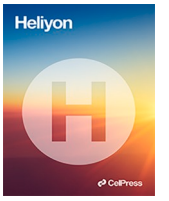
Heliyon 11 (2025) e42148

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Differential quantity of key bioactive compounds and their antioxidative potential in novel apple genotypes: A correlative study for potential therapeutics

Syed Mudassir Jeelani [*,1], Salwee Yasmin [1], Ashiq Hussain Lone [1], Javid Iqbal Mir [**], Mohammad Irfan, Vishal Dinkar, Wasim Hassan Raja, Sajad Un Nabi, Mahendra Kumar Verma, Geetika Malik, Om Chand Sharma

ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu & Kashmir, 191132, India

ARTICLE INFO

Keywords:
Apple genotypes
Bioactive compounds
Antioxidative potential
DPPH
Phenolics
PCA

ABSTRACT

Apples are among the most economically vital crops growing worldwide. The present study aimed to analyze the variation in the content of key bioactive principles and antioxidative potential of novel apple genotypes (developed through breeding techniques) along with their correlation for possible therapeutic insights. Using the HPLC method, the bioactive compounds of these apples were investigated, and their contribution to free radical scavenging activity by employing DPPH assay. HPLC analyses displayed concentration of bioactive compounds varies significantly among these genotypes with catechins, epicatechins, quercetin, and rutin were the key bioactive compounds. Principal Component Analysis results revealed a correlation between total phenolic content and antioxidative potential. It is also apparent that phenols are primary contributors to the antioxidant efficacy among the apple genotypes under investigation for potential therapeutic application. Besides, the study dispenses some valuable statistics for the production of novel apple genotypes having added phytochemicals for conventional and modern breeders.

1. Introduction

Apple is one of the substantial crops of economic importance, and breeders continue to develop new genotypes/varieties/cultivars with enhanced features. Due to their rich phenolic contents, apples and their byproducts are considered a potential source of natural antioxidants. However, the extraction process involved in the recovery of phenolics remain to be very vital. Apple (*Malus* × *domestica* Borkh.) belongs to the family Rosaceae, is one of the most vital fruits domesticated all over the temperate regions the world. It is a high-value nutritious crop growing the winter is near freezing regions [1]. Because of technological progress in the area of conservation and organoleptic properties, apples are consumed all through the year globally. In 2018, India produced about 3.02 % of the world's apple with a production of 2503 MT from an area of 314ha [2]. The Jammu and Kashmir contributes about 75 % to the total Indian apple production supporting about 4–5 lakh families. Apple industry provides the Jammu and Kashmir State exchequer with Rs. 8000 crores

* Corresponding author.
** Corresponding author.
E-mail addresses: sandrabi555@gmail.com (S.M. Jeelani), javidiqbal1234@gmail.com (J.I. Mir).
[1] Authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2025.e42148
Received 4 December 2024; Received in revised form 18 January 2025; Accepted 20 January 2025
Available online 21 January 2025

annually [3]. Not only from an economic point of view but looking at the health aspects of this crop, the importance it receives is worth mentioning. Apple is an imperative dietary component owing to various potentially active compounds namely sugars, minerals, ascorbic acid, vitamin C, and certain phenolic compounds known to be antioxidants [4] serving as a low-calorie fruit with high content of water [5]. These phenolics also maintain human health due to their preventive effect against numerous ailments, such as cardiovascular diseases, neuropathies, and diabetes [6]. The range of phytochemical compounds in apple thus being a potential health benefit and has made it a fruit of choice amongst the researchers as well as consumers. Several beneficial effects of apples are related to the number of polyphenols present. These are categorized as flavanols (catechins, epicatechin and procyanidins, quercetin glycosides), phenolic acids (chlorogenic, gallic and coumaric acids), dihydrochalcones (phloretin glycosides) and anthocyanins (cyanidin) [7]. At present, there is a great global interest in the bioactive compounds obtained from plants, and in turn, the extraction of such compounds from specific tissues is required [7].

The incidence of cancer and cardiovascular diseases whether due to genetic, environmental, or diet-related is huge worldwide. The nutritive affluent of apples cannot be overlooked because of their immense antioxidant potential which helps to stop the growth of cancer cells together with the reduction in the asthma, diabetes, and lowering of blood cholesterol levels [8,9]. Studies have shown that cancers can be prevented by adopting a healthy diet especially rich in antioxidants. To address this issue, consuming apples in the diet can pave the way forward since apples hold the distinction of one of the highest antioxidant potential of commonly consumed foods.

An in-depth understanding of Apple's health benefits has given it a status of par excellence in terms of trade gain. Owing to an upsurge in vegan healthy foods, an insight into the antioxidant potential will head towards new marketing opportunities. Hence, the present work intends to analyze the quantification of different bioactive compounds and their respective antioxidative potential in different apple genotypes growing under the same climatic and geographical conditions. The current study keeps up the recognition and systematic categorization of elite apple genotypes having biochemical profiles for better pharmacological/medicinal and marketable promises.

2. Materials and methods

2.1. Plant materials

Thirteen apple genotypes (Table 1) were obtained from ICAR-Central Institute of Temperate Horticulture (ICAR-CITH; 33.98° N, 74.79° E), Srinagar, Jammu and Kashmir, India. The genotypes were growing under the same climatic conditions but originating from different geographical areas. In order to homogenise the selection of the samples, the fruits of these genotypes were collected at an optimal ripening stage based on TSS, fruit size. The reagents used comprise Aluminum Chloride, Folin-Ciocalteau, and DPPH (2,2-diphenyl-2-picrylhydrazyl).

2.2. Pure compounds and chemicals

The investigated bioactive compounds viz. quercetin, rutin, catechin epicatechin, ferulic acid, chlorogenic, acid and sinapic acid were acquired from Sigma-Aldrich, St. Louis, USA (Fig. 1a–g). The rest of the chemicals and solvents were of analytical grade.

2.3. Process of extraction

Extractions were carried out following the protocol with modifications [10]. The fresh edible part of the apple i.e., fruit (10g) in liquid nitrogen was crushed to a fine powder in a mortar and pestle, after removing the seeds manually. A 3 g apple powder was moved to Oakridge tubes containing 15 mL of acetone or methanol in varying concentrations. This was followed by incubation at 10 °C for 10 min. At 10000 rpm for 10 min at 4 °C (Sigma 3–30 K, Munich, Germany), the mixture was centrifuged. Using a rotary evaporator (IKA, HB-10, Germany), the mixture was concentrated under vacuum at 4 °C. Using a sintered glass funnel, extracts were rapidly

Table 1
Information on accession numbers of different apple genotypes.

