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Evaluation of nutraceutical properties of finger millet genotypes from mid hills of northwestern Himalayan region of India

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Finger millet *Eleusine coracana* L., commonly called Ragi, is a rich source of phytochemicals and have number of health beneficial effects. The present study evaluated the total antioxidant activity (TAA), condensed tannins (CT), micronutrient content (Fe & Zn), diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, ferric reducing antioxidant power (FRAP) and phenolic compounds in 35 finger millet genotypes. The assayed genotypes showed 0.91-0.99 mg/g CT, 23.79-56.51 mM/kg TAA, 1.76-44.47 µM/g DPPH scavenging activity, 44.14-88.09 µg/mL ABTS activity, 100-463.53 µM FRAP value, 37.04-69.13 ppm Fe and 28.94-46.77 ppm Zn. HPLC analysis showed that gallic, tannic, ferulic, caffeic and o-coumaric acid to be major polyphenols in all genotypes. Principal component analysis (PCA) revealed significantly higher CT, TAA with relatively good amount of Fe and Zn in VL *Ragi* 146, VL *Mandua* 352, VL 336, VL 373, VL 325, VL 351, GPHCPB 7, GPHCPB 3, GPHCPB 52 and VR 708 genotypes. Agglomerative hierarchical cluster analysis classified the 35 genotypes into two clusters; Cluster I had higher CT, TAA, FRAP, DPPH, ABTS, while cluster II recorded higher Fe and Zn. This study clearly demonstrated the nutraceutical properties with higher antioxidant potential of identified genotypes, which can be suitably deployed for nutritional security, particularly in developing countries.

Keywords: ABTS, Condensed tannins, DPPH, *Eleusine coracana*, FRAP, nutritional security, Polyphenols, Principal component analysis (PCA), Ragi, Total antioxidant activity (TAA)

Finger millet (*Eleusine coracana* L.), commonly known as 'Ragi', is one of the important cereal (minor millets) of the Indian subcontinent[1]. It is a rich source of several phytochemicals, dietary fiber, polyphenols, vitamins and minerals[2] and offers several health benefits to its consumers[3]. It plays an important role in food and nutritional security in many developing countries because of its ability to grow under adverse climatic conditions[4]. Grains are highly nutritious and can be stored for several years without any damage[5]. Finger millet grains contain various phenolic compounds (bioactive secondary plant metabolites) including tannins[6], polyphenols and flavonoids that contribute to diverse physiological properties such as antimicrobial, antioxidant, anti-inflammatory, antitumor and anticarcinogenic[7]. These beneficial phytochemicals may significantly differ in different cultivars of the same plant[8]. Further, several phenolic compounds reported in fruits and vegetables have also been observed in millets[9].

Antioxidants are the substances that significantly delay or prevent the oxidation of the substrate at low concentrations as compared with other oxidizable substrate[10]. Researchers have shown bioactive compounds from plants and food materials with strong antioxidant activity to reduce the free radical production that causes cell damage in the biological process[11]. Similarly, genes responsible for various biotic and abiotic stresses have also been successfully identified and cDNA libraries have been characterized in millets[12].

Reactive oxygen species (ROS) are known to induce cellular damage and involved in several human diseases including cancer, arteriosclerosis, diabetic mellitus, hypertension and AIDS (Acquired immuno deficiency syndrome) and in aging processes[13]. Daily consumption of antioxidant substances results in effective action in various ways, including complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides, etc.[14]. Natural antioxidants or phenolic antioxidants may also protect DNA, protein, and membrane lipids from oxidative damage in biological systems, provide additional health benefits for

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disease prevention and may help to lower down the adverse effects related to aging[10].

Finger millets are reported to contain proanthocyanidins, which are also known as condensed tannins[15]. Proanthocyanidins are high-molecular weight polyphenols that consist of polymerized flavan-3-ol and/or flavan-3, 4-diol units. It has been observed that condensed tannins are more potent antioxidants than their corresponding monomers[16]. Several *in vivo* assays have demonstrated their anti-inflammatory, antiviral, antibacterial and antioxidant properties[15]. Chandrasekara and Shahidi have reported over 50 phenolic compounds from several whole millet grains, such as foxtail millet, proso millet, little millet and pearl millet using HPLC[9]. However, work on active compounds, antioxidant activity and the major phenolic acids present in finger millet genotypes of hill region are limited. Therefore in this study, we evaluated production of active compounds with antioxidant activity and the comparison of DPPH, FRAP and ABTS activities along with mineral nutrient analysis of methanolic extracts in the grains of finger millet.

Materials and Methods

Field-grown finger millet samples (n=35) comprising of advanced breeding lines of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora,, local collections from Uttarakhand hills and state as well as national released varieties were used in this study for nutritional analyses at quality laboratory, ICAR-VPKAS, Almora. Condensed tannins and total antioxidant properties were determined in finely threshed, cleaned and air dried powdered finger millet grain samples. Antioxidant activity was further evaluated by measuring DPPH activity, FRAP value and ABTS activity.

