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Chemo profiling and antioxidant activity of different genotypes of Brassica oleracea var acephala grown across Kashmir in different environments

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***Abstract*---**Green leafy vegetables are gaining significant interest due to their nutritional value and disease-preventing potential. The Kale (Brassica oleracea var. acephala) is known as the queen of vegetables in some parts of the world due to its higher nutritional value. The current comparative study evaluated total phenols, total flavonoids, glucosinolates, and antioxidant activity in thirty different genotypes of kale grown across the Kashmir valley in different environments. The leaves were collected at the edible stage, and the extracts were made using three different solvent systems (80% methanol, 80% ethanol, and water). The majority of the genotypes of kale indicated a difference in the content of bioactive compounds. Based on the accumulation of nutraceutical important phenols, flavonoids, alkaloids, and sterols, the 30 genotypes of kale indicated a difference in bioactive compounds' content. The aqueous extract of K-29 demonstrated the phenolic content (71.68mg/gallic acid/g DW) and 60.98µMol/g DW glucosinolate content. Moreover, the methanolic extract of the K-29 genotype also indicated the higher phenolic content (85.56 mg/gallic acid/g DW), flavonoid content (16.64 mg/quercetin/g DW) as well as antioxidant activity (92.56 µMol FRAP/g DW). The higher antioxidant potential of K-29 is corroborated by its higher metabolic content of phenols and flavonoids. Thus, this study underscores the advantage of the K-29 genotype in health-promoting phytochemicals as well as antioxidant activity. However, the study warrants further exploiting

the K-29 genotype in modulating adaptive cellular response pathways to promote health in different disease models involving oxidative stress.

***Keywords*---**flavonoids, glucosinolates, kale, phytochemistry.

Introduction

Edible plants, in particular green leafy vegetables, have served as a great source of nutrients that have influenced human health and well-being from times immemorial. The health benefits of *Brassica oleracea* vegetables are widely promulgated for their rich source of bioactive compounds such as phenolics and glucosinolates[1,2]. The brassica vegetables, especially those belonging to the acephala group, became very popular around 2010 in the USA and slowly across worldwide due to their tolerance to temperature fluctuations[3]. However, in Kashmir, commonly named "Hak," it has historically been in the list of good foods and cultural culinary[4] An increasing number of reports from different parts of the world suggest Kale is highly nutritious and possesses health-promoting phytochemicals, including glucosinolates, carotenoids, vitamin C, and phenolic compounds[5,6,7.]

Glucosinolates are the health-promoting sulfur-containing compounds that are well known in the brassica family[8]. They are diverse such as glucobrassicin, glucoiberin, glucoraphanin, sulforaphane, and sinigrin, and are classified into aliphatic, aromatic, and indole glucosinolates[9,10]. Glucosinolates and their degradation products from kale have been associated with reduced risk of neurodegenerative disorders, cardiovascular diseases, various types of cancers, diabetes, respiratory diseases, and atherosclerosis both *in vitro* and *in vivo* ([11,12]). Well-known carotenoids are efficient quenchers of singlet oxygen, can scavenge free radicals and thus prevent the development of various degenerative diseases like diabetes and cancer[7]. One of the most important vitamins, Vitamin C can scavenge superoxide and hydroxyl radicals and act as a chain-breaking antioxidant in lipid peroxidatio[13,14].The phenolics are the most extensively documented metabolites with myriad health benefits, such as inhibiting low-density lipoprotein oxidation, anticarcinogenic activity, managing neurodegenerative diseases, atherosclerosis, and type -2 diabetes[15,16,17,]. In brassica plants, phenolics are considered to contribute the majority of biological activity 18. The predominant flavonoids that are present in the kale are quercetin and kaempferol[19]. The content of these polyphenols that eventually also determines its antioxidant capacity in the kale depends on maturity stage, variety, environmental factors, and, more importantly, the location where it is grown. Thus, we need data from different places where it is grown and consumed to comprehend its biological activity. A growing number of reports suggest that foods rich in such phytochemicals contribute to the management and prevention of chronic diseases by reducing reactive oxygen species (ROS), free radicals and modulating adaptive response pathways [20.]