S. no.	Genotype	Accession Number	Status
I.	CITH-Ambri-1	IC-0638853	Existing
II.	Golden Delicious	EC-162914	Existing
III.	Mollies Delicious	EC-62373	Existing
IV.	Prima	EC-125416	Existing
V.	Red Delicious	EC-451348	Existing
VI.	Snow Drift	EC-390170	Existing
VII.	Top Red	IC-319088	Existing
VIII.	CITH-Ambrit	IC-0638855	New
IX.	CITH-Ammol	IC-0638854	New
X.	CITH-Golden Snow	IC-0638858	New
XI.	CITH-Priame	IC-0638857	New
XII.	CITH-Priator	IC-0638859	New
XIII.	CITH-Pride	IC-0638856	New

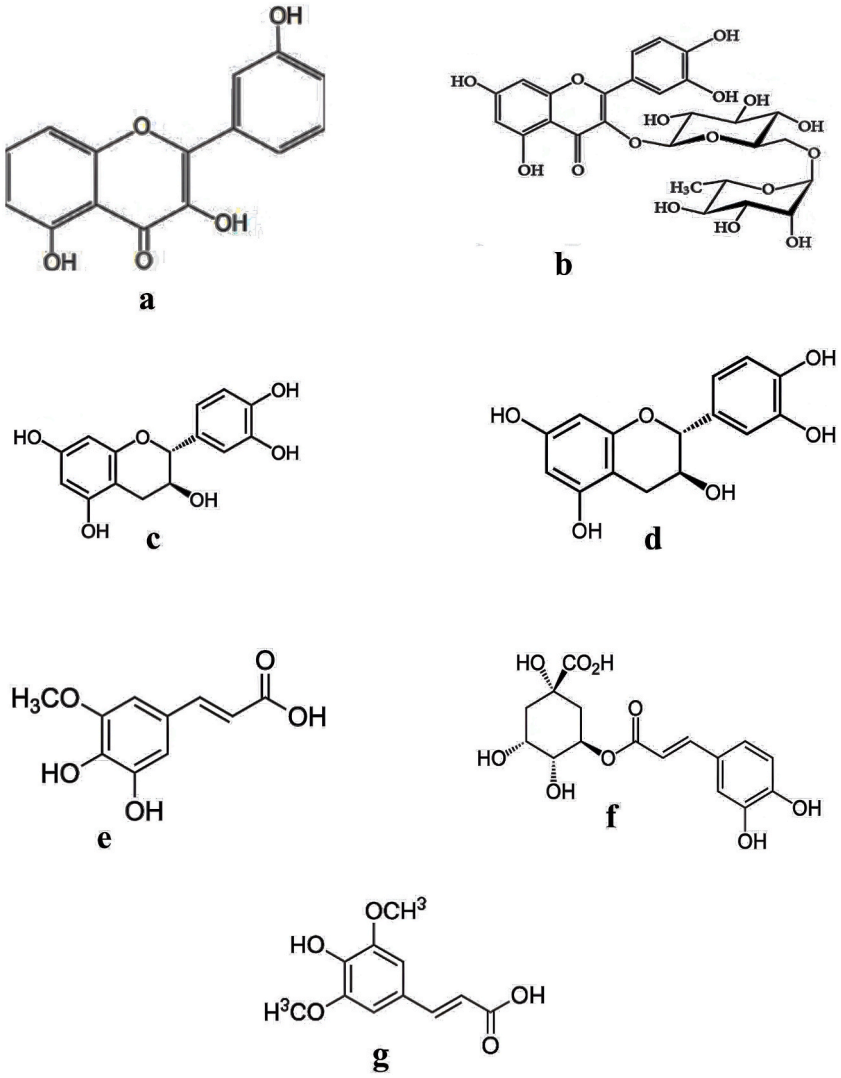


Fig. 1. Chemical structures of (a) Quercetin (b) Rutin (c) Catechin (d) Epicatechin (e) Ferulic acid (f) Chlorogenic acid (g) Sinapic acid.

Table 2
HPLC optimized operating conditions for the quantification various bioactive compounds.

Name of compound and $[M + H]^+$	Molecular formula/weight	Retention time (min)	Regression equation	R^2 Correlation coefficient	Linear range (mg/mL)	LOQ (mg/mL)	LOD (mg/mL)
Quercetin [303]	$C_{15}H_{10}O_7$/302	7.3	y = 123760x −44951	0.99	1.45–50	0.01	0.02
Rutin [610]	$C_{27}H_{30}O_{16}$/610	5.7	y = 9646.3x + 17674	0.99	0.39–100	0.54	1.42
Catechin [288]	$C_{15}H_{14}O_6$/290	4.9	y = 64767x + 12456	0.99	5–150	6.35	2.11
Epicatechin [288]	$C_{15}H_{14}O_6$/290	4.4	y = 72532x − 13465	0.99	5–150	6.21	2.07
Ferulic acid [193]	$C_{10}H_{10}O_4$/194	5.6	y = 2211.3x + 1527.02	0.99	0.05–50	0.92	0.24
Chlorogenic acid [355]	$C_{16}H_{18}O_9$/354	2.6	y = 1953.62 x − 99.25	0.99	0.10–50	1.23	0.35
Sinapic acid [223]	$C_{11}H_{12}O_5$/224	5.9	y = 1495.38x + 614.08	0.99	0.05–50	1.111	0.333

vacuum-filtered and kept under refrigerator conditions. The samples were reconstituted with 2 mL of 2.5 % acetic acid and methanol (3:1, v/v) and filtered through a 0.22 μm (Nylon, Mumbai, India) syringe filter (Moxcare, Haryana, India) and kept refrigerated until assayed.

2.4. Apparatus and chromatographic conditions

High Performance Liquid Chromatography (HPLC) was performed using Shimadzu HPLC, Kyoto, Japan armed through quaternary pumps, a degasser coupled to photo-diode-array (PDA) detector, and an injection valve (20 μL). Separation process was undertaken by an injection (20 μL volume) and a flow rate (1.0 mL per minute) with 30 min of run time. The system source settings recommended for the present study are given in Table 2.

2.5. Optimizations of chromatographic conditions for HPLC

On a C18 (250 × 4.6 mm) with a 5 μm column using a solvent system in gradient mode followed by an isocratic (methanol: water 1:1; v/v) run (Table 2), chromatographic separations were performed. The filtration of the mobile phase was carried using 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and exposed to 60 min ultrasonication. Instrument control, data acquisition, and data processing were done using Class WP software (version 6.1) from Shimadzu (Columbia, SC, USA). Considering the individual peak areas of standards at a particular retention time (RT) (Fig. 2), against concentration, quantitative determinations of investigated compounds (a–g) were made in units of mg/g of apple fruit. All the quantifications were made in triplicate.

2.6. Total phenolic content (TPC)

Total polyphenols were determined calorimetrically (Jenway 6505, UK) by the Folin-Ciocalteu procedure [11]. Covering the concentration (20–400 ppm), gallic acid (GAE) was employed as standard. Using a spectrophotometer (Shimadzu, Columbia, SC, USA), the absorbance was measured at 765 nm. The results were expressed as milligrams of GAE per 100 g of fresh plant material (FW) and calculated from the calibration curve: $Y = 0.001x+0.046$: $R2 = 1$. Here 'Y' is absorbance and 'x' is gallic acid equivalent in mg per 100 g respectively.

