GI value of PSPF accessions is 56.36 whereas the mean GI value of all the pigmented accessions is 61.29. Noticeably, all the UDAC accessions were found to have higher GI values than UBAC accessions (mean value 65.99). UDAC1, UDAC2, UDAC6, UDAC7, UDAC8, UDAC10 and UDAC12 have a GI value above 69 which is considered moderate to high, the highest being UDAC12 with a GI value of 91.65 (Table 1). Earlier reports suggested that the GI of pigmented potatoes is related to their polyphenol content, possibly mediated through an inhibitory action of anthocyanins on intestinal α-glucosidase [41].

The major use of potato tubers is in culinary activities and common white fleshed potatoes are considered unhealthy due to their high GI values. Potato shows variable GI in raw, boiled, baked and cooked forms [42,43]. Six popular Indian white potato varieties, viz. Kufri Jyoti, Kufri Pukhraj, Kufri Chipsona 1 and 3, Kufri Giridhari and Kufri Frysona were found to have very high GI ranging from 72 to 89 when boiled [4]. The native 'Badami' accessions in the current study showed significantly low GI in comparison to these cultivated white flesh table potato varieties. To date, the Carisma variety with a GI of 53 has been characterized as a low GI potato [44] and pigmented potato accessions in the present study show comparable GI with Carisma. Total dietary fibre (TDF), is another beneficial and healthy component of the tubers having a significant impact on human gut physiology. It enhances the fermentation process, provides nutrition to gut microflora, enriches the gut microbiome, and improves large bowel function. The recommended dose of dietary fibre in our diet is 25–30 g/day [45]. Pigmented potato accessions viz. UDAC4, UBAC5, UDAC9 and UDAC12 have excellent TDF content that varied significantly from 20.17 % to 27.34 % on a dry weight basis (mean value 17.86 %) indicating its better acceptability. The size of the tubers under study is very small with a diameter of 2–3 cm. The analysis was carried out using oven-dried whole potatoes with skin. The surface-to-volume ratio of these potatoes is high indicating samples contain a considerably high amount of skin which contributes to the increase in TDF. In white potatoes, the reported TDF in the skin is around 22 % and in the flesh 7.5 % [46]. Soluble fibre is reported to be 1.29 g per 100 g in white flesh potatoes whereas the pigmented potato was found to have higher

Table 1
Nutritional attributes of the pigmented potato tubers.