Sample preparation

Samples were extracted by a slight modification of the method of Rehman[17] and Demiray *et al*.[18] using aqueous methanol. The sample was homogenized in pestle and mortar at 32°C±5 with aqueous methanol (methanol: water, 70:30 v/v). Extracts were centrifuged at 4000 rpm for 30 min and the residues were re-extracted under the same conditions. Supernatants were pooled and combined and evaporated with a rotary evaporator. Extracts were stored at 4°C for biochemical studies. To maintain the quality of the data, all the experiments were carried out in triplicates and were validated by using standards wherever required.

Condensed tannins (CT)

The Vanillin-HCl method of Price *et al*.[19] was used to measure condensed tannin content. The extract and the vanillin reagent [4% HCl in methanol and 0.5% (w/v) vanillin in methanol] were maintained at 30°C in thermostat controlled water bath. Sample extract (1 mL) were mixed with 5 mL vanillin reagent in test tubes and then maintained at 30°C in the water bath for 20 min. Absorbance at 500 nm was measured. Catechin was used as the standard.

Total antioxidant activity (TAA)

Total antioxidant activity was estimated using the method of Prieto *et al*.[20] with slight modification. 1.23 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 20 µL of the extract and the contents were incubated at 90°C for 90 min, cooled to ambient temperature and the absorbance was measured at 695 nm. The antioxidant capacity was expressed as trolox (mM / 100 g of extract) equivalent.

Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

DPPH assay was used for determination of free radical scavenging of extracts according to the method of Chang *et al*.[21]. The scavenging effects on DPPH radicals were determined measuring the decrease in absorbance at 517 nm due to the DPPH radical reduction, indicating the antioxidant activity of the compounds in a short time. 10 µL of sample (5 mg/mL) was mixed with 90 µL of 50 mM Tris–HCl buffer (pH 7.4) and 200 µL of 0.1 mM DPPH-ethanol solution. When DPPH reacts with an antioxidant, that can donate hydrogen, it gets reduced and the resulting decrease in absorbance at 517 nm was recorded using a UV-Vis spectrophotometer (Thermo-Scientific, UV-Vis spectrophotometer). Trolox was used as a positive control. The results were expressed as trolox equivalents (µM per gm dw).

Ferric reducing activity power (FRAP) assay

The FRAP assay was carried out according to Stratil *et al*.[22] with slight modification using freshly prepared FRAP reagent. The 200 µL of methanolic extract of each sample was mixed in to 1.3 mL of the FRAP reagent. The tubes were vortexed and left at 37°C for 40 min, and the absorbance was measured at 595 nm. The absorbance changes in the text mixture were compared to those obtained from standard

mixture of trolox equivalent (0.1-1.0 µM/L). FRAP values were expressed as µM trolox equivalents per gram.

2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay

The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cation was compared to trolox standard[23]. A stock solution of ABTS radicals was prepared by mixing 5.0 mL of 7 mM ABTS solution with 88 µL of 140 mM potassium persulfate, and kept in the dark at room temperature for 12-14 h. An aliquot of stock solution was diluted with phosphate buffer (5 mM, pH 7.4) containing 0.15 M NaCl in order to prepare the working solution of ABTS radicals to an absorbance of 0.70±0.02 at 734 nm. A 65 µL aliquot of sample solution was mixed with 910 µL of ABTS radical working solution, incubated for 10 min at room temperature in the dark, and then absorbance was measured at 734 nm. The percent reduction of $ABTS^{+}$ to ABTS was calculated according to the following equation[24]:

$$\text{ABTS (\%)} = 1 - (\text{absorbance of sample/absorbance of control}) \times 100$$

Extraction of phenolic acids for HPLC analysis from *Finger millet*

One gram of dried, water soaked grains of finger millet was macerated separately in a pestle-mortar and finely grinded samples were suspended in 5 mL of methanol (HPLC grade) water (80:20; v/v). These samples were collected in screw-capped tubes and the suspension was subjected to centrifugation at 7500 rpm for 15 min. Supernatant was collected for HPLC analysis.

HPLC analysis

The methanolic extracts of the samples were filtered using pore size 0.45 µm, Millipore filters. 20 µL of the samples were injected into a loop injection valve of HPLC (Waters HPLC system) equipped with Photodiaode detector and analog pump connected to controller. Running conditions included mobile phase methanol-0.4 % acetic acid (80:20, v/v), flow rate 1.0 mL/min, injection volume 5 µL and detection at 290 nm. Tannic (TA), gallic (GA), protocatechuic (P-cat-A), caffeic (Caf-A), vanillic (VA), ferulic (FA), o-coumaric (o-Cou-A), cinnamic acid (CA) and chlorogenic (Chl-A) were used as internal and external standards. Phenolic compounds present in the sample were identified by comparing retention time (Rt) of standards, e.g., TA (Rt. 2.76 min), GA (Rt. 2.86 min), P-cat-A (3.04 min), Caf-A (Rt. 3.10 min), VA (Rt. 3.26 min), FA (Rt. 3.42 min), o-Cou-A (Rt. 3.58 min), Chl-A (Rt. 4.16 min) and CA (Rt. 4.45 min). The HPLC of samples was run at 290 nm using a reverse phase C-18 column. During the run, a flow rate of 1 mL/min was maintained using isocratic mode for 10 min.