2.7. Total flavanoid content (TFC) and flavanols

The total flavonoid content (TPC) of apple fruits was calculated using an Aluminum-Chloride colorimetric assay [12]. The absorbance was measured at 415 nm spectrophotometrically. The flavonoid amount was determined as a quercetin equivalent from the calibration curve of quercetin standard solutions and expressed as milligrams of quercetin per 100 g of fresh weight (FW). Similarly,

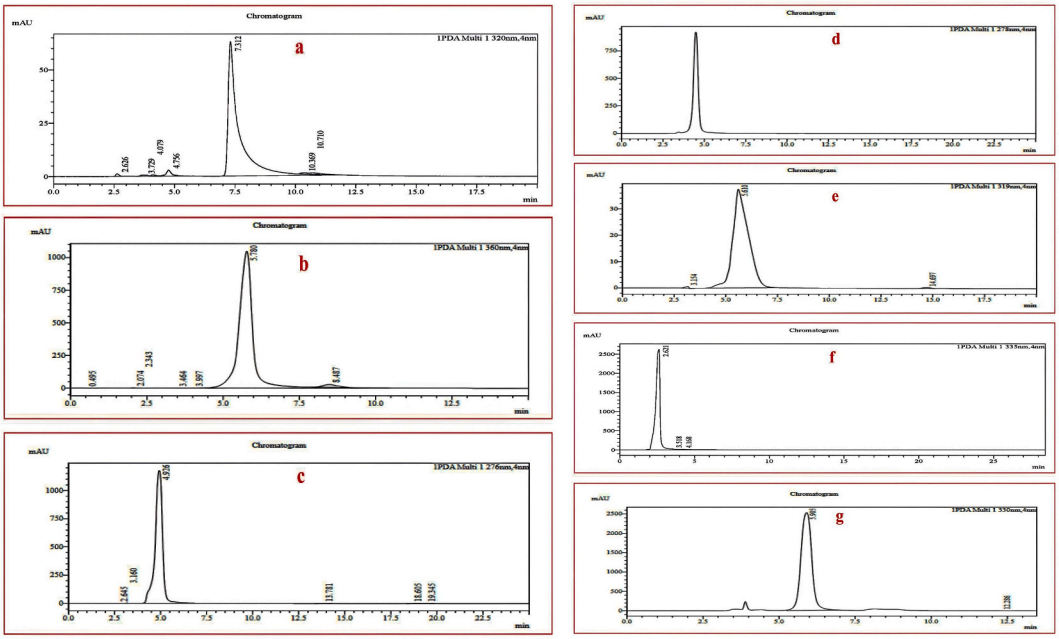


Fig. 2. HPLC chromatograms of investigated compounds (a) Quercetin (b) Rutin (c) Catechin (d) Epicatechin (e) Ferulic acid (f) Chlorogenic acid (g) Sinapic acid.

flavnols were calculated with an incubation period of 150 min and the absorbance was measured at 440 nm. The total flavanol content was also expressed as milligrams of quercetin per 100 g of fresh weight (FW).

2.8. Antioxidant properties

2.8.1. 2,2-Diphenyl-1-picrylhydrazyl assay

By employing the procedures with slight modifications, DPPH free radical scavenging activity was calculated [10,13]. The inhibition (%) was measured as (%IP) = [(At = 0 - At = 15)]/(At = 0) × 100. Here, At = 15 (absorbance of the test sample after 15 min) and At = 0 (absorbance of the control after 15 min).

The scavenging activity percentage (AA%) was calculated as below.

AA% = 100- {Abs sample - Abs control/Abs blank × 100}

Here, a blend of methanol and DPPH (1: 1) was used as blank, and a blend of standard i.e., ascorbic acid and DPPH (1: 1) was employed as control. 15 μg/mL concentration was used for both the test sample and the standard.

2.9. Statistical analyses

Statistical analyses were performed using ANOVA (analysis of variance) and the data was obtained in triplicate as mean ± SEM. Ducan's Multiple Range Test (DMRT) was used to find the significant differences among means. The statistical tests were carried out using OPSTAT software. Pearson's test was used to find the cluster analysis and correlation between DPPH vs phenols among genotypes.

3. Results and discussion

Many evidences suggest that apple rich diet decreases the probabilities of countless diseases. According to Manach et al. [14], dissimilar polyphenol molecule in apple might have diverse health benefits. However, the relationship amongst the potential health effects of apples and their flavour-aroma hitherto remains to be divisive. It would be worthwhile if the most flavoursome apple extract presented the most plentiful bioactive molecules with maximum antioxidant potential [15]. In terms of the overall quality and quantity of the extracts from different genotypes, the present study revealed diverse antioxidative potentials for different genotypes.

The varying concentration of major bioactive phytoconstituents in diverse apple genotypes presents a substantial perspective for their potential use in the modern drug industry and traditional medicinal systems. Hence, the present investigation provides an opportunity to evaluate elite apple genotypes with desirable chemical profiles and maximum antioxidative potential for pharmaceutical and commercial purposes.

3.1. Morphological study

Although all the thirteen apple genotypes were cultivated under similar climatic conditions, the appearances of mature fruits were remarkably dissimilar (Fig. 3). It is well known that apple varieties with characteristic morphology, color, texture, and taste have

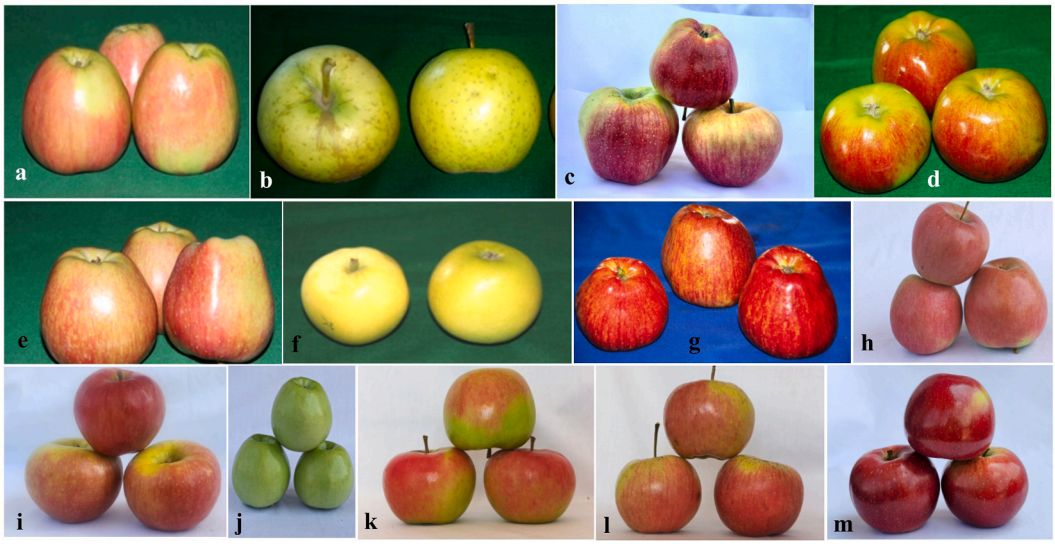


Fig. 3. The morphology 13 apple fruit genotypes. a) CITH-Ambri-1, b) Golden Delicious, c) Mollies Delicious, d) Prima, e) Red Delicious, f) Snow Drift, g) Top Red, h) CITH- Ambrit, i) CITH-Ammol, j) CITH-Golden Snow, k) CITH-Priame, l) CITH-Priator, m) CITH-Pride.

different polyphenol and bioactive composition [16].