Metabolomics is a vital approach employed in food and nutrition research to analyze various important metabolites [21]. The methodology describes various

methods for detecting different bioactive compounds such as glucosinolates, phenols, and flavonoids. For the detection of antioxidant activity under *in vitro* conditions, various methods have also been developed, including ORAC (Oxygen Radical Absorbance Capacity), FRAP (Fluorescence recovery after photobleaching), DPPH (2,2-diphenyl-1-picrylhydrazyl), etc. which have gained huge recognition globally. Sikora [22.] Also tested antioxidant activity in broccoli, kale, green and white cauliflower, Brussels sprouts. Among these, it has been discovered that kale had significantly higher antioxidant activity than other plants taken for study. Kale has also been demonstrated to get a preventive role upon this oxidation of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) in vitro, which may represent a preventive action towards an illness of cardiovascular disease[23]. These observations prompted us to pursue the metabolite level differences in various genotypes of Kale grown in Kashmir valley.
There are fewer or no comparative studies on the phytochemical profiles of 30-genotypes of kale genotypes grown in different parts of Kashmir. The current study attempted to characterize both the organic and aqueous extracts of the kale as it is mostly consumed in aqueous form. The work thus aimed to generate the metabolic profile of 30 different genotypes grown in Kashmir at different locations. Most of the genotypes displayed significant variance in phytochemical content when compared to each other, but the k-29 genotype underlined a significant richness in the phenolic and glucosinolate content that reflected its higher antioxidant potential as well in cell-free systems. Collectively, our data underscore the higher antioxidant potential of the k-29 genotype and other bioactive components that can be exploited to manage oxidative stress-related disorders.

Materials and Methods

Materials and Reagents

In the current study ethanol, methanol, sodium hydroxide, sodium carbonate, sodium phosphate (monobasic and dibasic), sodium chloride, TPTZ, sodium tetrachloropalladate were procured from Qualigens, (India) and Hi-Media Laboratories (India).

Collection of plant sample

In this study, 30 different genotypes of Kale (*Brassica oleracea* var *acephala*) were grown in Urban Technological Park, Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, Shalimar, Srinagar were investigated.

Extraction

All the genotypes were grown under identical environmental conditions, and leaf samples of each genotype were harvested at the edible stage, shade dried, powdered, and crushed to a fine powder. The extract (5-g) was prepared by using 80% methanol, 80% ethanol, and water .5-g of each Kale leaf sample was extracted in 75 mL of 80%mrethanol, 80%ethanol,distilled water.The mixture was homogenised and then filtered through Whatman filter paper 42. The extraction

was repeated thrice for each Kale leaf sample. The extracts were pooled and collected up to a final volume of 100 mL and stored in cryo vials at -20°C till further analysis.

Qualitative analysis of phytochemicals

The phytochemical screening of the aqueous extract of leaves of ten different *Brassica oleracea* var *acephala* genotypes was done using the following standardized protocols:

- Frothing test for Saponins
  For this, 1 mL of each aqueous extract of each Kale genotype was mixed with 2 mL of distilled water in a test tube. The test tube was plugged and shaken vigorously for 5 min. The shaken test tube was allowed to stand for 1 min. The appearance of honeycomb froth confirmed the presence of saponins in the extract.
- Salkowski's Test for Sterols
  To 2 mL of aqueous leaf extract, 2 mL chloroform and 2 mL concentrated H_2SO_4 were added. The presence of sterols in the test sample extract was indicated by the formation of a reddish-brown colored ring. Sterols in the presence of concentrated H_2SO_4 undergo a dehydration process producing the reddish-brown color.
- Liebermann's Test for Cardiac Glycosides
  2 mL of each aqueous crude extract was mixed with 2 mLof glacial acetic acid followed by the addition of 2 mLof chloroform. The mixture was allowed to stand for 1 min, and 1 mL of H_2SO_4 was added to each test tube. The presence of cardiac glycosides in the test leaf sample was indicated by the appearance of green color due to the reaction of acetic anhydride in chloroform with unsaturated glycosides in the presence of concentrated H_2SO_4.
- Salkowski's Test for Terpenoids
  To 100 µl of each leaf extract, 1ml of chloroform and 1mL of sulphuric acid were added. The formation of a reddish-brown precipitate indicated the presence of terpenoids in the test extract. Again, the dehydration reaction of terpenoids in the presence of concentrated H_2SO_4 gives out the reddish-brown color
- Mayer's test for Alkaloids
  To 2 mL of each test extract, 2 mL of hydrochloric acid was added, followed by a few drops of Mayer's reagent. The presence of alkaloids in the test solution was indicated by the presence of green color or white precipitate. Most of the alkaloids are precipitated from the aqueous extract by Mayer's reagent. Mayer's reagent test solution: Solution A: 1.36-g of Mercuric chloride was dissolved in 60 mL of water. Solution B: 5-g of Bi potassium iodide was dissolved in 10 mL of water. These two solutions (A and B) were mixed, and the final volume was made up to 100 mL with water.

Quantification of bioactive components

- Estimation of total Phenolics:

The total phenolic content of different Kale ecotypes (*Brassica oleraceae*) was determined by the modified method reported by Malick and Singh [24]. To determine the phenolic content, 0.1mL of each immediate kale extract was combined with water followed by the addition of 500µL Folin ciocalteau reagent (FCR) and incubated at for 3min at room temperature. After this, 2mL sodium carbonate (20%) was added to each, vortexed, and then kept in a boiling water bath for 1min. The absorbance was measured at 650nm for each sample against blank. A standard curve was drawn using catechol as standard and the concentration of phenol in each sample was determined accordingly. For each sample, three replicates were analyzed.