For quantitative determination of various peaks, the integration area values of different standard polyphenols with known concentration were compared with the sample peaks and the polyphenol content was calculated accordingly.

Statistical analysis

The statistical analyses were performed using Statistical Analysis System (SAS) JMP software version 9.0. The analysis of CT, TAA, DPPH scavenging capacity, FRAP, ABTS, Fe and Zn of each finger millet genotype was based on two replications and the results are expressed as mean values ± standard error (SE). Significance of each group was tested with one-way analysis of variance followed by Duncan's multiple range test (P <0.05). For multi-factorial comparison, principal component analysis (PCA) and two way agglomerative hierarchical clustering (AHC) were used to display the correlations between the various antioxidant parameters and nutrients *viz.* Fe and Zn of 35 finger millet genotypes.

Result and Discussion

Analysis of variance (ANOVA) revealed significant variation among the genotypes for the studied antioxidant parameters and nutrients parameters. Finger millet genotypes showed 0.90-0.99/100 mg genotype catechin equivalents of condensed tannins and the genotype VL 336 showed significantly higher amount of condensed tannins (0.99/100 mg catechin equivalents) (Table 1). Siwela[25] also reported 0.60-1.32 mg catechin equivalents/100 mg of condensed tannins in finger millet genotypes.

In order to determine the relationship between the level of condensed tannins and free radical scavenging activity, we have estimated the total antioxidant activity from the methanolic extract. Among genotypes, the higher total antioxidant activity (Table 1) of the methanolic extract was observed in the genotypes VL 373 (56.51 mM/g) followed by GPHCPB 7 (56.48 mM/g), VL 370 (56.42 mM/g) and HR 374 (56.42 mM/g). Similar results were also reported by Banerjee *et al*.[26] in

Table 1 — Major phenolic acid identified in finger millet genotypes through HPLC

Genotypes	Tannic acid	Gallic acid	Caffeic acid	Vanillic acid	Coumaric acid	Ferulic Acid	Chlorogenic acid
VL 146	7.16	18.06	0.21	1.43	0.10	-	0.17
VL 149	5.02	15.69	-	1.83	0.11	-	-
VL 201	4.00	13.38	-	-	0.15	-	-
VL 204	4.00	12.31	-	-	0.11	-	0.64
VL 283	4.30	15.23	-	-	0.14	-	-
VL 305	4.03	13.39	-	-	0.05	-	-
VL 312	4.52	12.44	-	-	0.11	0.04	0.88
VL 315	3.96	13.97	-	-	0.08	-	-
VL 324	4.10	12.99	-	-	0.07	-	-
VL 325	3.90	12.23	0.47	-	0.09	-	-
VL 328	3.73	12.96	-	-	0.10	-	-
VL 330	4.99		-	-	0.07	-	-
VL 332	-	13.29	-	-	0.12	-	-
VL 333	5.14	14.41	-	-	0.10	-	-
VL 336	4.83		-	-	0.12	-	-
VL 340	4.80	15.54	0.09	-	0.08	-	-
VL 342	-	13.57	-	-	0.08	-	0.75
VL 348	4.06		-	-	0.04	-	-
VL 351	4.56	13.66	0.18	-	0.10	-	-
VL 352	4.89	67.65	-	1.27	0.08	-	-
VL 357	4.26	13.19	-	-	0.12	-	-
VL 361	4.75	14.36	-	1.16	0.06	-	0.80
VL 367	4.96	12.08	-	1.12	0.06	-	-
VL 369	-	-	-	-	0.10	-	0.89
VL 370	5.10	13.54	-	-	0.10	-	-
VL 373	4.84	16.38	-	1.06	0.15	-	-
VL 374	5.50	11.91	-	1.95	0.09	-	-
GE 86	4.80	14.69	-	-	0.14	-	-
GE 440	4.49	14.30	-	-	0.07	-	-
GPHCPB 7	4.45	13.68	-	-	0.07	-	-
GPHCPB 3	3.98	9.08	0.09	-	0.08	-	-
GPHCPB 52	4.33	13.70	-	-	0.07	-	-
GPU 45	4.40	12.12	-	-	0.08	-	-
HR3 74	-	13.69	0.15	-	0.12	-	-
VR 708	5.00	11.50	0.07	-	0.05	-	-

[Values are expressed as µg/g. – Not detected]

finger millet genotypes. They reported that the polar solvent had the higher antioxidant activities.