3.2. Phytochemical studies

3.2.1. Bioactive compounds of plant extracts

The identification and quantification of major bioactive compounds in different apple genotypes were reported previously [10, 17–20]. However, this is the first study of its kind wherein individual quantification of seven major bioactive compounds was corroborated with antioxidative potential. The occurrence of six new apple genotypes along with already existing one's makes the study imperative (Table 1). In this study, total phenolics (TPC), total flavonoids (TFC), flavanols, and antioxidant activity (DPPH) varied significantly. A total of thirteen apple genotypes (Fig. 3) were scrutinized phytochemically to find the concentration levels of principal bioactive constituents, i.e., quercetin, rutin, catechin, epicatechin, ferulic acid, chlorogenic acid, sinapic acid through High-Performance Liquid Chromatography (HPLC). The results indicated that catechins (53.55 %), quercetin (21.36 %), epicatechins (12.19 %), and rutin (11.66 %) were the chief bioactive compounds in all the investigated apple genotypes followed by sinapic acid (5.23 %) ferulic acid (2.74 %), and chlorogenic acid (2.23 %) with variable concentrations individually (Table 3). Comparatively, the concentration of catechins in all the studied apple genotypes was maximum. Earlier, investigations also reported variations in the concentration and composition of phenols and other bioactive compounds of apples from different geographical areas [10,21]. The bioactive compounds quantity depends on factors such as climate, soil, water core, genotypes, season, harvest date, geographic region, storage, bitter pit and, irradiation, as well as other conditions [19]. Additionally, metabolite buildup is the interaction of genetic constitution and environmental factors [22,23]. However, genetic variations were recognized as key factors that affect the contents of active constituents [22,24–26] and well applicable to the presently studied novel genotypes showing higher concentration of bioactive compounds.

3.2.2. Quantification of individual bioactive compounds

The individual quantified values of different bioactive compounds of all the studied apple genotypes are listed in Table 3. Among the thirteen apple genotypes, CITH-Pride exhibited the maximum (2.078 ± 0.212^{a} mg/g) quercetin content whereas, Snow Drift showed a minimum (0.046 ± 0.061^{e} mg/g) concentration. Similarly, the maximum (1.614 ± 0.131^{a} mg/g) and minimum (0.101 ± 0.053^{e} mg/g) concentrations of rutin were found in CITH-Priator and Mollies Delicious, respectively. CITH-Priame revealed the highest (6.072 ± 0.211^{b} mg/g) and Top Red exhibited the lowest (0.252 ± 0.102^{f} mg/g) quantity of catechins correspondingly. Epicatechins also varied widely among the studied apple genotypes. CITH-Ambrit revealed the topmost (1.552 ± 1.496^{a} mg/g) concentration whereas, Top Red showed the lowermost content (0.086 ± 0.067^{a} mg/g). The highest (0.531 ± 0.035^{a} mg/g) sinapic acid concentration was noticed in CITH-Golden Snow and the lowest (0.107 ± 0.021^{a} mg/g) in Mollies Delicious, congruently. At present as the total concentration of ferulic acid was recorded lesser than sinapic acid, but it also showed variability among different genotypes. Overall, the concentration of chlorogenic acid was recorded lowest among the all studied bioactive compounds (Table 3).

Table 3
Variability in concentration (mg/g) of various analysed bioactive compounds of different apple genotypes.

S. No.	Genotypes	Quercetin	Rutin	Catechin	Epicatechin	Ferulic acid	Chlorogenic acid	Sinapic acid
I.	CITH-Ambri-1	0.457 ± 0.017^{bcd}	0.143 ± 0.047^{e}	1.352 ± 0.567^{e}	0.109 ± 0.038^{a}	0.059 ± 0.071^{b}	0.095 ± 0.032^{cde}	0.148 ± 0.341^{a}
II.	Golden Delicious	0.142 ± 0.032^{de}	0.282 ± 0.046^{e}	0.632 ± 0.082^{ef}	0.162 ± 0.117^{a}	0.112 ± 0.069^{ab}	0.078 ± 0.0453^{de}	0.315 ± 0.251^{a}
III.	Mollies Delicious	0.286 ± 0.02^{bcde}	0.101 ± 0.053^{e}	0.686 ± 0.226^{ef}	0.176 ± 0.116^{a}	0.065 ± 0.025^{b}	0.071 ± 0.081^{de}	0.107 ± 0.021^{a}
IV.	Prima	0.459 ± 0.065^{bcd}	0.175 ± 0.117^{e}	3.176 ± 0.178^{d}	0.736 ± 0.687^{a}	0.067 ± 0.0232^{b}	0.015 ± 0.043^{e}	0.144 ± 0.066^{a}
V.	Red Delicious	0.276 ± 0.024^{cde}	0.252 ± 0.049^{e}	0.278 ± 0.174f	0.116 ± 0.058^{a}	0.258 ± 0.166^{ab}	0.128 ± 0.103^{bcd}	0.122 ± 0.032^{a}
VI.	Snow Drift	0.046 ± 0.061^{e}	0.724 ± 0.044^{d}	0.632 ± 0.184^{ef}	0.166 ± 0.109^{a}	0.253 ± 0.214^{ab}	0.068 ± 0.021de	0.112 ± 0.072^{a}
VII.	Top Red	0.477 ± 0.103^{bcd}	0.234 ± 0.048^{e}	0.252 ± 0.102^{f}	0.086 ± 0.067^{a}	0.059 ± 0.025^{b}	0.058 ± 0.032^{de}	0.193 ± 0.041^{a}
VIII.	CITH-Ambrit	2.188 ± 0.087^{a}	0.924 ± 0.146^{c}	5.591 ± 0.991^{a}	1.552 ± 1.496^{a}	0.079 ± 0.0331^{ab}	0.162 ± 0.051^{abc}	0.267 ± 0.129^{a}
IX.	CITH-Ammol	0.623 ± 0.155^{b}	1.056 ± 1.225^{bc}	4.816 ± 0.322^{c}	1.062 ± 0.926^{a}	0.140 ± 0.062^{ab}	0.172 ± 0.023^{abc}	0.469 ± 0.0543^{a}
X.	CITH-Golden Snow	0.561 ± 0.071^{bc}	1.210 ± 0.058^{b}	3.784 ± 0.134^{d}	0.811 ± 0.668^{a}	0.121 ± 0.054^{ab}	0.175 ± 0.032^{abc}	0.531 ± 0.035^{a}
XI.	CITH-Priame	0.582 ± 0.035^{bc}	0.622 ± 0.157d	6.072 ± 0.211^{b}	1.244 ± 1.112^{a}	0.185 ± 0.051^{ab}	0.215 ± 0.025^{a}	0.377 ± 0.032^{a}
XII.	CITH-Priator	0.396 ± 0.284^{bcd}	1.614 ± 0.131^{a}	5.062 ± 0.096^{c}	1.102 ± 0.942^{a}	0.16 ± 0.093^{ab}	0.118 ± 0.07^{bcd}	0.461 ± 0.064^{a}
XIII.	CITH-Pride	2.078 ± 0.212^{a}	0.747 ± 0.126^{d}	4.778 ± 0.212^{c}	1.129 ± 0.975^{a}	0.341 ± 0.068^{a}	0.192 ± 0.061^{ab}	0.381 ± 0.038^{a}