- Estimation of total flavonoids:
  Lalliansawna [24] modified method was used to determine the total flavonoid content of aqueous leaf extracts of ten different *Brassica oleracea* var *acephala* genotypes. 75 µl of 5% $NaNO_2$ solution was added to 1.5 mL of each aqueous leaf extract. This solution was incubated for 6 minutes following which 150 µL of 10% $AlCl_3.6H_2O$ was added to it and again incubated at room temperature for 6 minutes. 0.5 mL of 1M NaOH was added to each tube, and deionized water was used to make up the final volume of this solution to 2.5mL. This solution was mixed thoroughly and the absorbance was measured at 510 nm immediately against a reagent blank. Quercetin was used as a standard for the preparation of the standard calibration curve. The total flavonoid content of each sample was calculated and expressed as mg equivalents of quercetin (QE) per 100-g leaf sample.
- Estimation of total glucosinolate content:
  Total glucosinolate content of all the 30 genotypes was done according to the standardized protocol of Ibandalin and Mawlong25. 100-mg defatted each sample was extracted with 80% methanol. To 100 µl of this methanolic extract, 0.3 mL distilled water and 3 mL of 2 mM sodium tetrachloropalladate (58.8 mg of Sodium tetrachloropalladate and 170- µl concentrated HCl and 100 mL distilled water) was added, followed by incubation at room temperature for 60min. After this, the absorbance of each sample was measured at 425 nm against the reagent blank. A standard graph was established using sinigrin, and glucosinolate content was calculated accordingly.
- Determination of total antioxidant activity:
  Ferric reducing antioxidant (FRAP) assay as reported by Benzie and the strain (26) was used to determine the antioxidant activity in leaf extract of 30 different *Brassica oleracea* var *acephala* genotypes. 10 mM 2, 4, 6-tripyridyl -S-triazine (TPTZ), 40Mm HCL and 20mM ferric chloride prepared in 300 mM sodium acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v) are the constituents of FRAP reagent. 500-µl of each aqueous leaf extract was added to 3mL of FRAP reagent and mixed properly. The absorbance (0 min) was immediately recorded at 593 nm followed by incubation for 4 min at room temperature. Again, absorbance (4 mins) was recorded at 593 nm. Ammonium ferrous sulphate was used as a standard to plot a standard graph. This graph was used to determine the total antioxidant activity of each sample, expressed as µM FRAP g^{-1} of the sample on a dry weight basis.

Statistical analysis

All values were expressed as Mean ± S.E computed using GraphPad Prism 5 software. $p < 0.05$ in each experiment was considered as the reference for statistical significance. The statistical analysis was performed by one-way ANOVA that is followed by Tukey's test for multiple comparisons.

Results

Phytochemical Composition (Qualitative analysis)

Phytochemical screening was carried out from the leaves of *brassica oleracea var acephala*. Table 1 depicts the presence or absence of various phytochemicals such as alkaloids, sterols, terpenoids saponins and cardiac glycosides.

Table 1
Qualitative analysis of phytochemicals in leaves of 30 Kale genotypes

Genotypes	Alkaloids	sterols	Terpenoids	saponins	Cardiac glycosides
K1	−	+	+	+	+
K2	−	−	+	+	+
K3	+	−	−	+	+
K4	−	+	+	+	+
K5	−	+	+	+	+
K6	−	−	−	+	+
K7	+	−	_	−	+
K8	−	+	_	+	+
K9	−	+	+	+	+
K10	−	+	+	+	_
K11	+	+	_	+	_
K12	+	+	_	+	_
K13	_	+	+	+	_
K14	+	+	+	+	+
K15	_	+	+	+	+
K16	_	+	+	+	+
K17	+	+	+	+	+
K18	_	+	+	+	+
K19	+	+	+	+	+
K20	_	+	_	+	+
K21	+	+	+	+	+
K22	+	+	+	+	+
K23	_	+	+	+	+
K24	+	+	+	+	+
K25	_	+	+	+	+
K26	+	_	+	_	+
K27	+	_	_	_	_
K28	_	+	-	-	_
K29	+	+	+	+	+
K30	+	+	+	+	+

Phenotypic variation

The data generated from the current study revealed that 30 genotypes differed significantly from one another; and broadly classified them in to six groups viz., curly group (SH- K-1, SH- K-2, SH- K-3 and SH- K-4),ornamental group (SH- K-5, SH- K-6, SH- K-7, SH- K- 8, SH- K-9 and SH- K-10), perennial group (SH- K-11, SH- K-12, SH- K-13, SH- K-14, SH- K-15 and SH- K-16), collard group (SH- K-17, SH- K-18, SH- K-19, SHK-20, SH- K-21, Jumadari, SH- K-23, SH- K-24,SH- K-25, SH- K-35, SH-K-38 and SH- K-39) and tall group (Khanyari, SH- K-27, SH- K-28, SH- K-29,SH- K-30, Kawadari, SH- K-32, SH- K-33, SH- K-34, SH- K-36,SHK-37 and SH- K-40).