Accession	STA (% FW)	AMY (% FW)	AMYL (% FW)	AMYl/ AMY	RES (mg/ 100 g FW)	NRS (mg/ 100 g FW)	TSS (mg/ 100 g FW)	PRO (% FW)	GI	TDF (% DW)	TAC (mg/ 100gFW)	VIT C (mg/ 100gFW)	VIT A (mg/ 100gFW)
UBAC1	21.94 ± 1.67 ^{bcde}	18.12 ± 0.92 ^{lm}	81.89 ± 0.92 ^{de}	4.52	105.9 ± 1.61 ^{cdef}	263.04 ± 2.9 ^{de}	368.77 ± 4.44 ^{gh}	2.37 ± 0.1 ^{ij}	54.21 ± 2.99 ^{mn}	14.4 ± 0.44 ^{op}	83.74 ± 0.66^{c}	28.11 ± 0.56^{e}	0.89 ± 0.07 ^{defg}
UDAC1	19.47 ± 1.13 ^{gh}	16.31 ± 1.03^{n}	83.7 ± 1.03^{c}	5.13	96.5 ± 0.83 ^{hi}	262.54 ± 3.37 ^{de}	358.19 ± 3.99^{j}	2.47 ± 0.14 ^{ij}	69.36 ± 0.03^{c}	15.62 ± 0.35^{n}	23.38 ± 0.72^{l}	25.65 ± 0.85^{f}	0.88 ± 0.11 ^{defg}
UBAC2	18.46 ± 0.18^{h}	19.73 ± 0.73^{l}	80.28 ± 0.73^{e}	4.07	104.98 ± 0.69 ^{ef}	266.65 ± 4.08^{d}	371.68 ± 4.53^{g}	3.1 ± 0.18 ^{fg}	63.26 ± 0.1^{f}	19.58 ± 0.33^{f}	84.24 ± 1.19^{c}	24.77 ± 0.3 ^{fg}	0.86 ± 0.09 ^{defgh}
UDAC2	23.43 ± 0.3^{a}	65.07 ± 2.28 ^{bc}	34.94 ± 2.28 ^{no}	0.54	99.08 ± 0.69^{g}	254.6 ± 5.25^{f}	352.63 ± 3.32^{k}	1.75 ± 0.09^{l}	65.7 ± 0.02 ^{de}	19.81 ± 0.22 ^{ef}	40.4 ± 1.13^{j}	36.22 ± 0.81^{b}	0.82 ± 0.1 ^{efgh}
UBAC3	17.08 ± 0.39^{i}	24.45 ± 0.79^{k}	75.56 ± 0.79^{f}	3.09	112.52 ± 0.77^{b}	300.88 ± 2.23^{a}	413.48 ± 2.43^{a}	2 ± 0.15 ^{kl}	52.46 ± 2.08 ^{no}	17.21 ± 0.06^{k}	82.99 ± 0.53^{c}	20.67 ± 0.22^{h}	0.77 ± 0.1 ^{fgh}
UDAC3	21.52 ± 0.22 ^{de}	49.14 ± 1.24^{f}	50.87 ± 1.24^{k}	1.04	89.5 ± 0.48^{j}	241.89 ± 2.2^{g}	329.26 ± 1.74^{n}	1.74 ± 0.16^{l}	60.84 ± 0.04 ^{gh}	16.12 ± 0.13^{m}	44.65 ± 1.23^{i}	32.55 ± 0.57^{c}	0.95 ± 0.07 ^{bcde}
UDAC4	23.58 ± 0.02^{a}	19.4 ± 2.27^{l}	80.61 ± 2.27^{e}	4.16	104.83 ± 1.33 ^{ef}	261.17 ± 1.42^{e}	365.87 ± 2.9 ^{hi}	2.38 ± 0.16 ^{ij}	58.51 ± 0.27 ^{ij}	22.45 ± 0.33^{b}	24.36 ± 2.21^{l}	28.49 ± 0.52^{e}	0.86 ± 0.07 ^{defgh}
UBAC4	21.8 ± 0.28 ^{cde}	39.64 ± 1.53^{i}	60.37 ± 1.53^{h}	1.52	97.7 ± 0.25^{h}	239.84 ± 0.19^{g}	337.59 ± 0.05^{m}	3.51 ± 0.19 ^{cde}	60.21 ± 0.03 ^{hi}	16.26 ± 0.11^{m}	57.55 ± 1.09^{f}	27.62 ± 0.94^{e}	0.71 ± 0.06^{h}
UBAC5	19.1 ± 0.35 ^{gh}	16.98 ± 0.7 ^{mn}	83.03 ± 0.7 ^{cd}	4.89	105.9 ± 0.78 ^{cdef}	281.3 ± 1.95 ^{bc}	387.25 ± 2.42^{f}	3.38 ± 0.16 ^{def}	54.76 ± 0.29 ^{lm}	20.17 ± 0.05^{d}	51.38 ± 0.94^{g}	32.57 ± 0.85^{c}	0.91 ± 0.05 ^{cdef}
UBAC6	19.64 ± 0.92 ^{gh}	19.23 ± 1.36^{l}	80.78 ± 1.36^{e}	4.20	105.39 ± 0.74 ^{def}	266.62 ± 1.93^{d}	372.06 ± 2.58^{g}	4.76 ± 0.19^{a}	55.25 ± 0.1 ^{klm}	18.59 ± 0.11^{h}	91.73 ± 1.68^{a}	24.62 ± 0.96 ^{fg}	0.79 ± 0.05 ^{fgh}
UBAC7	16.79 ± 0.5^{i}	14.14 ± 0.4^{o}	85.87 ± 0.4^{b}	6.07	113.56 ± 1.24^{b}	302.49 ± 1.89^{a}	416.1 ± 1.75^{a}	3.58 ± 0.23 ^{cd}	52.33 ± 0.5 ^{no}	16.31 ± 0.26^{m}	82.67 ± 1.07^{c}	24.49 ± 0.85 ^{fg}	0.85 ± 0.09 ^{defgh}
UDAC5	21.08 ± 0.29 ^{ef}	73.27 ± 1.59^{a}	26.74 ± 1.59^{p}	0.36	104.99 ± 0.17 ^{ef}	263.01 ± 3.42 ^{de}	368.04 ± 3.64 ^{ghi}	4.28 ± 0.15^{b}	50.16 ± 0.61^{p}	17.86 ± 0.05^{j}	29.77 ± 0.76^{k}	16.44 ± 0.48^{j}	0.82 ± 0.08 ^{efgh}
UBAC8	19.2 ± 0.43 ^{gh}	45.7 ± 2.44^{g}	54.31 ± 2.44^{j}	1.19	107.19 ± 0.1^{c}	279.55 ± 1.29^{c}	385.95 ± 1.07^{f}	2.2 ± 0.16 ^{jk}	64.25 ± 0.06 ^{ef}	16.81 ± 0.16^{l}	83.67 ± 1.41^{c}	20.61 ± 0.92^{h}	0.77 ± 0.09 ^{fgh}
UBAC9	19.19 ± 0.31 ^{gh}	64.14 ± 2.09^{c}	35.87 ± 2.09^{n}	0.56	112.37 ± 0.74^{b}	282.05 ± 1.36 ^{bc}	394.47 ± 0.71 ^{cd}	3.23 ± 0.17 ^{ef}	60.99 ± 0.23 ^{gh}	13.87 ± 0.16^{q}	80.35 ± 0.99^{d}	40.59 ± 0.98^{a}	0.84 ± 0.1 ^{defgh}
UDAC6	19.49 ± 0.82 ^{gh}	29.63 ± 2.06^{j}	70.38 ± 2.06^{g}	2.38	105.85 ± 0.39 ^{cdef}	241.89 ± 2.16^{g}	346.22 ± 0.79^{l}	2.48 ± 0.22 ^{ij}	66.38 ± 0.02^{d}	15.51 ± 0.19^{n}	16.56 ± 0.82^{o}	28.52 ± 0.74^{e}	0.74 ± 0.1 ^{gh}
UBAC10	19.2 ± 0.41 ^{gh}	43.28 ± 1.39^{h}	56.73 ± 1.39^{i}	1.31	106.57 ± 0.83 ^{cd}	284.74 ± 1.74^{b}	391.36 ± 2.35 ^{de}	2.06 ± 0.22^{k}	55.63 ± 0.02 ^{klm}	18.9 ± 0.13^{g}	62.42 ± 0.74^{e}	20.52 ± 0.59^{h}	0.98 ± 0.12 ^{bcd}
UBAC11	19.74 ± 1.04^{g}	51.88 ± 2.37^{e}	48.13 ± 2.37^{l}	0.93	105.26 ± 1.5 ^{def}	263.45 ± 2.63 ^{de}	368.98 ± 3.7 ^{gh}	2.6 ± 0.18 ^{hi}	52.22 ± 0.57^{o}	18.28 ± 0.22^{i}	88.68 ± 0.99^{b}	32.17 ± 0.73^{c}	0.78 ± 0.07 ^{fgh}
UDAC7	18.99 ± 0.31 ^{gh}	18.12 ± 2.46 ^{lm}	81.89 ± 2.46 ^{de}	4.52	106.42 ± 0.85 ^{cde}	284.13 ± 4.24^{b}	388.41 ± 1.29 ^{ef}	1.93 ± 0.12 ^{kl}	68.71 ± 0.06^{c}	14.57 ± 0.09 ^{op}	18.56 ± 0.7^{n}	30.53 ± 0.5^{d}	0.84 ± 0.07 ^{defgh}
UBAC12	22.91 ± 0.62 ^{abc}	73.23 ± 2.04^{a}	26.78 ± 2.04^{p}	0.37	97.32 ± 0.77^{h}	262.45 ± 1.7 ^{de}	359.83 ± 1.97^{j}	2.87 ± 0.24 ^{gh}	56.63 ± 0.11 ^{jkl}	16.24 ± 0.13^{m}	84.48 ± 0.7^{c}	28.57 ± 0.58^{e}	0.8 ± 0.06 ^{efgh}
UDAC8	20.14 ± 0.5 ^{fg}	61.78 ± 2.65^{d}	38.23 ± 2.65^{m}	0.62	104.42 ± 0.61^{f}	264.49 ± 2.36 ^{de}	369 ± 1.67 ^{gh}	2.84 ± 0.17 ^{gh}	73.02 ± 0.03^{b}	14.68 ± 0.23^{o}	49.62 ± 1.02^{h}	24.18 ± 0.6^{g}	0.88 ± 0.06 ^{defg}
UDAC9	22.5 ± 0.67 ^{abcd}	9.07 ± 2.38^{p}	90.94 ± 2.38^{a}	10.03	105.54 ± 0.35 ^{def}	266.18 ± 0.61^{d}	371.77 ± 0.6^{g}	3.73 ± 0.21^{c}	62.59 ± 0.23 ^{fg}	27.34 ± 0.3^{a}	5.36 ± 1.21^{p}	36.48 ± 0.62^{b}	1.31 ± 0.06^{a}
UDAC10	20.09 ± 0.7 ^{fg}	66.58 ± 0.67^{b}	33.43 ± 0.67^{o}	0.50	95.35 ± 1.28^{i}	303.43 ± 3.34^{a}	397.28 ± 2.59^{c}	2.89 ± 0.21 ^{gh}	68.65 ± 3.73^{c}	14.34 ± 0.03^{p}	21.33 ± 0.98^{m}	12.53 ± 0.66^{k}	1.05 ± 0.1 ^{bc}
UDAC11	23.06 ± 0.86 ^{ab}	7.7 ± 1.74^{p}	92.31 ± 1.74^{a}	12.00	121.09 ± 0.73^{a}	281.51 ± 1.22 ^{bc}	402.64 ± 1.83^{b}	3.51 ± 0.18 ^{cde}	56.94 ± 0.05 ^{jk}	19.78 ± 0.19 ^{ef}	51.04 ± 0.41 ^{gh}	24.65 ± 0.57 ^{fg}	0.87 ± 0.11 ^{defg}
UBAC13	23.49 ± 0.34^{a}	12.95 ± 1.99^{o}	87.06 ± 1.99^{b}	6.73	122.17 ± 0.71^{a}	243.95 ± 3.39^{g}	364.28 ± 0.69^{i}	1.37 ± 0.17^{m}	58.3 ± 2.06 ^{ij}	19.95 ± 0.07 ^{de}	89.47 ± 1.03^{b}	32.55 ± 0.16^{c}	0.81 ± 0.08 ^{efgh}
UDAC12	22.59 ± 0.64 ^{abcd}	46.22 ± 1.78^{g}	53.79 ± 1.78^{j}	1.16	104.42 ± 0.13^{f}	223.82 ± 2^{h}	326.1 ± 1.93^{n}	1.73 ± 0.13^{l}	91.06 ± 0.25^{a}	22.09 ± 0.08^{c}	5.38 ± 0.67^{p}	17.67 ± 0.21^{i}	1.08 ± 0.06^{b}
SE(d)	0.535	1.438	1.438		0.684	2.138	2.049	0.139	0.929	0.165	0.863	0.545	0.065
CV	3.187	4.861	2.761		0.795	0.979	0.674	6.183	1.857	1.131	1.953	2.484	9.111
F value	27.753	468.363	468.321		231.347	178.873	267.106	75.27	177.763	719.038	2,359.29	298.233	7.724