In diverse genotypes/varieties/cultivars, the study of biochemical differences becomes of greatest importance as a plant's genotype plays a prime role in the determination of its phytochemical profile [27]. The noticeable build-up of bioactive elements (catechins, epicatechins, quercetin, and/or rutin) in CITH-Ambrit, CITH-Priame, CITH-Ammol, CITH-Priator, CITH-Pride, and CITH-Golden Snow (Fig. 4) make these apples amongst utmost prudent genotypes. Previously, such results were reported for some apple genotypes with rutin and catechin concentrations being maximum [10]. To exchange desirable characters in other genotypes/cultivars/species, these genotypes may play the role of parents of choice. Such genotypes can also help in breeding programs and will depict a significant part in the expansion of new genetic material having higher quantities of bioactive compounds. Further, the identification of molecular markers that are related to traits viz. maximum concentration of bioactive molecules will also help for marker-assisted breeding programs in apples.

3.2.3. Total phenol content (TPC)

Fruits are an imperative source of antioxidants as they contain phenolic compounds. By using the Folin-Ciocalteu's reagent, the TPC was calculated as milligrams of Gallic Acid Equivalent per 100 g. The thirteen investigated apple genotypes showed substantial variance TPC ranging from 148.86 ± 0.728^{l} to 376.233 ± 0.888^{a} mg GAE/100g Fw (Fig. 5). In contrast to grape extracts (a drink famous for its TPC), this content of TPC is higher. In literature, diverse phenolic constituents from different plant extracts have been recognized [28–30]. According to Moure et al. [31], higher levels of polyphenolics are often produced when the solvent is more polar. The TPC is also influenced by the ethanol concentration and the interaction between extraction time and solvent volume [32]. Additionally, the concentration of phenols may be influenced by genotype, extrinsic and agronomic factors, light exposure, soil as well as seasonality.

3.2.4. Total flavonoid content (TFC) and flavanols

Flavonoids are powerful antioxidants and radical scavengers to promote good health besides acting as pigments responsible for the colours of plants [33]. The TFC of thirteen apple genotype extracts is shown in Fig. 6. The maximum TPC was reported in CITH--Ambri-1(15.5 ± 0.173^{a} mg QE/100g Fw) and minimum in Top Red (1.197 ± 0.031^{i} mg QE/100g Fw). Likewise, the flavanol content varied from 1.25 ± 0.13^{j} to 4.533 ± 0.044^{a} mg QE/100g Fw with CITH-Ambri-1 exhibited highest quantity and Top Red having lowest quantity (Fig. 7). The differences in TFC and flavanols among the presently studied apple genotypes may be due to different genetic makeups, different environmental conditions, storage of maturity, varietal differences, or soil fertilization as has been previously reported [34–36].

3.3. Biological evaluation

The phytochemical scrutiny evaluated here for different apple genotypes showed disparities in the quantity of bioactive compounds. Due to this erraticism in the accretion of bioactive constituents, all such genotypes were assessed for biological activity profile.

3.3.1. Antioxidative potential of different apple genotypes

The antioxidative potential of thirteen apple genotypes showing scavenging activity was calculated using DPPH assay. The results indicated that DPPH radical scavenging activity was markedly influenced by the genotype. The DPPH radical scavenging activity of apple genotypes ranged from 19.397 ± 0.592^{h} μmol AAE/g Fw in CITH-Priator to 45.347 ± 0.709^{a} μmol AAE/g Fw in Prima (Fig. 8). In particular, Prima had the strongest antioxidant capacities with a DPPH free radical scavenging rate of 45.347 ± 0.709^{a} μmol AAE/g

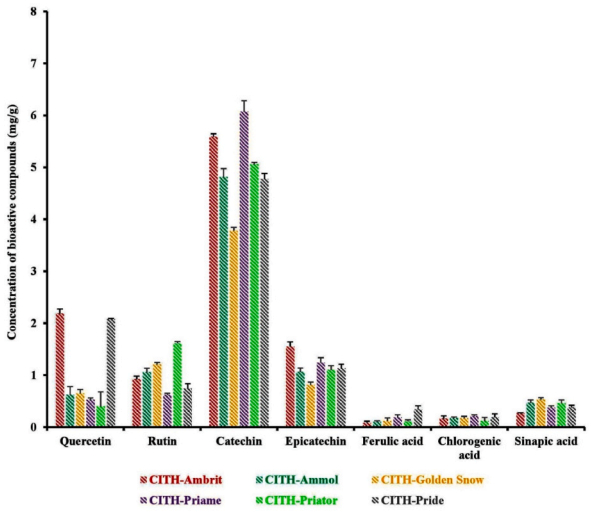


Fig. 4. Concentration of key bioactive compounds in six potential apple genotypes from the study.

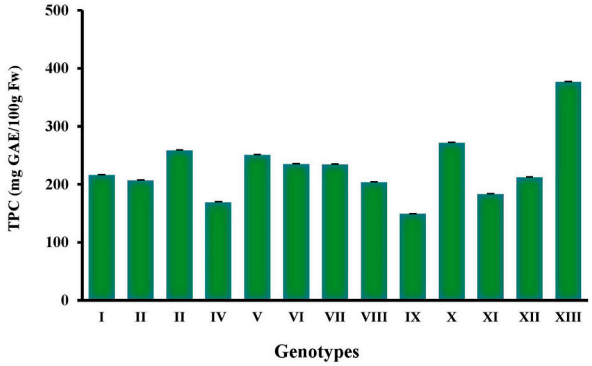


Fig. 5. Concentration of total phenolic content (TPC) of 13 apple fruit extracts measured as Gallic Acid Equivalent (GAE) in mg/100 gm Fw. (See Table 1 for I-XIII plant extracts).