Bioactive compounds and correlation with glucosinolate content

As shown in table 1-3 the various bioactive compounds varied significantly with respect to each other ($p<0.0001$). The leaves of 30 genotypes were prepared by using 80% methanol, 80%ethanol and water. The total phenol content in methanolic extract ranged from 11.7-85.56-mg /gallic acid with_k-29 having higher content (Table 1, Fig 1a). The flavonoids ranged from 2.44 to 7.1 mg Quercetin/g DW with K29 genotype having the highest content (Table 1, Fig 1b). The total glucosinolate content also ranged from 20.17 µMol/g to 60.57 µMol/g and K29 genotype having highest content(Table 1, Fig 1c). The FRAP activity also ranged from 52.733µM FRAP/g DW to 92.56 µM FRAP/g DW (Table 1, Fig 1d).

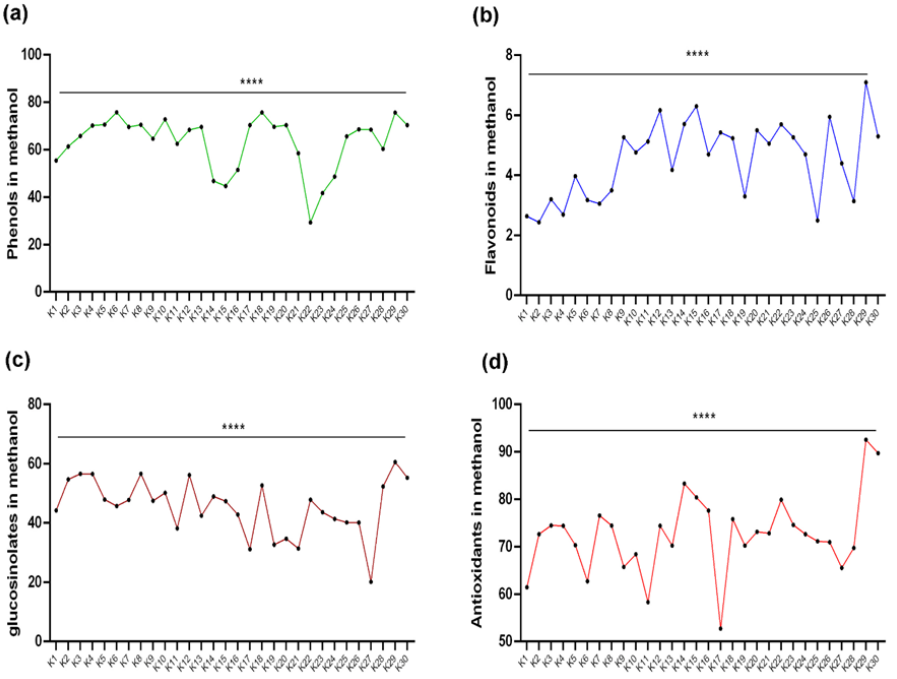

Fig 1. Representation of 30 genotypes for their comparative analysis of metabolites in methanolic extract ($p<0.0001$). a. Phenols in methanol b. Flavonoids in methanol c. Glucosinolates in methanol d. Antioxidants in methanol

Table 2
Comparisons of individual kale genotypes in methanol for total phenols, flavonoid, antioxidant activity, and total glucosinolate content. Values represent means± standard errors