FW: on fresh weight basis, DW: on dry weight basis, Different alphabets in superscript of the mean value in each column indicate significant difference between the accessions for that parameter.

dietary fibre (13–27 %) as also supported by earlier reports [47].

3.2.4. TAC, Vit C and Vit A content and inter-relationship of nutritional parameters

Phenolic compounds in food are believed to have beneficial effects on human health and are considered as healthy [48]. Anthocyanin is the major phenolic component that gives the signature red-to-violet colour of the potato [49]. Anthocyanins are also known to have good antioxidant properties. The highest total anthocyanin content (TAC) was found in UBAC6 with 91.73 mg/100 g (mean value of UBAC 78.72 mg/100 g) (Table 1). The UDAC accessions have relatively less anthocyanin content, the lowest being UDAC9 and UDAC12 with 5.36 mg and 5.37 mg respectively (mean value of UDAC 27.53 mg/100 g). The Vit C content in these twenty-five accessions varied from 40.58 mg to 12.53 mg/100 g on a fresh weight basis (mean value 26.87 mg/100 g). On the other hand, UDAC accessions have exhibited higher Vit A content (mean value 0.92 mg/100 g) compared to other pigmented genotypes.

Coloured Potatoes contain important antioxidant compounds and other phytochemicals, which have beneficial effects on the human body [50]. It has been well-documented that purple potatoes have more phenolic content than white or yellow-coloured potato genotypes. The high polyphenolic content leads to high antioxidant activities of these genotypes [51,52]. Recently few traditional small potato cultivars of north eastern region of India were characterized for nutritional and quality traits. The authors have reported that these cultivars surpassed India's commercial tetraploid potato varieties in terms of dry matter, starch, ascorbic acid, β carotene, and total phenol content [53]. In India, the purple skin-coloured specialty potato variety Kufri Neelkanth with anthocyanin content of 83 mg per 100 g has been developed [54]. Spearman Ranks correlation analysis was performed among different nutritional and biochemical parameters to understand the impact of these nutritional attributes on each other (Fig. 3). Interestingly, GI showed a significant negative correlation with TAC ($r = -0.58, p < 0.05$), It was observed that UBAC accessions (PSPF, RSPF) have high TAC with low GI compared to UDAC accessions (RSWF, YSWF) depicting UBAC accessions to be healthier among these landraces supporting earlier findings [41].