In this study, the range of antioxidant activity (23.79-56.58 mM/g) exhibited by the finger millet genotypes was higher and comparable to maize and wheat[27], and such higher activity by the methanolic extracts may be due to the presence of gallic acid, ferulic acid, hydroxybenzoic acid and their derivatives[28].

HPLC analysis showed that the genotypes VL 325, VL 340, VL 351 and VR 708 possess considerable amount of all studied phenolic acids other than ferulic acid (Table 2). The genotype VL *Mandua* 352 showed significantly higher content of gallic acid (67.65 µg/g). It was found that tannic acid, gallic acid and o-coumaric acids are the most common phenolics present in almost all the genotypes other than VL 348, VL 369 and VL 342. Other phenolic acids such as caffeic acid, ferulic acid, chlorogenic acid, protocatechuic acid and vanillic acid were also present in some genotypes in detectable amount. Among all the studied phenolics the gallic acid was observed in higher quantity mostly in all genotypes. In this study, since most of the finger millet genotypes contained gallic acid, which is highly anti-inflammatory in high amount, it is advisable for its daily consumption to those suffering from joint pains or inflammation of the body. Ferulic acid was detected only in the genotypes VL *Mandua* 347 and VL 312. However, McDonough and Rooney[29] have reported ferulic, *p*-coumaric and cinnamic acids as the major phenolics in finger millet. Ferulic acid and coumaric acid in cereals are known to express high antioxidant activity. Few peaks remained unidentified in all the chromatograms, which could be flavonoids,

Table 2 — Antioxidant activity and mineral content in thirty five finger millet genotypes