Genotypes	Total phenols(mg Gallicac./g DW	Total flavonoids(mg Quercetin/g DW	Total antioxidant(µMol FRAP/g DW)	Total glucosinolates (µMol/g DW
K1	49.13±0.0	2.64±0.012	61.47±0.012	44.25±0.012
K2	41.59±0.012	2.44±0.012	72.64±0.0	54.69±0.0
K3	75.53±0.088	3.2±0.115	74.5±0.115	56.58±±0.017
K4	28.77±0.012	2.69±0.012	74.4±0.115	56.54±0.019
K5	34.95±0.012	3.97±0.012	70.3±0.117	47.91±0.0
K6	54.06±0.018	3.18±0.012	62.7±0.115	45.69±0.0
K7	69.96±0.015	3.05±0.012	76.6±0.116	47.797±0.012
K8	47.6±0.115	3.5±0.115	74.43±0.012	56.62±0.012
K9	43.4±0.115	5.27±0.012	65.73±0.0	47.467±0.016
K10	57.6±0.0	4.77±0.012	68.4±0.115	50.19±0.0
K11	68.7±0.114	5.13±0.012	58.3±0.115	38.17±0.013
K12	55.04±0.019	6.17±0.012	74.4±0.115	56.16±0.0
K13	85.28±0.0	4.193±0.007	70.25±0.012	42.46±0.017
K14	76.4±0.115	5.7±0.115	83.3±0.117	48.93±0.018
K15	51.3±0.115	6.3±0.115	80.4±0.115	47.39±0.012
K16	65.19±0.0	4.7±0.115	77.6±0.116	42.83±0.016
K17	62.59±0.044	5.43±0.011	52.73±0.012	31.17±0.012
K18	49.16±0.0	5.24±0.012	75.83±0.023	52.7±0.015
K19	28.37±0.016	3.3±0.115	70.247±0.0	32.67±0.013
K20	34.93±0.016	5.5±0.115	73.13±0.018	34.69±0.012
K21	52.35±0.0	5.06±0.012	62.833±0.011	31.39±0.012
K22	65.5±0.115	5.7±0.115	79.93±0.012	47.83±0.016
K23	11.7±0.115	5.27±0.012	74.62±0.0	43.64±0.012
K24	53.63±0.0	4.7±0.115	72.66±0.0	41.347±0.016
K25	44.7±0.115	2.5±0.115	71.16±0.0	40.22±0.012
K26	38.4±0.115	5.94±0.012	70.95±0.0	40.14±0.012
K27	51.6±0.115	4.4±0.115	65.53±0.0	20.17±0.012
K28	61.4±0.114	3.14±0.012	69.75±0.012	52.35±0.0
K29	85.56±0.0	7.1±0.115	92.56±0.019	60.57±0.019
K30	79.3±0.117	5.3±0.115	89.73±0.0	55.29±0.019

The total phenol content in ethanolic extract ranged from 19.64-78.56 mg/ gallic acid with K29 genotype having higher content (Table 2, Fig 2a). The flavonoids ranged from 12.05-16.64mg Quercetin/gDW with K30 genotype having highest content (Table 2, Fig 2b). The total glucosinolate content also ranged from in 15.60 µMol/g to 58.457 µMol/g in K29 genotype possessing highest content (Table 2, Fig 2c). The FRAP activity also ranged from 35.36µM FRAP/g DW to 75.64µM FRAP/g DW (Table 2, Fig 2d).

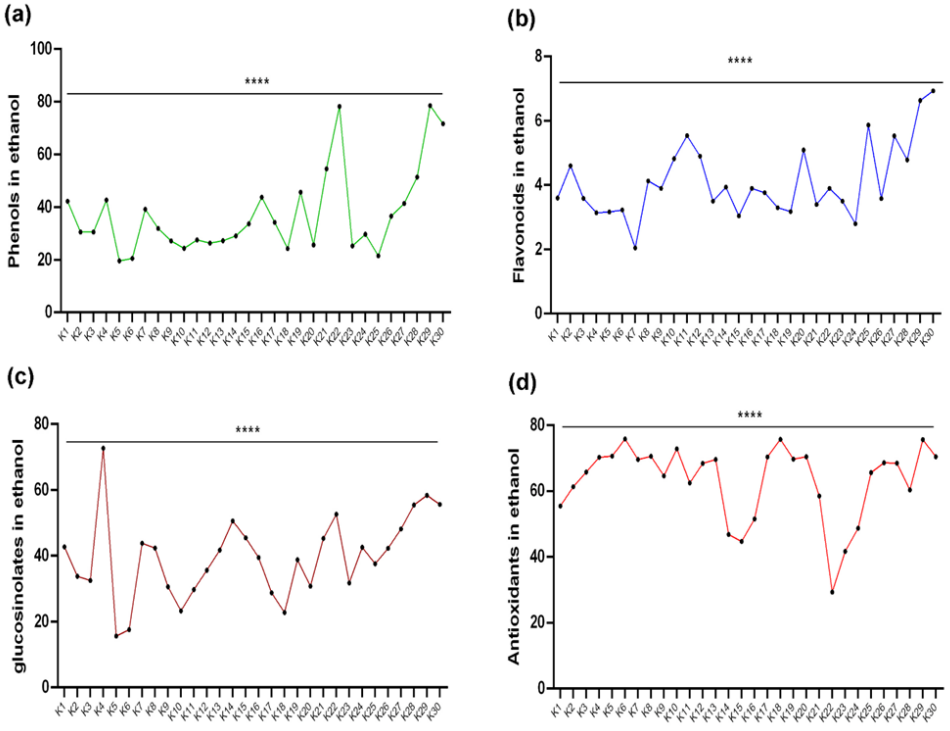

Fig 2. Representation of 30 genotypes for their comparative analysis of metabolites in ethanolic extract ($p<0.0001$). a. Phenols in ethanol b. Flavonoids in ethanol c. Glucosinolates in ethanol d. Antioxidants in ethanol.