3.3. Profiling of anthocyanins from pigmented potato tubers

Coloured potatoes may serve as a potential source of natural anthocyanins [55]. To rank the genotypes based on GI, and anthocyanin content Spearman ranking analysis was performed. UBAC12 (PSPF) was selected as the best among the pigmented accessions. The extract of the UBAC12 tuber was further analyzed to identify the bioactive pigments present. Non-targeted LC-MS data analysis revealed the presence of diverse bioactive natural compounds including anthocyanins, phenolic compounds, flavonoid derivatives, alkaloids etc. in the extract (Table 2, Fig. S2). Within the anthocyanin group, an anthocyanidin glycoside of delphinidin 3-O-(6-caffeoyl-beta-D-glucoside) ($C_{30}H_{27}O_{15}^{+}$), petunidin 3-(6″-p-coumaroyl-glucoside) ($C_{31}H_{29}O_{14}^{+}$), pelargonidin 3-rutinoside-5-glucoside ($C_{33}H_{41}O_{19}^{+}$), and an aglycone of cyanidin 3-O-(6″-acetyl-glucoside) ($C_{23}H_{23}O_{12}$), are the major compounds detected through LC-MS analysis. There are three coumarin compounds namely 7-methoxy-4-methylcoumarin ($C_{11}H_{10}O_3$), scopoletin ($C_{10}H_8O_4$) and 6-Hydroxy-4-methylcoumarin ($C_{10}H_8O_3$), flavonoids like quercetin ($C_{15}H_{10}O_7$), phenolics like quinic acid ($C_7H_{12}O_6$), salsolidine ($C_{12}H_{17}NO_2$) along with few amino compounds like cytisine, loline, dopamine was also detected in the analysis. Their potential biological activities are listed in Table 2. Four naturally occurring pure anthocyanins, namely delphinidin, cyanidin, petunidin and pelargonidin were estimated from the methanolic extracts of accessions which showed promising total anthocyanin content earlier. The standard curve for delphinidin ($y = 4463.7x + 5313.48$; $R^2 = 0.99$), cyanidin ($y = 3797.4x\text{-}124.04$; $R^2 = 0.99$), petunidin ($y = 2064.9x + 3335.36$; $R^2 = 0.99$), and pelargonidin ($y = 6059.9x + 1603.11$; $R^2 = 0.99$) was constructed and validated. Retention Times (RT) of the authentic standards and their UV spectra pattern were recorded (Fig. 4A). The RT of the compounds was recorded on 7.47

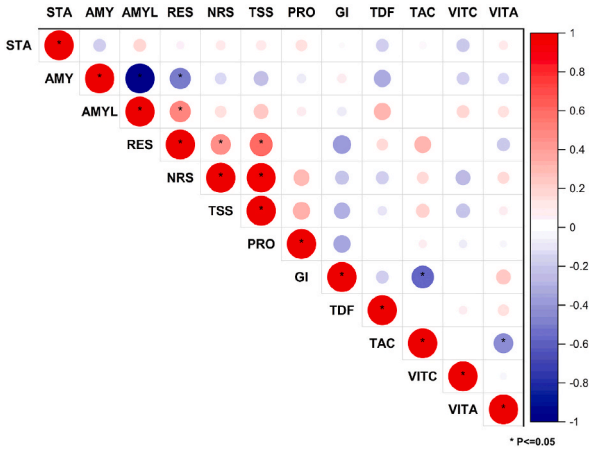

Fig. 3. Spearman rank's correlation matrix among biochemical and nutritional attributes of the small pigmented potato accessions using Origin pro, 2024. The red circles indicate a positive correlation and the blue circles indicate a negative correlation. Astrix in the circle suggests a level of significance with a *P* value < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
LC-MS profiling of bioactive compounds from pigmented potato tubers.

Group	Compound	Formulae	Biological Activity
Anthocyanins	Delphinidin 3-O-(6-caffeoyl-beta-D-glucoside)	$C_{30}H_{27}O_{15}^{+}$	Antioxidant, anti-inflammatory, anticancer, cardio, osteo, hepato and neuro protective
	Petunidin 3-(6″-p-coumaroyl-glucoside)	$C_{31}H_{29}O_{14}^{+}$	Antioxidant, anti-hypersensitive, Lipid oxidation inhibition
	Cyanidin 3-O-(6″-acetyl-glucoside)	$C_{23}H_{23}O_{12}$	Antioxidant, Anti inflammation, improves glucose metabolism
	Pelargonidin 3-rutinoside-5-glucoside	$C_{33}H_{41}O_{19}^{+}$	Antioxidant, anti-inflammatory
Phenolic compounds	Quinic acid	$C_7H_{12}O_6$	Antioxidant, anti-diabetic, anticancer, antimicrobial, antiviral, anti-aging, anti-nociceptive and analgesic
	Salsolidine	$C_{12}H_{17}NO_2$	Salsolidine is a tetrahydroisoquinoline alkaloid, acts as a stereoselective competitive Monoamine oxidase inhibitor.
	DL-Methionine sulfoxide	$C_5H_{11}NO_3S$	Biomarker for oxidative stress
	Quercetin	$C_{15}H_{10}O_7$	Antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities
	3-Hydroxy-3′,4′-Dimethoxyflavon	$C_{17}H_{14}O_5$	Antioxidant, antifungal
	Dopamine	$C_8H_{11}NO_2$	Neurotransmitter
	3,4-Dihydroxy-L-phenylalanine	$C_9H_{11}NO_4$	antiparkinson drug, a dopaminergic agent,
Coumarin derivatives	7-methoxy-4-methylcoumarin	$C_{11}H_{10}O_3$	Antibacterial
	Scopoletin	$C_{10}H_8O_4$	Antimicrobial, anticancer, anti-inflammation, anti-angiogenesis, anti-oxidation, antidiabetic, antihypertensive, hepato and neuroprotective properties and immunomodulatory effects
	6-Hydroxy-4-methylcoumarin	$C_{10}H_8O_3$	Antioxidant, anticarcinogenic
Alkaloids	Cytisine	$C_{11}H_{14}N_2O$	smoking cessation, reducing drinking behavior, anti-tumor, cardiovascular protection, blood sugar regulation, neuroprotection, osteoporosis prevention and treatment
	Loline	$C_8H_{14}N_2O$	Insecticidal
	(S)-(−)-Perillic acid	$C_{10}H_{14}O_2$	antineoplastic agent
	1,2-dimethylnaphthalene	$C_{12}H_{12}$	Antimicrobial
	Methyloctanoic acid	$C_9H_{18}O_2$	Antifungal, antibacterial
	alpha,beta-Dehydrocurvularin	$C_{16}H_{18}O_5$	Acetylcholinesterase inhibition