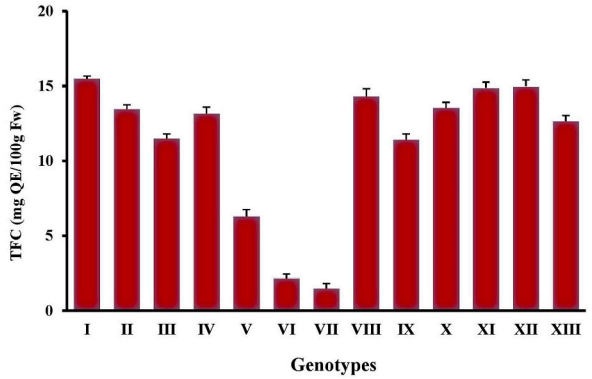


Fig. 6. Concentration of total flavanoid content (TFC) of 13 apple fruit extracts measured as Quercetin Equivalent (QE) in mg/100 gm Fw.

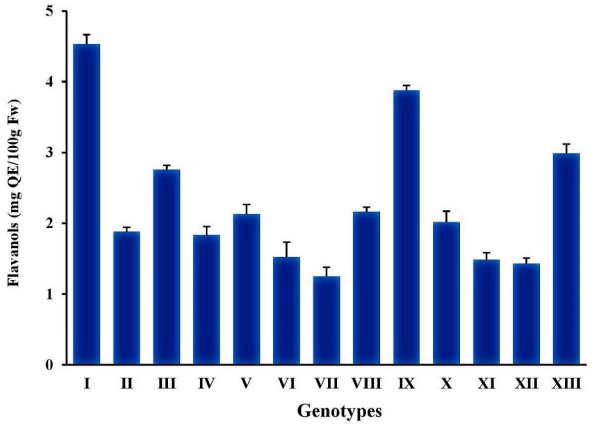


Fig. 7. Concentration of flavanols of 13 apple fruit extracts measured as Quercetin Equivalent (QE) in mg/100 gm Fw.

Fw. The antioxidant activity of Golden Delicious was weaker than any of the other polyphenols. At present, the DPPH activity was closely persistent in most of the apple genotypes. These results are in agreement with previous ones [37,38]. According to Chinnici et al. [39], the radical scavenging activity of apple extracts depends on their phenolic composition in qualitative and quantitative means. In the literature, it is also mentioned that apple extracts exhibit the highest contents of phenolic compounds and have the maximum antioxidant capacity in comparison with other extracts [40,41]. Also, the synergistic interactions between antioxidants are responsible for antioxidant activity. While considering the potential biological consequences for apple plants and human health, synergism is very important. The current study also enables the relationship between apple phenols and their antioxidative potential.

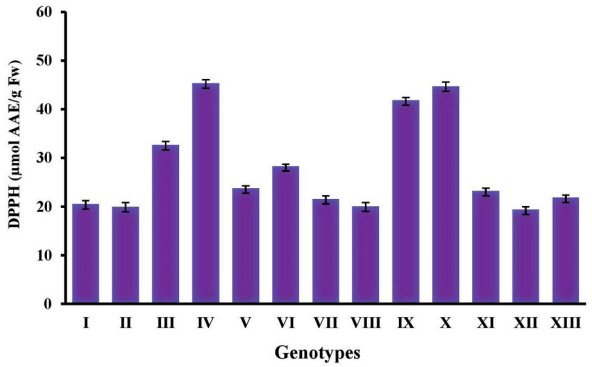


Fig. 8. DPPH antioxidative activity of 13 apple fruit extracts measured as µmol AAE/g Fw.

Antioxidants boost an active therapeutic role in enhancing the valuable effects of apple and their derivatives that in turn have an enormous number of therapeutic benefits. However, the relationship between bioactive compounds and antioxidant activity may vary depending on the kind of bioactive ingredient present naturally.

3.4. Principal component analysis (PCA)

It is a mathematical method that reduces the number of degrees in data and makes it conceivable to find the correlations among dissimilar kinds of data. The standardized data of evaluated parameters for all the thirteen diverse apple genotypes were observed under PCA. The PCA disclosed the strong variance of the different apple genotype extracts based on bioactive constituents and their antioxidative properties (Fig. 9a). The eigenvalues of three principal components i.e., PC1, PC2, and PC3 were greater than 1 (Fig. 9b). Overall, nine components were observed with the first three components contributing 73.93 % (PC1 = 40.68 %, PC2 = 21.41 %, and PC3 = 11.83 %) of the total variation. But only first two components contributed adequately to the total variance. Fig. 9a shows the direction of each arrow, and all the variables are vectored substantially and positive along PCA1 (40.70 %) excluding flavanols (FL) which is vectored positive along PCA2 (21.42 %).

3.4.1. Correlation between bioactive compounds and antioxidant potential of apple extracts

The correlation between bioactive compound extracts (TPC, TFC, and flavanols) and antioxidative potential (DPPH) is mentioned in Fig. 9c. A significant correlation existed for TPC and DPPH. As mentioned in the above results in general the antioxidant activity is directly influenced by the accumulation of polyphenolic metabolites besides the nature of genotype, which was also revealed by PCA.

4. Conclusion

The present study reveals that the extracts of thirteen different apple genotypes contain copious volumes of phenolic constituents and exhibit high antioxidative potential. A defined relationship existed between total phenolic content and antioxidant activity (DPPH). Some genotypes (CITH-Ambrit, CITH-Priame, CITH-Ammol, CITH-Priator, CITH-Pride, and CITH-Golden Snow) with a rich repository of desirable bioactive metabolites make them suitable for trade and industry. The extracts of Prima, CITH-Ammol, and CITH-Golden Snow revealed the maximum antioxidant activity. Additionally, the PCA certified that the major bioactive compounds

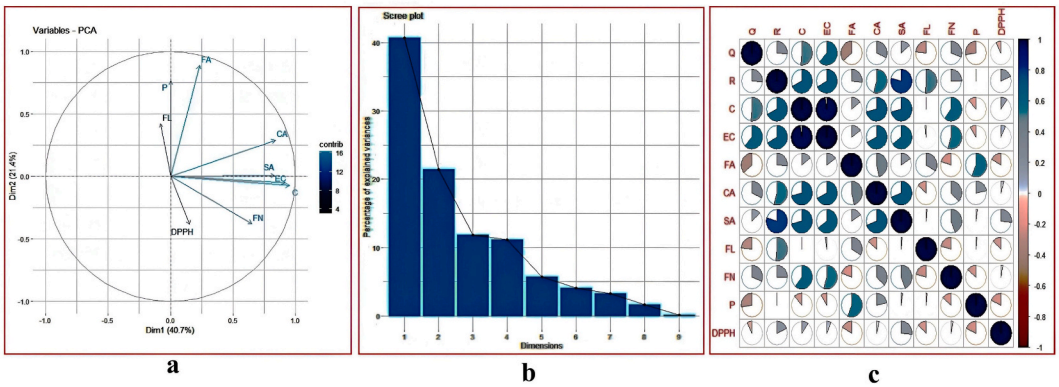


Fig. 9. Principal component analysis (PCA): a) variables of apple genotypes plotted along first two components. b) scree plot. c) correlation matrix among numerous components.

and total polyphenols are principal contributors to antioxidant efficacy in these apple genotypes. Furthermore, the phenolic compound profile and the antioxidant activity physiognomies of dissimilar apple extracts motivate the widespread usage of these products in the food industry for novel dietary products. The study has also the prospect of bringing elite high-yielding apple genotypes for future breeding programs on a commercial scale.