Table 3
Comparisons of individual kale genotypes in ethanol for total phenols, flavonoids, antioxidant activity and total glucosinolate content. Values represent means± standard errors

Genotypes	Total phenols(mg Gallic ac./g DW	Total flavonoids(mg Quercetin/g DW	Total antioxidant(µMol FRAP/g DW)	Total glucosinolates (µMol/g DW
K1	42.25±0.012	13.6±0.116	61.47±0.012	42.7±0.115
K2	30.57±0.012	14.6±0.116	55.34±0.012	33.8±0.058
K3	30.53±0.013	13.59±0.011	51.77±0.012	32.5±0.115
K4	42.7±0.115	13.14±0.012	70.24±0.018	72.7±0.114
K5	19.64±0.010	13.17±0.012	43.64±0.012	15.603±0.205
K6	20.56±0.012	13.23±0.012	45.8±0.115	17.6±0.115
K7	39.19±0.012	12.05±0.011	69.6±0.116	43.8±0.058
K8	31.95±0.013	14.14±0.012	68.54±0.0	42.34±0.012
K9	27.15±0.012	13.57±0.237	62.64±0.012	30.6±0.115
K10	24.36±0.011	14.83±0.012	55.13±2.434	23.3±0.115
K11	27.57±0.012	15.54±0.011	68.5±0.115	29.77±0.014
K12	26.31±0.012	14.57±0.237	62.4±0.114	35.6±0.115
K13	27.24±0.012	13.5±0.115	69.58±0.022	41.77±0.012
K14	29.07±0.012	13.94±0.012	74.8±0.117	50.6±0.115

K15	33.67±0.013	13.04±0.011	44.7±0.115	45.5±0.115
K16	43.76±0.014	13.57±0.237	41.5±0.115	39.5±0.115
K17	34.25±0.012	13.77±0.011	35.36±0.012	28.77±0.114
K18	24.28±0.013	13.3±0.115	31.7±0.116	22.8±0.057
K19	45.69±0.0	13.18±0.011	59.7±0.115	38.8±0.058
K20	25.69±0.011	15.13±0.091	51.4±0.114	30.8±0.057
K21	54.58±0.016	13.4±0.116	58.56±0.067	45.3±0.115
K22	78.26±0.0	13.57±0.237	69.3±0.117	52.6±0.115
K23	25.3±0.115	13.5±0.115	41.7±0.115	31.8±0.057
K24	29.67±0.012	12.8±0.115	48.7±0.115	42.6±0.057
K25	21.5±0.115	15.87±0.012	45.6±0.115	37.6±0.115
K26	36.6±0.115	13.59±0.011	68.6±0.116	42.3±0.115
K27	41.4±0.115	15.53±0.012	68.5±0.115	48.14±0.012
K28	51.4±0.114	14.79±0.011	54.36±0.012	55.47±0.012
K29	78.56±0.0	16.64±0.011	75.64±0.0	58.4±0.114
K30	71.7±0.114	16.94±0.011	70.4±0.115	55.6±0.115

Total phenol content in aqueous extract ranged from 28.22-71.68 mg/ gallic with K29 genotype having higher content (Table 3, Fig 3a). The flavonoids ranged from 2.05-7.5mg Quercetin/g DW with K30 genotype having highest content (Table 3, Fig 3b). The total glucosinolate content also ranged from in 15.60 µMol/g to 58.457 µMol/g in K29 genotype possessing highest content (Table 3, Fig 3c). The FRAP activity also ranged from 51.13µM FRAP/g DW to 85.5 µM FRAP/g DW (Table 3, Fig 3d).