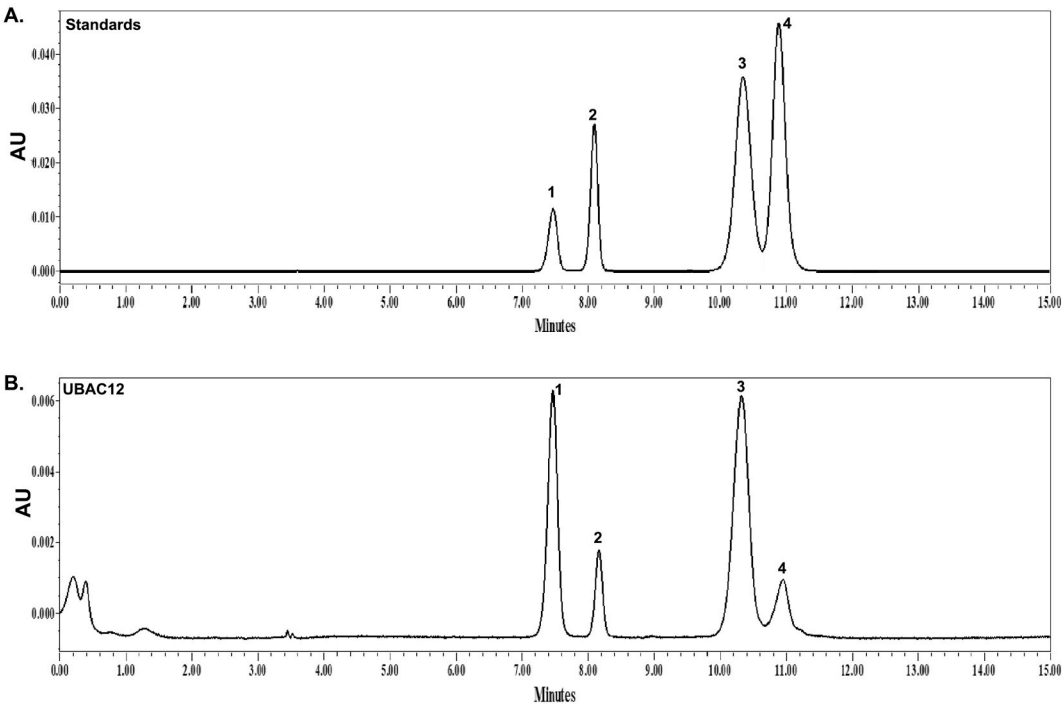

Fig. 4. Quantification of different anthocyanins from pigmented small potato tubers using High-Performance Liquid Chromatography (HPLC). A. HPLC chromatogram generated at 517 nm from a mixture of HPLC grade standards of delphinidin 3, 5 di glucosides (1), cyanidin 3, 5 diglucoside (2), pelargonidin chloride salt (3) and petunidin chloride salt (4), B. HPLC chromatogram generated from methanolic extract of UBAC12 (PSPF).

min for delphinidin, 8.09 min for cyanidin, 10.34 min for pelargonidin and 10.88 min for petunidin respectively. Quantification of anthocyanin performed through HPLC showed linearity in values of both LOD and LOQ for all selected standards attained from prepared calibration curves (10–100 μg/mL, $R^2 = 0.99$). The LOD and LOQ values for all standards were calculated as 0.28–0.34 μg/mL and 0.85–1.04 μg/mL, respectively. Previous reports support a similar trend of LOD (0.98 μg/mL) and LOQ (2.97 μg/mL) for anthocyanin estimation using C_{18} column with a run time of 25 min in a similar experimental setup [56]. The majority of anthocyanin compounds were detected from UBAC accessions (PSPF, RSPF) (Fig. 4B–Table 3), whereas in most UDAC accessions only delphinidin was present except UDAC7 (RSWF) and UDAC12 (YSWF) where petunidin was detected along with delphinidin. UBAC12 was the only accession where all four anthocyanins' presence could be detected. In percentage, delphinidin was the most predominant anthocyanin detected in these accessions revealed by HPLC quantification (Fig. 5) with UBAC8 having the highest content (834.09 μg/g). Out of the other three anthocyanins, petunidin is detected in eleven accessions, namely UBAC2, UBAC3, UBAC4, UBAC6, UBAC7, UBAC8, UBAC10, UDAC7, UBAC12, UBAC13 and UDAC12 with UBAC13 having the highest content (1710.35 μg/g) (Table 3 and Fig. 5) whereas cyanidin content was highest in UBAC13 (147.10 μg/g) and UBAC6 accession showed highest pelargonidin content (146.94 μg/g) (Table 3 and Fig. 5). The major anthocyanins identified in purple flesh potato cultivars are petunidins and malvidins [57]. A considerable number of genes and transcription factors are involved in anthocyanin biosynthesis in pigmented potato tubers [58]. The majority of the pigmented potatoes in this study were found to have very high anthocyanin content (>80 mg/100 g). Generally, the delphinidin glycosylates provide purple pigment in fruits and berries whereas cyanidins are responsible for the reddish purple colour of vegetables. petunidin is a methylated anthocyanidin which provides water-soluble dark red or purple pigment and pelargonidin is known to provide a pink hue in vegetables [59]. Multifaceted health benefits have been reported for delphinidin including antioxidant, anti-inflammation, anti-diabetic, anti-cancerous and cardiovascular protection [60]. Parallelly, cyanidin and pelargonidin are reported to reduce genotoxic stress-induced DNA damage and protect against carcinogenic nitrosation [61]. Previously potato anthocyanins from pigmented cultivars was reported to inhibit key enzymes involved in diabetes [62]. The presence of these compounds in these native pigmented potato accessions makes these landraces elite, health-promoting and unique and can be recommended for consumption to patients suffering from lifestyle diseases.

3.4. Detection and estimation of phenolics

The accession UBAC12 exhibited lowest RES, a low GI and the highest TAC with all four anthocyanins. To further investigate its health-promoting potential, quantification of a few phenolic compounds was attempted from this accession (Fig. 6, Table 4). For the method establishment of five phenolics standards viz. gallic acid, vanillic acid, caffeic acid, cinnamic acid and quercetin; calibration curves (10–100 μg/mL, $R^2 = 0.99$) were prepared through HPLC. For quantification of the phenolics; the LOD and LOQ values were recorded to be within the range of 0.18–0.28 μg/mL and 0.54–0.85 μg/mL respectively (Table 4). Earlier, Moo-Huchin et al. (2019) [63] reported a similar range of LOD (0.14–0.31 μg/mL) and LOQ (0.63–0.92 μg/mL) while studying the effect of different solvent-mediated extraction on the phenolic compounds. The result suggests the presence of gallic acid, cinnamic acid, caffeic acid,

Table 3
Detection and quantification of different anthocyanins using HPLC.