Genotypes	CT	TAA	DPPH	ABTS	FRAP	Fe	Zn
VL 146	0.97± 2.10^{ab}	37.02± 0.55^{d}	22.20±1.20^{fg}	81.70±1.38^{ed}	232.17±30.8^{mno}	48.71±0.60^{fghijk}	28.94±1.18^{no}
VL 149	0.91±1.01^{bc}	31.22± 0.40^{efgh}	8.14±0.58^{klm}	65.39±1.18^{l}	299.02±20.12^{ghij}	58.18±3.20^{bcd}	32.19±1.76^{jklmn}
VL201	0.95±3.51^{abc}	34.53±3.04^{ed}	1.76±1.02^{n}	44.14±0.99^{q}	190.09±1.09^{pqr}	61.36±1.04^{b}	31.35±0.68^{klmn}
VL 204	0.94±0.50^{abc}	42.09± 3.16^{bc}	16.08±1.75^{hi}	84.76±0.99^{c}	100.54±2.41^{s}	49.84±0.39^{efghijk}	43.33±3.22^{ab}
VL 283	0.95±2.38^{abc}	32.11±0.06^{efg}	15.77±1.01^{hi}	69.14±0.37^{ij}	188.97±1.48^{pqr}	52.98±0.94^{cdefg}	35.11±1.01^{ghijk}
VL 305	0.92±0.45^{abc}	26.74± 0.03^{hi}	14.15±1.63^{ij}	66.55±0.57^{kl}	165.84±15.06^{qr}	54.91±0.93^{bcdefg}	38.47±1.15^{cdefg}
VL 312	0.92±0.46^{abc}	29.29± 2.39^{fgh}	4.79±2.19^{mn}	59.68±0.42^{no}	285.47±1.64^{hijkl}	43.56±3.22^{klmno}	32.99±1.21^{ijklmn}
VL 315	0.96±3.32^{abc}	31.22± 0.40^{efgh}	9.75±1.02^{jklm}	57.60±0.59^{o}	259.95±29.78^{jklm}	37.94±0.95^{no}	26.41±1.30^{o}
VL 324	0.91±0.95^{bc}	26.65± 0.12^{hi}	6.70±2.07l^{mn}	54.97±0.82^{p}	329.96±8.29^{efgh}	45.05±1.06^{ijklm}	31.07±1.24^{klmn}
VL 325	0.94±1.71^{abc}	37.70±0.12^{cd}	31.06±2.38^{bcd}	88.00±0.01^{ab}	332.57±30.69^{defg}	59.90±2.30^{bc}	35.75±1.53^{fghij}
VL 328	0.92±0.45^{abc}	23.79±4.69^{i}	23.55±2.99^{efg}	82.44±1.45^{d}	152.81±3.08^{r}	55.16±0.17^{bcdef}	34.91±2.02^{ghijkl}
VL 330	0.95±2.98^{abc}	45.68±0.12^{h}	11.19±0.41^{ijkl}	61.38±0.40^{mn}	306.59±5.92^{ghi}	52.07±0.74^{defghi}	29.98±0.14^{mno}
VL 332	0.92±0.45^{abc}	38.06±0.06^{cd}	15.45±0.79^{hi}	86.12±0.16^{abc}	307.34±6.65^{ghi}	46.88±1.88^{hijkl}	40.90±0.58^{bcd}
VL 333	0.94±1.98^{abc}	45.71±0.09^{h}	22.72±1.05^{fg}	79.75±0.41^{ef}	258.76±8.90^{klm}	56.14±0.16^{bcde}	30.84±0.17l^{mn}
VL 336	0.99±0.21^{a}	45.68±0.12^{h}	22.36±0.38^{fg}	86.65±0.36^{abc}	297.57±7.59^{ghijk}	45.21±1.33^{ijklm}	30.80±0.91l^{mn}
VL 340	0.95±3.52^{abc}	27.26±0.06^{ghi}	5.74±0.76l^{mn}	60.28±0.49^{n}	220.99±11.01^{nop}	56.65±2.67^{bcde}	46.77±1.00^{a}
VL 342	0.97±1.75^{ab}	32.22±4.93^{ef}	27.34±1.35^{cdef}	85.69±1.01^{bc}	308.91±9.49^{fghi}	59.90±0.69^{bc}	32.88±0.24^{ijklmn}
VL 348	0.90±2.02^{c}	27.87± 1.11^{fghi}	15.62±0.49^{fhi}	71.43±1.28^{h}	319.35±1.26^{fghi}	51.33±0.71^{defghij}	31.28±0.85^{klmn}
VL 351	0.95±2.82^{abc}	27.44± 0.68^{fghi}	16.43±0.75^{hi}	68.51±0.06^{ijk}	183.79±12.2 ^{pqr}	39.99±0.76^{mno}	39.41±1.08^{bcdef}
VL 352	0.94±0.55^{abc}	38.16± 0.58^{fg}	34.47±1.4^{b}	73.56±0.48^{g}	369.62±18.64^{cd}	37.04±0.06^{o}	31.23±1.12^{klmn}
VL 357	0.92±0.45^{abc}	38.19±0.06^{cd}	32.61±2.29^{bc}	80.11±0.44^{ef}	347.68±2.70^{cdef}	47.96±0.94^{ghijkl}	39.91±0.80^{bcde}
VL 361	0.93±0.08^{abc}	32.11±0.06^{efg}	23.19±2.70^{fg}	66.05±0.93^{l}	406.66±5.87^{b}	61.31±1.08^{b}	38.86±1.09^{cdefg}
VL 367	0.95±3.52^{abc}	32.11±0.06^{efg}	4.10±1.88^{mn}	27.13±0.70^{s}	204.81±4.02^{opq}	44.46±1.24^{jklmn}	29.16±0.95^{no}
VL 369	0.92±0.45^{abc}	37.27± 0.31^{d}	8.29±0.69^{klm}	63.32±0.32^{m}	271.01±15.03^{ijkl}	41.57±1.25^{lmno}	34.25±2.25^{hijkl}
VL 370	0.94±3.38^{abc}	56.42±0.18i^{a}	13.32±0.32^{ijk}	59.60±0.06^{no}	287.28±5.39^{hijkl}	56.97±0.65^{bcd}	34.94±1.05^{ghijkl}
VL 373	0.91±0.05^{bc}	56.51±0.09i^{a}	28.95±0.84^{bcde}	85.69±0.20^{bc}	333.18±5.08^{defg}	51.52±1.52^{defghij}	33.62±0.51^{ijklm}
VL 374	0.93±0.38^{abc}	36.96± 0.61^{d}	7.59±2.30^{klm}	44.70±1.38^{q}	182.42±13.3^{pqr}	59.38±8.38^{bc}	38.84±1.61^{cdefg}
GE 86	0.94±2.04^{abc}	26.83±0.06^{hi}	4.09±0.47^{mn}	44.28±0.59^{q}	302.89±4.85^{ghi}	69.13±4.56^{a}	31.37±0.59^{klmn}
GE 440	0.95±2.27^{abc}	32.08±0.09^{efg}	9.78±0.33^{jklm}	39.00±0.11^{r}	249.49±3.52^{lmn}	47.73±0.97^{hijkl}	39.63±0.63^{bcdef}
GPHCPB 7	0.97±2.01^{ab}	56.48±0.12i^{a}	25.06±1.95^{efg}	86.27±0.28^{abc}	364.20±5.30^{cd}	55.40±1.55^{bcdef}	36.82±1.37^{efghi}
GPHCPB 3	0.95±1.00^{abc}	45.74±0.06^{b}	44.47±5.48^{a}	78.80±0.69^{f}	368.92±0.88^{cd}	57.75±0.41^{bcd}	35.52±0.52^{fghij}
GPHCPB 52	0.92±0.45^{abc}	45.77±0.03^{b}	20.63±1.09^{gh}	70.01±0.02^{hi}	350.53±4.63^{cde}	51.33±1.01^{defghij}	42.12±1.33^{bc}
GPU4 5	0.95±1.97^{abc}	27.23±0.09^{ghi}	32.54±2.55^{bc}	88.09±0.20^{a}	463.53±2.47^{a}	49.89±0.44^{efghijk}	35.77±0.78^{fghij}
HR 374	0.95±2.97^{abc}	56.42±0.18^{ia}	26.47±0.36^{def}	86.80±0.24^{abc}	386.31±6.72^{bc}	61.60±1.15^{b}	38.01±0.44^{defgh}
VR 708	0.95±3.07^{abc}	45.71±0.09^{h}	9.66±1.12^{jklm}	67.36±0.64^{jkl}	277.42±3.88^{ijkl}	48.62±0.64^{fghijk}	35.57±0.92^{fghij}