CRediT authorship contribution statement

Syed Mudassir Jeelani: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Salwee Yasmin:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Ashiq Hussain Lone:** Writing – original draft, Validation, Methodology, Data curation. **Javid Iqbal Mir:** Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Mohammad Irfan:** Formal analysis. **Vishal Dinkar:** Supervision. **Wasim Hassan Raja:** Supervision. **Sajad Un Nabi:** Supervision. **Mahendra Kumar Verma:** Supervision. **Geetika Malik:** Supervision. **Om Chand Sharma:** Supervision.

Ethical statement

Not applicable.

Data availability statement

All the pertinent data produced during the study are included within the manuscript in the form of tables, figures and supplementary data.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are grateful to the Indian Council of Agriculture Research (ICAR), New Delhi, India for supporting this research work.

Appendix. ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42148.

References

[1] Z. Fathizadeh, M. Aboonajmi, S.R. Hassan-Beygi, Non-destructive methods for determining the firmness of apple fruit flesh, Inf. Process. Agric. 8 (2021) 515–527, https://doi.org/10.1016/j.inpa.2020.12.002.
[2] National horticulture board. www.nhb, 2023.
[3] S.A. Wani, S. Kumar, F. Naqash, F. Shaheen, F. Potential of apple cultivation in doubling farmer's income through technological and market interventions: an empirical study in Jammu & Kashmir, Indian J. Agric. Econ. 76 (2021) 278–291, https://doi.org/10.63040/25827510.2021.02.005.
[4] N.P. Bondonno, C.P. Bondonno, N.C. Ward, J.M. Hodgson, K.D. Croft, The cardiovascular health benefits of apples: whole fruit vs. isolated Compounds, Trends Food Sci. Technol. 69 (2017) 243–256, https://doi.org/10.1016/j.tifs.2017.04.012.
[5] Z. Ergün, Determination of biochemical contents of fresh, oven-dried, and sun-dried peels and pulps of five apple cultivars (Amasya, Braeburn, Golden Delicious, Granny Smith, and Starking), J. Food Qual. 2021 (2021) 1–11, https://doi.org/10.1155/2021/9916694.
[6] A. Wojdyło, P. Nowicka, I.P. Turkiewicz, K. Tkacz, F. Hernandez, F. Comparison, Of bioactive compounds and health promoting properties of fruits and leaves of apple, pear and quince, Sci. Rep. 11 (2021) 20253. https://10.1038/s41598-021-99293-x.
[7] J. Patocka, K. Bhardwaj, B. Klimova, E. Nepovimova, Q. Wu, M. Landi, K. Kuca, M. Valis, W. Wu, Malus domestica: a Review on nutritional features, chemical composition, traditionaland medicinal value, Plants 9 (2020) 14089, https://doi.org/10.3390/plants9111408.
[8] S.J. Kim, N.H. Anh, C.W. Jung, N.P. Long, S. Park, Y.H. Cho, Y.C. Yoon, E.G. Lee, M. Kim, E.Y. Son, T.A. Kim, Y. Deng, J. Lim, S.W. Kwon, Metabolic and cardiovascular benefits of apple and apple-derived products: a systematic review and meta-analysis of randomized controlled trials, Front. Nutr. 9 (2022) 766155, https://doi.org/10.3389/fnut.2022.766155.
[9] E. Šahinović, S. Murtić, A comparison of antioxidant properties of two apple cultivars, Contemp. Agric. 68 (2019) 60–64, https://doi.org/10.2478/contagri-2019-0010.
[10] W. Shafi, S. Mansoor, S. Jan, D.B. Singh, M. Kazi, M. Raish, P. Ahmad, Variability in catechin and rutin contents and their antioxidant potential in diverse apple genotypes, Molecules 24 (2019) 943, https://doi.org/10.3390/molecules24050943.
[11] X. Wang, C. Li, D. Liang, Y. Zou, P. Li, F. Ma, Phenolic compounds and antioxidant activity in red-fleshed apples, J. Funct.Foods 18 (2015) 1086–1094, https://doi.org/10.1016/j.jff.2014.06.013.
[12] C.C. Chang, M.H. Yang, H.M. Wen, J.C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Anal. 10 (2002) 178–182, https://doi.org/10.38212/2224-6614.2748.
[13] K.H. Musa, A. Abdullah, B. Kuswandi, M.A. Hidayat, A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity, Food Chem. 141 (2013) 4102–4106, https://doi.org/10.1016/j.foodchem.2013.06.112.
[14] C. Manach, A. Scalbert, C. Morand, C. Rémésy, Jiménez, L. Polyphenols: food sources and bioavailability, Am. J. Clin. Nutr. 79 (2004) 727–747, https://doi.org/10.1093/ajcn/79.5.727.