Accession Name	Delphinidin 3,5-diglucoside (μg/g)	Cyanidin 3,5-diglucoside (μg/g)	Pelargonidin Chloride (μg/g)	Petunidin (Cl salt) (μg/g)	Total (μg/g)
UBAC1	187.72 ± 3.75^{b}	37.82 ± 1.86^{c}	–	–	225.54
UDAC1	98.76 ± 6.2^{i}	–	–	–	98.76
UBAC2	152.76 ± 1.11^{e}	–	181.09 ± 3.22^{b}	–	333.85
UDAC2	118.54 ± 2.77^{fg}	–	–	–	118.54
UBAC3	201.76 ± 3.53^{a}	–	127.65 ± 3.75^{f}	–	329.41
UDAC3	71.82 ± 2.08^{k}	–	–	–	71.82
UDAC4	56.71 ± 2.52^{l}	–	–	–	56.71
UBAC4	162.54 ± 5.8^{cd}	–	171.81 ± 2.03^{c}	–	334.35
UBAC5	112.51 ± 3.28^{gh}	–	–	–	112.51
UBAC6	187.12 ± 1.59^{b}	–	261.51 ± 7.48^{a}	97.23 ± 0.46^{a}	545.86
UBAC7	121.67 ± 1.87^{f}	–	78.31 ± 1.42^{g}	–	119.98
UDAC5	98.38 ± 1.04^{i}	–	–	–	98.38
UBAC8	156.09 ± 6.75^{de}	–	124.55 ± 4.76^{f}	87.31 ± 1.23^{d}	367.95
UBAC9	187.31 ± 2.5^{b}	67.34 ± 0.63^{b}	–	95.01 ± 2.75^{b}	349.66
UDAC6	87.91 ± 1.39^{j}	–	–	–	87.91
UBAC10	125.76 ± 4.02^{f}	–	161.8 ± 2.96^{d}	78.31 ± 4.09^{e}	365.87
UBAC11	154.09 ± 13.71^{e}	100.65 ± 3.01^{a}	–	86.27 ± 0.84^{d}	341.01
UDAC7	87.21 ± 0.72^{j}	–	34.83 ± 1.18^{h}	–	122.04
UBAC12	165.68 ± 0.35^{c}	21.328 ± 1.82^{d}	178.55 ± 3.97^{b}	89.625 ± 3.3^{c}	455.183
UDAC8	–	–	–	–	–
UDAC9	–	–	–	–	–
UDAC10	109.61 ± 1.16^{h}	–	–	–	109.61
UDAC11	78.56 ± 2.83^{k}	–	–	–	78.56
UBAC13	169.72 ± 4.03^{c}	101.38 ± 0.75^{a}	142.93 ± 3.94^{e}	–	414.03
UDAC12	16.43 ± 0.61^{m}	–	7.65 ± 0.23^{i}	–	24.08

Different alphabets in superscript of the mean value in each column indicate significant difference between the accessions for that parameter.

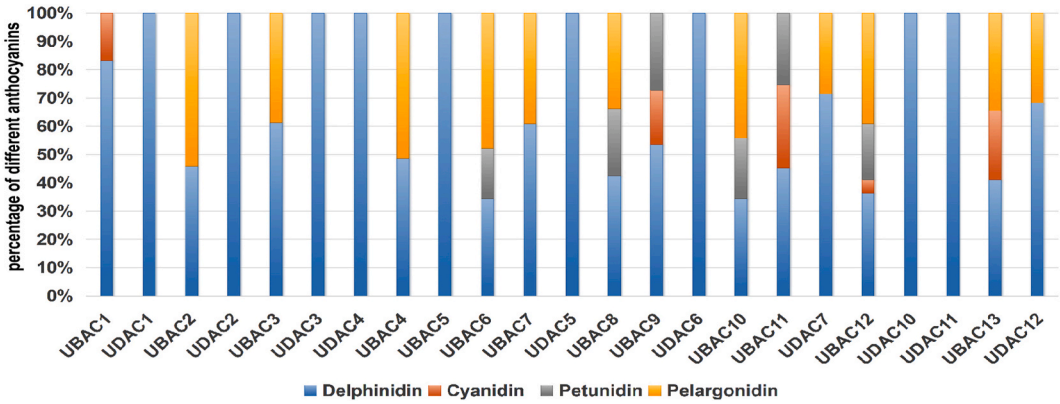

Fig. 5. Percentage bar diagram of different anthocyanins present in pigmented potato accessions.

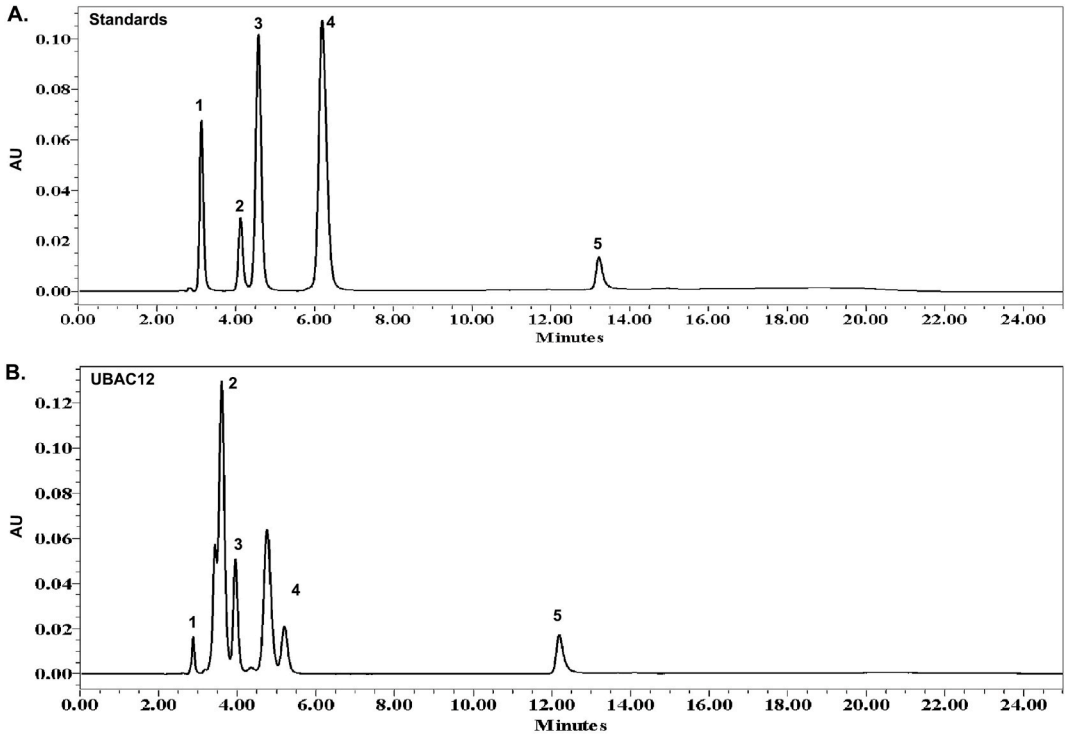

Fig. 6. Detection and quantification of different phenolic compounds from UBAC12 using High-Performance Liquid Chromatography (HPLC). A. HPLC chromatogram generated at 280 nm from a mixture of HPLC grade standards of caffeic acid (1) followed by gallic acid (2) and vannilic acids (3). cinnamic acid (4) and quercetin (5) B. HPLC chromatogram generated from extract of UBAC12 (PSPF).