[Values are expressed as means ± S.E. of triplicate measurements. Values with different letters indicate significant difference (P <0.05, ANOVA). Condensed tannin content in mg of catechin equivalents/100 mg dry wt.; Antioxidant activity in mM of trolox equivalents/kg dry wt.; DPPH activity in µM of trolox equivalents/g dry wt.; ABTS scavenging capacity in µg/mL towards quenching of ABTS; FRAP in µM of trolox equivalents; and Fe & Zn in ppm]

anthocyanins or even anthocyanidins, besides phenolics (Fig 1 A and B). The finger millet genotypes used in the present study therefore, are rich source of phenolics, which have been already reported as the wound healing agent in finger millet and Kodo millet[30]. Hence, it can be concluded that intake of finger millet in daily diets will act as a therapeutic agent.

In order to determine the relation between anti-oxidant activity and phenolics the DPPH free radical scavenging activity, FRAP value and ABTS were determined. The free-radical scavenging potential of the methanolic extract was analyzed by the DPPH method and the results are shown in Table 1. Free radical scavenging activity of the genotypes VL *Mandua* 352, VL *Ragi* 146 and GPHCPB 3 was recorded higher among all genotypes having the values of 34.47, 27.20 and 44.47 µ*M* of trolox equivalents/g, respectively. Fardet *et al*.[31] also reported that cereal products have significant antioxidant potentials and average antioxidant activity through DPPH assay in cereals and cereal products varies between 12 to 35 µ*M* of trolox equivalents/g. We obtained higher values in finger millet genotypes indicating higher free radicals scavenging potential of finger millet in comparison to major cereals.

The ABTS scavenging activity of finger millet extract indicated that the genotypes *viz*., GPU 45, VL 325, VL 373, VL 336 and VL *Ragi* 146 had highest scavenging capacity (88.09, 88.00, 85.69, 86.65 and 81.70 % inhibition, respectively) towards quenching

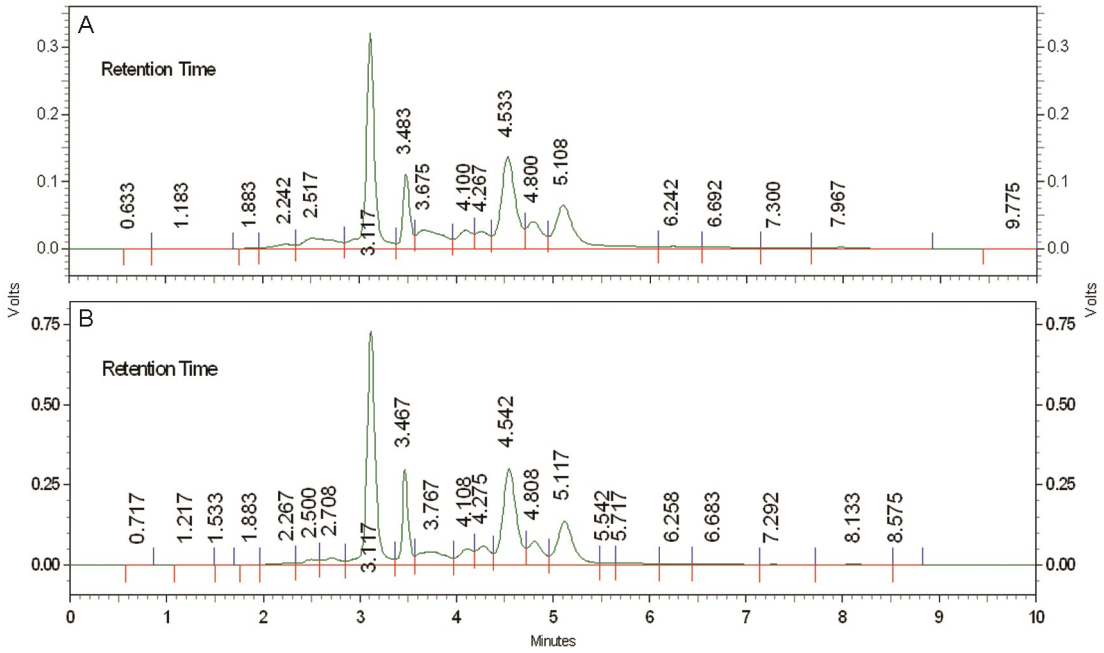

Fig. 1 — HPLC chromatogram of major phenolic acid in (A) VL *Ragi* 146;and (B) VL *Mandua* 352 finger millet genotype.

of ABTS, whereas the genotypes GE 440, VL 367, VL *Ragi* 149 and VL 374 had lowest percent inhibition. Amadou[24] also reported higher ABTS scavenging activity (60-70% inhibition) in millets.