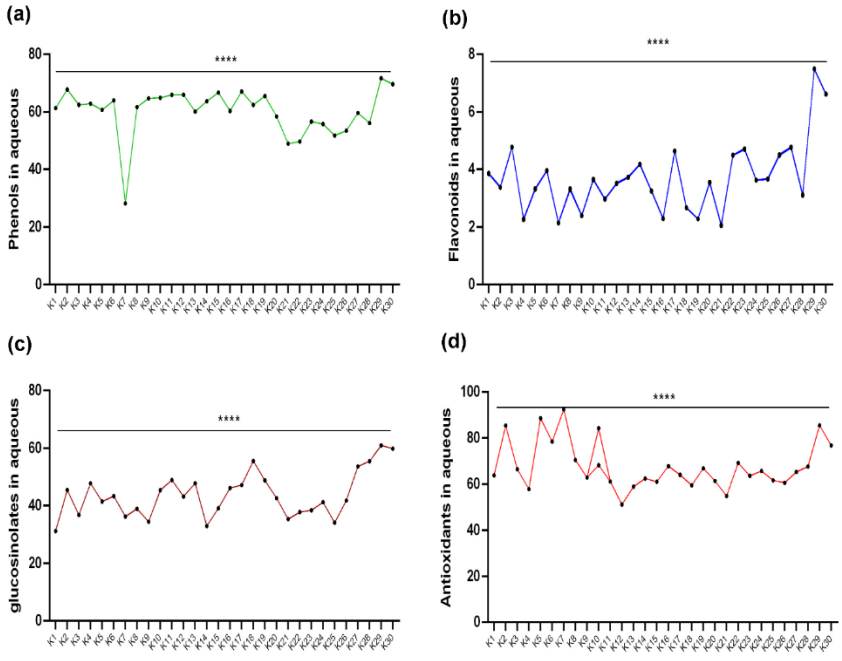

Fig 3. Representation of 30 genotypes for their comparative analysis of metabolites in aqueous extract ($p<0.0001$). a. Phenols in aqueous b. Flavonoids in aqueous c. Glucosinolates in aqueous d. Antioxidants in aqueous

Table 4
Comparisons of individual kale genotypes in aqueous for total phenols, flavonoids, antioxidant activity, and total glucosinolate content. Values represent means ± standard errors

	Total phenols(mg Gallic ac./g DW	Total flavonoids(mg Quercetin/g DW	Total antioxidant(µMol FRAP/g DW)	Total glucosinolates (µmol/g DW
K1	61.35±0.0	3.86±0.012	63.84±0.012	31.25±0.012
K2	67.78±0.0	3.39±0.012	85.44±0.0	45.47±0.012
K3	62.45±0.012	4.78±0.012	66.47±0.0	36.78±0.012
K4	62.89±0.0120.012	2.27±0.007	57.84±0.012	37.8±0.115
K5	60.69±0.0	3.33±0.007	88.6±0.116	43.47±0.012
K6	63.98±0.677	3.96±0.007	78.47±0.0	41.36±0.012
K7	28.22±0.012	2.15±0.007	82.47±0.0	46.25±0.012
K8	61.69±0.0	3.32±0.01	70.48±0.0	38.97±0.012
K9	64.71±0.017	2.4±0.067	62.88±0.0	34.46±0.016
K10	64.91±0.0	3.65±0.007	78.93±0.012	45.38±0.0
K11	65.91±0.0	2.97±0.007	61.11±0.012	41.98±0.012
K12	65.98±0.0	3.52±0.003	51.13±0.0	40.25±0.012
K13	60.14±0.012	3.73±0.012	58.93±0.019	45.79±0.018
K14	63.69±0.0	4.18±0.012	62.46±0.019	46.98±0.012
K15	66.74±0.017	3.25±0.012	61.05±0.012	39.14±0.012
K16	60.29±0.019	2.3±0.115	67.83±0.022	46.16±0.017
K17	67.1±0.016	4.64±0.012	64.05±0.021	45.18±0.017
K18	62.42±0.015	2.68±0.012	59.48±0.012	41.58±0.015
K19	65.52±0.017	2.28±0.012	66.85±0.017	48.39±0.012
K20	58.45±0.012	3.55±0.012	61.43±0.019	42.67±0.007
K21	48.98±0.012	2.06±0.012	54.84±0.012	35.35±0.115
K22	49.69±0.0	4.5±0.115	69.2±0.114	37.8±0.115
K23	56.65±0.017	4.71±0.012	63.61±0.012	36.45±0.012
K24	55.76±0.014	3.63±0.012	65.72±0.0	41.25±0.012
K25	51.82±0.018	3.67±0.012	61.65±0.017	34.15±0.115
K26	53.47±0.012	4.5±0.115	60.64±0.012	31.8±0.115
K27	59.67±0.014	4.77±0.012	65.29±0.0	53.67±0.014
K28	56.12±0.012	3.12±0.012	67.66±0.0	55.46±0.019
K29	71.68±0.012	7.5±0.115	85.5±0.115	60.98±0.012
K30	69.65±0.0	6.62±0.012	76.85±0.018	59.87±0.012