Table 4
Quantification of different phenolic compounds from tubers of pigmented potato accession UBAC12.

Sl no.	*Phenolic compound*	Regression equation	R^2	LOD (μg/ml)	LOQ (μg/ml)	Amount (μg/g)
1	*Gallic acid*	y = 41020x + 175429	0.99	0.178	0.539	48.89 ± 3.26[b]
2	*Vannilic acid*	y = 1454.3× - 12277	0.99	0.230	0.698	36.71 ± 1.37[c]
3	*Caffeic acid*	y = 14528× - 17853	0.99	0.237	0.720	64.08 ± 2.59[a]
4	*Cinnamic acid*	y = 3379.2x + 8691.8	0.99	0.280	0.850	18.61 ± 0.6[e]
5	*Quecertin*	y = 37926× - 264114	0.99	0.208	0.631	22.89 ± 1.78[d]

vanillic acid and quercetin in the extract of UBAC12. Among the phenolics, the highest quantity was found in caffeic acid (64.08 μg/g) followed by gallic acid (48.89 μg/g) and vannilic acids (36.71 μg/g). cinnamic acid (18.61 μg/g) and quercetin (22.89 μg/g) was found in the lowest quantity. Major phenolic compounds detected in native Andean pigmented potatoes are chlorogenic acid and caffeic acid derivatives [64]. High amounts of quercetin derivatives were also reported in pigmented potatoes [65]. The presence of these health benefitting phenolics emphasizes the abundant health benefits of consuming these pigmented potatoes over white fleshed table potatoes.

3.5. Evaluation of genetic diversity and association of markers with nutritional traits

The selected small pigmented accessions under the current study are grown in a small and confined geographical location as clonal accessions since ancient times, but the extent of their genetic diversity is not known. The genetic diversity of these potato accessions was studied using microsatellite-based molecular markers. Seven simple sequence repeat (SSR) and eighteen inter simple sequence repeat (ISSR) markers were selected based on stable, accurate, and polymorphic PCR amplicons produced (Table S4, Table S5). A total of 59 alleles were found among which 51 were polymorphic and displayed 86.44 % polymorphism. The ISSR markers, namely ISSR 14 and ISSR 9, had the greatest PIC value and heterozygosity (H = 0.499) among the marker combinations. The highest discriminating power and resolving power were found in ISSR 10 (D = 0.99) and ISSR 5 (R = 2.96) respectively (Table 5). Six phylogenetic clusters were identified in UPGMA-based clustering where clusters I and II predominantly consisted of UDAC accessions with oval RSWF tubers with lower TAC. Cluster III and cluster IV exhibited UBAC accessions (PSPF) with oblong-shaped tubers containing comparatively higher TAC (Fig. 7A). Cluster V presented round-shaped RSWF accessions with one YSWF accession (UDAC12). The other YSWF accession (UDAC9) was also present close to UDAC12 in the phylogenetic cluster VI, a mixed cluster of UDAC and UBAC genotypes. UBAC13, an accession from Nagaland did not fall in any major cluster. The population structure of the potato accessions was analyzed from the binary data retrieved using polymorphic markers and was calculated using the Bayesian approach. The results revealed that an optimal number of K = 3 signifies the presence of three major clusters among the accessions (Fig. 7B) which were denoted by the colours red, blue, and green. Allelic data revealed that population I (predominantly red) and III (admixture consisting of all colours) have five and nine accessions respectively, while population II has comparatively pure ten accessions (predominant green). The structure analysis suggested that the majority of the accessions studied had a common ancestor, few potato accessions, especially UBAC7, UDAC3, UBAC2, UDAC4, UBAC3, UDAC5, UDAC2, and UBAC8, have very pure genetic makeup (green) and share a similar origin. Accessions UBAC12, UDAC7, UDAC8, UDAC11, and UDAC9 may have common ancestors in the past and have a discrete origin than population II. Accessions such as UBAC11, UDAC10, UBAC6, UBAC5, and UBAC10 represent a combination of three colours which indicates the mixing of different populations during their evolution in the past. The pigmented accessions showed superiority concerning their TAC and GI. Single marker association (SMA) analysis with these two major nutritional attributes was carried out. The results of the SMA studies are presented in a Manhattan plot (Fig. 7C). The results depict a 500 bp band of the satellite repeat motif $(AGG)_6$ is tightly linked with TAC (Log10 (*P*-value) > 3) (Fig. 7C and D). A log of *P* value > 2 indicates a strong correlation of the marker with the trait [30]. A 300 bp band with the motif $(GGC)_5TA$ was found to be linked with GI although with a low level of significance (Log10 (*P*-value) > 1.6) (Fig. S3). The normal distribution pattern of both nutritional traits with the markers was confirmed through the linear trend of Q-Q plot (Fig. S3). This is the first report where the genetic diversity of these landraces has been unravelled and linked markers for crucial nutritional traits were identified. Earlier the genetic diversity and structure analysis of conventional white-fleshed potato populations from Korea [66], USA [67], Columbia [68] and India [69] were reported but genetic diversity analysis using the elite, nutritionally rich, healthy, pigmented accessions is rarely found.

4. Conclusion

Pigmented potato cultivars offer numerous health-promoting phytonutrients and excellent matrix for developing functional foods and nutraceuticals [70]. Pigmented potato landraces from the North-Eastern *sub-Himalayan* plains of India are a unique agricultural heritage of these region offering excellent nutritional potential and other healthy attributes than the popular white-fleshed potato cultivars. This is the first report where the nutritional potential of these Indian landraces has been explored in terms of starch quality, glycemic index, phenolics and anthocyanin compound characterization, and dietary fibre content. Considering all the parameters, the accession UBAC12 stands out with its high TAC and a bouquet of anthocyanin and phenolic compounds presence. The dark purple colour of the boiled UBAC12 tubers and low GI and RES value will also attract consumers' attention. The present study will help conserve and popularize these landraces and encourage their production, consumption, and multifaceted uses by attracting consumers worldwide.