FRAP value was observed highest in GPU 45 (463.53 µ*M*) and VL 361 (406.66 µ*M*), while lower values was recorded in VL 204 (100.54 µ*M*) and VL 328 (152.81 µ*M*). However, rest of the genotypes were having FRAP value in the range of 165.84-463.53 µ*M*. Singh[32] also observed similar results, where the FRAP values ranged from 170 to 370 µ*M* of Fe equivalents, respectively.

The estimated value of Fe (ppm) content was observed higher in the genotypes VL 374 (59.38 ppm), GPHCPB 7 (55.40 ppm), VL 373 (51.52 ppm) and VL *Ragi* 146 (48.71 ppm), while lower Fe values was recorded in VL *Mandua* 352 (37.04 ppm). The Zn (ppm) content was higher in all the genotypes. Zinc was highest in the genotype VL 340 (46.77 ppm), followed by GPHCPB 52 (42.12 ppm) while lowest value was observed in VL *Mandua* 315 (26.41 ppm), which is higher that the reported Zn values by Shashi[33] in finger millet. The overall results obtained in this study are in agreement with the earlier reports on Fe (36-73.01 ppm) and Zn (17.62-43.70 ppm) by Malleshi[5], Fernandez *et al*.[4] and Shashi *et al*.[33] and Glew *et al*.[34].

The results of the *in vitro* antioxidant assays reveal potent antioxidant and free radical scavenging activity in the grains of evaluated finger millet genotypes, equivalent to that of standard trolox and catechin. This potent antioxidant activity may be attributed to its high phenolic and tannin contents.

Principal component analysis

Principal component analysis (PCA) is a useful statistical technique which has found application in reduction of the original variables (condensed tannins, DPPH scavenging capacity, FRAP, ABTS, TA, Fe and Zn) to a smaller number of underlying variables (principal components) in order to reveal the interrelationships between the different variables and to determine the optimum number of extracted principal components. The first principal component (PC1) had the highest eigen value of 2.4 and accounted for 34.3% of the total variation in the data set, while the second principal component (PC2) with Eigen value of 1.25 explained 17.9% of the variation. The projections of genotypes and traits are shown in PC1 and PC2 biplot (Fig. 2). In PCA, the length, direction and the angles between the lines indicate correlation between the variables or between variables and principal component axes (e.g. $\alpha=0^0$ and/or 180° and r=1; α=90° and r=0). The longer the line, the higher is the variance. The cosine of the angle between the lines approximates the correlation between the variables they represent. The closer the angle is to 90 or 270 degrees, the smaller the correlation. An angle of 0 or 180 degrees reflects a correlation of 1 or −1, respectively[35]. All parameters

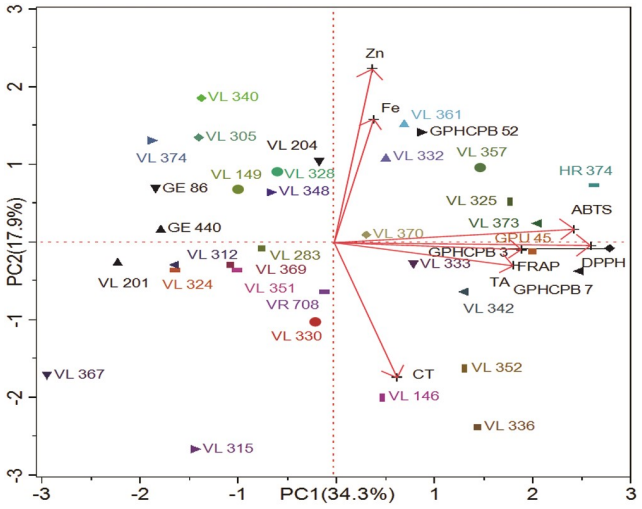

Fig. 2 — Multivariate comparison of finger millet genotypes and biochemical parameters using principal component analysis (PCA).

occupied the right side of the biplot. Zn, Fe and ABTS were observed on the right upper side of the biplot with high positive loading for both PC1 and PC2, while DPPH, FRAP, TA and CT were grouped together on the right lower side of the biplot with positive loadings for PC1 and negative loadings for PC2. Significant positive correlation was observed between TA and DPPH, TA and ABTS, and DPPH and ABTS. FRAP and DPPH also showed significant positive correlation (r=0.523) with each other. Nonsignificant negative correlation was observed among CT, FRAP, Fe and Zn. This indicates that the genotypes having high values of Fe and Zn were low in CT and *vice versa*.