[15] A. Francini, L. Sebastiani, Phenolic compounds in apple (*Malus* x *domestica* Borkh.): compounds characterization and stability during postharvest and after processing, Antioxidants 2 (2013) 181–193, https://doi.org/10.3390/antiox2030181.
[16] X. Li, X. Wu, J. Bi, X. Liu, X. Li, C. Guo, Polyphenols accumulation effects on surface color variation in apple slices hot air-drying process, LWT 108 (2019) 421–428, https://doi.org/10.1016/j.lwt.2019.03.098.
[17] P. Kumar, S. Sethi, P.R. Sharma, S. Singh, S. Saha, V.K. Sharma, M.K. Verma, S.K. Sharma, Nutritional characterization of apple as a function of genotype, J. Food Sci. Technol. 55 (2018) 2729–2738, https://doi.org/10.1007/s13197-018-3195-x.
[18] J. Oszmiański, S. Lachowicz, E. Gławde, T. Cebulak, I. Ochmian, Determination of phytochemical composition and antioxidant capacity of 22 old apple cultivars grown in Poland, Eur. Food Res. Technol. 244 (2018) 647–662, https://doi.org/10.1007/s00217-017-2989-9.
[19] L. Pavun, S. Uskoković-Marković, M. Jelikić-Stankov, D. Đikanović, Determination of flavonoids and total polyphenol contents in commercial apple juices, Czech J. Food Sci. 36 (2018) 233–238, https://doi.org/10.17221/211/2017-CJFS.
[20] A.H. Elhakem, M.M. Almatrafi, N. Benajiba, M.Y. Koko, R. Sami, Comparative analysis of bioactive compounds, antioxidant and anti-inflammatory activities of apple varieties, Asian J. Plant Sci. 20 (2021) 61–66. https://scialert.net/abstract/?doi=ajps.2021.61.66.
[21] M. Vasile, A. Bunea, C.R. Ioan, B.C. Ioan, S. Socaci, M. Viorel, Phytochemical content and antioxidant activity of Malus domestica Borkh peel extracts, Molecules 26 (2021) 7636, https://doi.org/10.3390/molecules26247636.
[22] S.M. Jeelani, U. Farooq, A.P. Gupta, S.K. Lattoo, Phytochemical evaluation of major bioactive compounds in different cytotypes of five species of *Rumex* L, Ind. Crops Prod. 109 (2017) 897–904, https://doi.org/10.1016/j.indcrop.2017.09.015.
[23] S.K. Lattoo, R.S. Dhar, A.K. Dhar, P.R. Sharma, S.G. Agarwal, Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea*, Flavour Fragr. J. 21 (2006) 817–821, https://doi.org/10.1002/ffj.1733.
[24] S.M. Jeelani, J. Singh, A. Sharma, G.A. Rather, S.A. Ali, A.P. Gupta, S. Singh, S.K. Lattoo, In-vitro cytotoxicity in relation to chemotypic diversity in diploid and tetraploid populations of *Gentiana kurroo* Royle, J. Ethnopharmacol. 274 (2021) 113966, https://doi.org/10.1016/j.jep.2021.113966.
[25] S.M. Jeelani, M. Shahnawaz, A.P. Gupta, S.K. Lattoo, Phytochemical diversity in relation to cytogenetic variability in *Inula racemosa* Hook.f., an endangered medicinal plant of Himalayas, Chem. Biodivers. 19 (2022) e202200486, https://doi.org/10.1002/cbdv.202200486.
[26] V.G. Do, Y. Lee, J.H. Kim, Y.S. Kwon, J.T. Park, S. Yang, J. Park, N.M. Win, S. Kim, The synergistic effects of environmental and genetic factors on the regulation of anthocyanin accumulation in plant tissues, Int. J. Mol. Sci. 24 (2023) 12946, https://doi.org/10.3390/ijms241612946.
[27] B. Farneti, M.S. Gutierrez, B. Novak, N. Busatto, D. Ravaglia, F. Spinelli, G. Costa, Use of the index of absorbance difference (IAD) as a tool for tailoring postharvest 1-MCP application to control apple superficial scald, Sci. Hortic. 190 (2015) 110–116, https://doi.org/10.1016/j.scienta.2015.04.023.
[28] N. Aissani, F. Albouchi, H. Sebai, Anticancer effect in human glioblastoma and antioxidant activity of *Petroselinum crispum* L. methanol extract, Nutr. Cancer 73 (2021) 2605–2613, https://doi.org/10.1080/01635581.2020.1842894.
[29] M. Calderón-Jurado, O. Cruz-Álvarez, P. Juárez-López, O.A. Hernández-Rodríguez, L.R. Orozco-Meléndez, E.R. Morales-Maldonado, D.L. Ojeda-Barrios, I. Alia-Tejacal, Bioactive compounds, minerals and antioxidants of edible flowers of peach and apple, Int. J. Food Prop. 26 (2023) 1855–1866, https://doi.org/10.1080/10942912.2023.2236325.
[30] W. Yeddes, A. Chalghoum, W. Aidi-Wannes, R. Ksouri, M.S. Tounsi, Effect of bioclimatic area and season on phenolics and antioxidant activities of rosemary (*Rosmarinus officinalis* L.) leaves, J. Essent. Oil Res. 31 (2019) 432–443, https://doi.org/10.1080/10412905.2019.1577305.
[31] A. Moure, J.M. Cruz, D. Franco, J.M. Domínguez, J. Sineiro, H. Domínguez, J.C. Parajó, Natural antioxidants from residual sources, Food Chem. 72 (2001) 145–171, https://doi.org/10.1016/S0308-8146(00)00223-5.
[32] I. Solaberrieta, A. Jiménez, M.C. Garrigós, Valorization of aloe vera skin by-products to obtain bioactive compounds by microwave-assisted extraction: antioxidant activity and chemical composition, Antioxidants 11 (2022) 1058, https://doi.org/10.2139/ssrn.4042463.
[33] N.C. Cook, S. Samman, Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources, J. Nutr. Biochem. 7 (1996) 66–76, https://doi.org/10.1016/S0955-2863(95)00168-9.
[34] L. Mindaugas, V. Pranas, K. Darius, R. Raimondas, J.A. Valdimaras, Comparative study of phenolic content in apple fruits, Int. J. Food Prop. 18 (2015) 945–953, https://doi.org/10.1080/10942912.2014.911311.
[35] S.K. Kim, D.S. Kim, D.Y. Kim, C. Kim, Variation of bioactive compounds content of 14 oriental strawberry cultivars, Food Chem. 184 (2015) 196–202, https://doi.org/10.1016/j.foodchem.2015.03.060.
[36] Y. Li, H. Sun, J. Li, S. Qin, Z. Niu, X. Qiao, B. Yang, Influence of genetic background, growth latitude and bagging treatment on phenolic compounds in fruits of commercial cultivars and wild types of apples (Malus sp.), Eur. Food Res. Technol. 247 (2021) 1149–1165, https://doi.org/10.1007/s00217-021-03695-0.
[37] J. Bouayed, L. Hoffmann, T. Bohn, Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: bioaccessibility and potential uptake, Food Chem. 128 (2011) 14–21, https://doi.org/10.1016/j.foodchem.2011.02.052.
[38] M.S.M. Rufino, R.E. Alves, E.S. de Brito, J. Perez-Jimenez, F. Saura-Calixto, J. Mancini-Filho, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil, Food Chem. 121 (2010) 996–1002, https://doi.org/10.1016/j.foodchem.2010.01.037.
[39] F. Chinnici, A. Bendini, A. Gaiani, C. Riponi, Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition, J. Agric. Food Chem. 52 (2004) 4684–4689, https://doi.org/10.1021/jf049770a.
[40] D. Granato, A.R. Karnopp, S.M. van Ruth, Characterization and comparison of phenolic composition, antioxidant capacity and instrumental taste profile of extracts from different botanical origins, J. Sci. Food Agric. 95 (2015) 1997–2006, https://doi.org/10.1002/jsfa.6910.
[41] K. Mishra, H. Ojha, N.K. Chaudhury, Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results, Food Chem. 130 (2012) 1036–1043, https://doi.org/10.1016/j.foodchem.2011.07.127.