Data availability statement

Data included in article/supp. material/referenced in the article.

CRediT authorship contribution statement

Jammugani Vinod Kumar: Writing – original draft, Validation, Software, Investigation, Data curation. **Riman Saha Chaudhury:** Writing – original draft, Resources, Investigation. **Prudveesh Kantamraju:** Methodology, Investigation. **Subir Dutta:** Methodology, Investigation. **Kumaresh Pal:** Project administration, Methodology, Investigation. **Srinjoy Ghosh:** Methodology, Investigation.

Table 5
Polymorphism statistics calculated with 25 different SSR and ISSR markers for the pigmented potato accessions.

Name of the marker	Motif	H	PIC	MI	D	R
ISSR 1	(GAC)5	0.49	0.37	0.01	0.81	2.88
ISSR 2	(GTGC)4	0.36	0.30	0.01	0.43	0.96
ISSR 3	(GACA)4	0.32	0.27	0.00	0.96	0.80
ISSR 4	(AGG)6	0.48	0.36	0.01	0.85	0.80
ISSR 5	(GA)9T	0.49	0.37	0.01	0.81	2.96
ISSR 6	T(GA)9	0.46	0.35	0.01	0.87	2.08
ISSR 7	(GTG)5	0.25	0.22	0.00	0.98	0.88
ISSR 8	(GGA)4	0.36	0.30	0.00	0.95	0.48
ISSR 9	(GGC)5AT	0.50	0.37	0.01	0.78	0.96
ISSR 10	(AAG)5 GC	0.18	0.16	0.00	0.99	0.40
ISSR 11	(AAG)5 TG	0.15	0.14	0.14	0.16	0.00
ISSR 12	(AAG)5CC	0.32	0.27	0.00	0.96	1.60
ISSR 13	(AGC)5CA	0.49	0.37	0.01	0.83	2.16
ISSR 14	(AGC)5CG	0.50	0.37	0.01	0.73	2.64
ISSR 15	(GGC)5 TA	0.32	0.27	0.00	0.96	1.60
ISSR 16	(AGC)5 GA	0.47	0.36	0.01	0.85	0.80
ISSR 17	(AAG)5CG	0.33	0.28	0.01	0.38	1.28
ISSR 18	CCA(GTG)4	0.08	0.07	0.00	1.00	0.16
PM0398	TA(2*12)	0.00	0.00	0.00	0.00	0.00
SSR0675	AT(2*14)	0.00	0.00	0.00	0.00	0.00
SSR0707	(AGA)8	0.08	0.07	0.00	0.08	0.08
ST10012	(ATT)n	0.00	0.00	0.00	0.00	0.00
ST10032	(GGA)n	0.00	0.00	0.00	0.00	0.00
STG0016	(CAA)3	0.00	0.00	0.00	0.00	0.00
STM2022	TTTAAC(6*4)	0.00	0.00	0.00	0.00	0.00

[H= Heterozygosity index; PIC= Polymorphic information content; MI = Marker index; D = Discriminating power; R= Resolving Power].

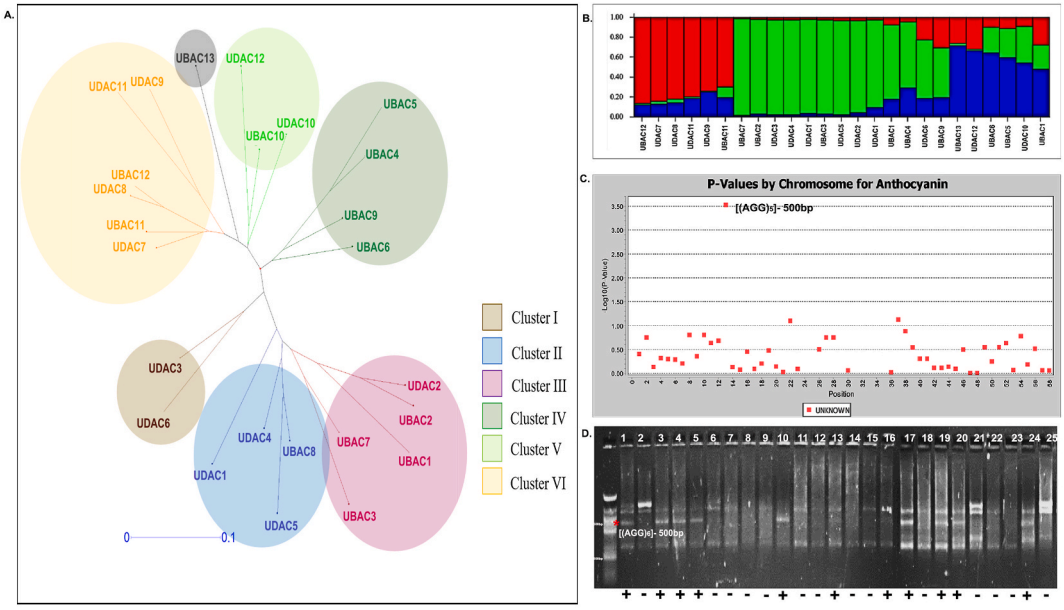

Fig. 7. Genetic evaluation of potato accessions with ISSR and SSR markers. A. Genetic diversity analysis of twenty-five small potato accessions generated utilizing dissimilarity matrix and UPGMA clustering. B. The population structure was analyzed using STRUCTURE 2.3.4 platform. C. Manhattan plot is generated using Single Marker Analysis (SMA) indicating associated markers with total anthocyanin content generated using TASSEL program. D. The 2 % agarose gel picture with asterisks points to a 500 bp band for the repeat motif $(AGG)_5$ which shows strong linkage with anthocyanin content of the genotypes. The presence (+) and absence (−) of the band is indicated in the panel below the gel.

Simanta Das: Methodology, Investigation. **Rupsanatan Mandal:** Methodology, Investigation. **Suchand Datta:** Resources, Project administration, Conceptualization. **Ashok Choudhury:** Resources, Project administration. **Somnath Mandal:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation. **Nandita Sahana:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36730.

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