Cluster analysis

Two way clustering analysis of 35 genotypes resulted into two major clusters (Fig. 3). Cluster I consisted of 18 genotypes in two groups, which was further divided into subgroups. Group I included VL *Ragi* 146, VL 336, VL *Mandua* 352, HR 374, GPHCPB 7, VL 373, VL 333, VR 708, VL 330, VL 370 and Group II included VL 325, VL 342, GPHCPB 3, GPU 45, VL 361, VL 332, GPHCPB 52 and VL 357. Seventeen genotypes in cluster II were subdivided into two groups. The first group in cluster II contained VL 351, VL 204, VL 305, VL 328, VL 283, VL 374, GE 440, VL 340, VL 201, GE 86, while genotypes VL *Mandua* 315, VL 367, VL *Ragi* 149, VL 348, VL *Mandua* 324, VL 312 and VL 369 constituted the second group. The genotypes in cluster I had higher CT, TA, FRAP, DPPH and

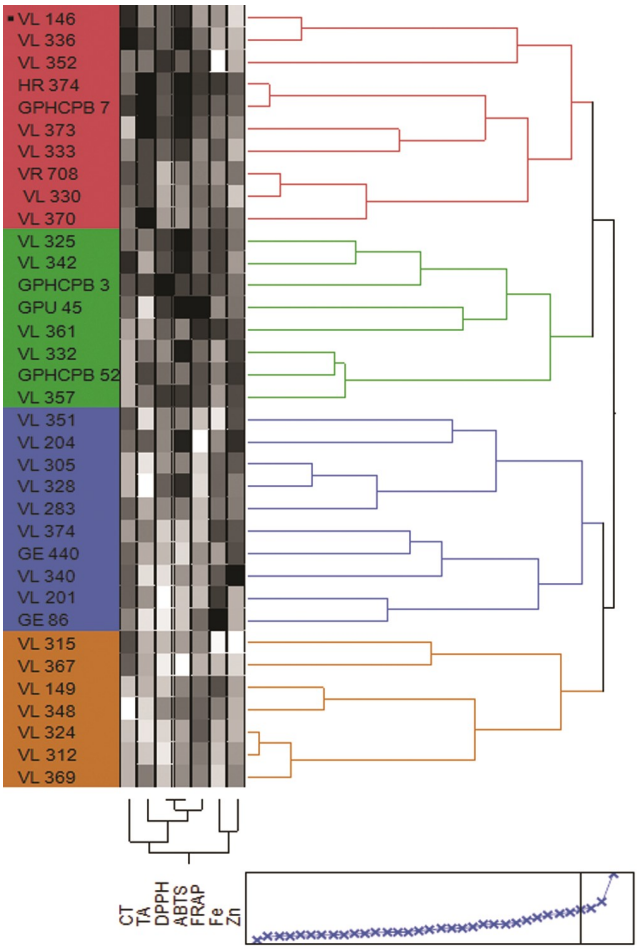

Fig. 3 — Two way clustering between thirty five finger millet genotypes on the basis of seven biochemical parameters.

ABTS, while genotypes in cluster II recorded higher Fe and Zn.

It was observed from the cluster analysis that GE 86 and VL 340 recorded higher Fe and Zn content while, VL *Mandua* 315, VL *Ragi* 149, VL *Mandua* 324, VL *Mandua* 352 and VL 348 showed lower values. GPHCPB 7, GPHCPB 52 showed highest DPPH, FRAP and ABTS activity and were observed in cluster 1.

Conclusion

From the present study, it may be concluded that finger millet is a good source of phytochemicals and showed significantly higher DPPH, ABTS free radical scavenging activity, FRAP value, condensed tannins, phenolic content and micronutrient (Fe & Zn). The genotypes VL *Ragi* 146, VL *Mandua* 352, VL 336, VL 373, VL 325, VL 351, GPHCPB 7, GPHCPB 3, GPHCPB 52, VR 708 and HR 374 were identified

with high amount of total antioxidant activity and condensed tannins. VL 374, GPHCPB 7, VL 373 and VL 340, GPHCPB 52 showed significantly higher amount Fe and Zn content. Major phenolics identified through HPLC were gallic, tannic, ferulic, caffeic, vannilic, protocatechuic and coumaric acids. The genotypes VL *Ragi* 149 and VL 373 recorded higher amount of tannic and gallic acid whereas, VL *Ragi* 146, VL 340, VL 325, VL 351 and VR 708 showed relatively good amounts of caffeic and coumaric acids including tannic and gallic acids.

It is significant to note that these higher antioxidant activities and phenolic acids in finger millet genotypes are known to offer several health benefits such as antidiabetic, antioxidant, hypocholesterolemic, antimicrobial effects and protection from diet related chronic diseases to its regular consumers. These results clearly indicate that finger millet is a good source of micronutrients, which could alleviate the wide spread micronutrient malnutrition in India.

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