Discussion

Vegetables are an essential part of the human diet globally that play a primary role in nutrition due to numerous phytonutrients, including vitamins, minerals, nutrients, nutritional fiber, and phytochemicals [27,28]. The phytochemicals present in vegetables are robust antioxidants and are considered to decrease the danger of persistent disease by directly acting as the free radicle scavengers in the target cells besides modulating the vital adaptive response pathways[17]. Among the green leafy vegetables, cruciferous vegetables including Kale, cabbage, broccoli, and brussels sprouts are rich in various bioactive phytochemicals and vitamins. Kale also possesses a high fiber, protein, sulfur, and calcium content compared to

broccoli. Kale is grown in various world regions and has been reported to be a rich source of different nutrients, phytochemicals, and bioactive compounds[29] .As far as J&K is concerned, kale is a unique crop that supplements the nutritional needs of the local population in the Kashmir valley, both during the summer and winter months, thus making this vegetable as one of the most widely grown crops in the area. As far as the kale genotypes grown in the Kashmir Valley are concerned, very scant literature is available on their chemical composition. As such, these genotypes have unfortunately remained highly unexplored. Therefore, immediate steps need to be taken to generate a way to minimize the lessening of its biodiversity and availability. In this direction, the current investigation was undertaken to evaluate the nutraceutical, photochemical, antioxidant, and antidiabetic potential of different kale genotypes grown in Kashmir valley for their better use.

The data in this study, upon analysis for various metabolites, revealed that most genotypes have a difference in the contents of different phytochemicals ($p < 0.0001$). These metabolites included glucosinolates, phenols, and flavonoids in different solvent systems relevant to consumption modes. The nutraceutical importance in plant-based compounds is attributed to the presence of higher content of phenols and flavonoids [30]. Plant-based foods are usually rich sources of phenols, are widely distributed, and are associated with their antioxidant and nutritional properties. These phenols play an important role in antiseptic, anti-cancerous, antibacterial, and antidiabetic activity .Besides it, they are highly desired for antioxidant activities [31]. The total phenol content was seen highest in methanolic fraction in k-29 with 71.68 mg/gallic acid. Agarwal[32] also reported phenolic content in Kale genotypes and thus corroborate well with the current study. The analyzed genotypes, especially Khanyari, can act as a great source of natural antioxidants in the form of phenols to cope up with various oxidative stress-related diseases. Flavonoids are also reported for antioxidant effects that render cells from oxidative damage[33]. Therefore, keeping in view the diverse role of flavonoids, in the current investigation, the leaf samples of 30 genotypes of Kale evaluated for their total flavonoid content was reported highest in aqueous extract of k-29 with concentration of 85.5m g/100g DW of quercetin (Table 3, Fig 3b). Glucosinolates and their degradation products from kale have been associated with reduced risk of different diseases (e, f, repeated references). The total glucosinolate content was seen highest in aqueous extract of k-29 with 60.98(µMol/g DW (Table 3, Fig 3c).

Phenolic compounds in the plant kingdom are associated with their redox properties, which allow them to act directly as singlet oxygen scavengers and reducing agents [34]. This was in accordance with the results observed in the FRAP activity of different genotypes of Kale in different mediums. The data indicated significant variation among the selected genotypes that ranged from 52.73µM FRAP/g DW to 92.56 µM FRAP/g DW. Among the 30 Kale genotypes analyzed in the current study, Khanyari demonstrated the highest antioxidant activity (92.56µM FRAP/g) and thus represented the best antioxidant source as dietary kale grown in the Kashmir valley. The antioxidant capacity of these plant base compounds depends on the number and arrangement of -hydroxyl groups and activates antioxidative systems[35]. The other studies also point to the content of phenols and flavonoids determines the fate of antioxidant capacity. Podsek[36]

reported that among cruciferous vegetables, Kale and other species, which mainly consist of *Brassica oleracea* cultivars and some cultivars of *Brassica napus*, have the maximum antioxidant potential due to the presence of a good amount of antioxidants, especially carotenoids and xanthophylls content like zeaxanthin and lutein[37].

Although we obtain the bulk of antioxidants mainly from food, substantial damage still occurs daily. Thus for the protection of such cellular damage and other oxidative stress-related disorders, the development of nutraceuticals is of prime importance. To our knowledge, it is the first report to highlight the comparison of various metabolites in 30-genotypes of kale that are grown in Kashmir at different places. The k-29 genotype indicated the presence of a higher amount of compounds of nutraceutical importance. This is attributed to lower temperatures and high light intensity that induces higher antioxidant contents [38]. Although this in part explains the higher content of phenols, flavonoids, and antioxidative capacity of the k-29 genotype, the data on the genetics of these genotypes and the effect of different environmental conditions are minimal and warrants further studies.

Conclusion

This is the first study of a kind that reports the comparison of 30 genotypes kale in their phenol, flavonoid, glucosinolates, and antioxidant potential in cell-free systems. The majority of the genotypes displayed a significant difference in the metabolite content. The metabolite content of phenols and flavonoids in the K-29 genotype known as "Khanyar hak" was higher, contributing to its higher antioxidative potential. Our results suggest that the environment might have played a role in the higher concentration of metabolites in the K-29 genotype

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