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Decoding Variation among the Phenolic Composition and Antioxidant Properties of Some Finger Millet [*Eleusine coracana* (L.) Gaertn.] Landraces Grown in Maharashtra, India

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ABSTRACT

Background: The profiles of phenolic compounds and antioxidant capacities of ten finger millet landraces harvested from the northern Western Ghats of Maharashtra were investigated.

Methods: The antioxidant activity of methanol extracts of ten landraces were assessed using H_2O_2, FRAP and Phosphomolybdenum assays.

Result: Results of the H_2O_2 scavenging assay exhibit variation in their inhibition activity (59 -90.12%). The highest H_2O_2 scavenging activity was shown by T1, followed by T2, T3, T5, T8, T10 and T29 (90.12, 82, 79.8, 82.9, 79 and 85.78 respectively) when compared the control sample T had significantly lower activity (55.87%). While the reducing power was in the range of 1.02-1.76 mM of Fe (II)/gm and the reducing capacity was in the range of 73.86-158.71 equivalents of ascorbic acid in µg/gm of extract. The highest reducing power was shown by T2, T15 and T8 as 1.98, 1.94 and 1.89 mM of Fe (II)/gm respectively; while lower values were recorded in T19 (0.89 mM of Fe (II)/gm) and T1 (1.02 mM of Fe (II)/gm). The total antioxidant activity of the methanolic extract was calculated as ascorbic acid equivalents (AAE) per gram. Based on the results, T2, T5, T8 and T10 had a high reducing potential (158.71, 143.2, 149.5 and 152.65 AAE of a sample, respectively) with reducing capacities ranging from 73.86 to 158.71 AAE. A total of seventeen phenolic compounds were identified in the extracts including seven ûavonoids, with catechin, Iso-orientin and Iso-vitexin being the predominant flavonoids. Ten phenolic compounds were identified in the extracts, with p-hydroxybenzoic acid, genistic acid and gaillic acid being the predominant ones. In conclusion, based on the antiradical activities, the landraces T2, T5, T8, T10 and T29 could be potential cultivars and certainly effective sources of natural antioxidants for applications in the food and pharmaceutical industries.

Key words: Antioxidant activity, Correlation, Finger millet landraces, Phenolic profiling.

INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.] has a considerable historical, cultural and nutritional significance, notably in Asia and Africa (Pokharia *et al*., 2014). More than 25 countries on the African and Asian continents farm it, making up 12% of the world's total millet area (Vetriventhan *et al*., 2016). In Maharashtra, locally known as *ragi*, *nachani* and *nagli*, are mostly cultivated in hilly areas of Western Ghats. Potentially climate-resistant and nourishing, finger millet has strong nutraceutical and antioxidant qualities (Kumar *et al*., 2016). It supplies a steady supply of food, making it an essential crop in certain environments. The crop yields food grain as well as straw, which is an important source of animal feed (Rurinda *et al*., 2014). In addition to being gluten-free, finger millet grain is high in calcium, fibre and iron, as well as having great malting capabilities and a low glycemic index (GI). As a result of these characteristics, finger millet grain is a preferred diet for diabetics (Padhan *et al*., 2010).

Additionally, it has gained importance because of a higher amount of calcium (344 mg/100 gm), an excellent source of natural iron 3.9 mg (Gopalan *et al*., 2009), phenolic compounds (Geetha *et al*., 1990) and its functional components, such as slowly digestible starch, protein (5-8%), carbohydrates (65-75%), dietary fibre (15-20%) and minerals (2.5-3.5%) (Chethan and Malleshi, 2007). However, the mille*t al*so contains phytates (0.48%), polyphenols, tannins (0.61%), trypsin inhibitory factors and dietary fibre, which were once considered "anti-nutrients"

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due to their metal chelating and enzyme inhibition activities (Thompson, 1993). Apart from nutritional studies, several therapeutic properties of finger millet including antibacterial, antifungal, antidiabetic, antioxidant activity, anti-inflammatory, anti-carcinogenic and anti-cardiovascular activities have been reported (Devi *et al*., 2014).

Finger millet exhibits great genetic diversity as it grows in a wide range of environmental conditions. Native landraces, on the other hand, are more often associated with traditional and subsistence agriculture (Joshi *et al*., 2021; Ceasar *et al*., 2023). Local farmers select seeds based on empirical criteria such as the high yield, flavour and sustainability of the local environment. Native varieties may be capable of providing currently undiscovered benefits, making germplasm banks necessary to preserve diversity. Research into unknown characteristics of local landraces may produce findings that provide economic incentives for farmers to continue using native landraces. For example, if a local landrace proves to have high antioxidant properties, its commercial viability would surely improve (Syedd-León *et al*., 2020). Different landraces of finger millet are grown in different regions of Maharashtra based on the suitability of the local environment and its use. Previously, we investigated the morpho-agronomic diversity, nutritional value, polyphenol content and DPPH radical scavenging assay of finger millet landraces grown in various regions (Auti *et al*., 2017, Kazi *et al*., 2021; Kazi *et al*., 2022). Following our investigation into finger millet landraces, we present the results of antioxidant activity and LC-MS phenolic compound characterization, thereby adding value to this crop as a nutraceutical source and generating data that could be used in future breeding programmes.

MATERIALS AND METHODS

Chemicals

The standards including ascorbic acid, quercetin, catechol and tannic acid were obtained from Sigma, Mumbai. The solvents employed, Folin-Ciocalteu reagent, Phosphomoly bdenum reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrozine, hydrogen peroxide and aluminium chloride were of analytical grade and purchased from Hi Media, Mumbai. The LC-MS grade methanol, acetonitrile, water and formic acid for analysis were obtained from Sigma, Mumbai.

Collection of landraces

Ten finger millet (*Eleusine coracana*) landraces were collected in November 2018 from different locations in the Nashik and Dhule districts of Maharashtra, India and accession numbers were given (T1-T29). In addition to that, a known variety (Dapoli-1) rose by Balasaheb Konkan Krishi Vidyapeeth Dapoli, was considered as a control (Table 1). The whole finger millet grains harvested from the plants were cleaned to remove soil and other particles and ground using mortar and pestle to obtain a fine powder. The obtained powder was placed in plastic containers and stored at room temperature (20°C) for further analysis.

Preparation of the extracts

The whole seed samples of landraces (0.5 g) were homogenized in 10 ml of 1% HCl-methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated three more times with 1% HCl-methanol. The supernatants were filtered using filter paper (Whatman 1 Sigma, Mumbai) and collected in a round flask. The obtained polyphenol-rich extracts were then used for the subsequent analysis.

In vitro antioxidant activity

Hydrogen peroxide scavenging activity

The ability of finger millet to scavenge H_2O_2 was determined as per the method suggested by Halliwell and Whiteman (2004). Samples were extracted as mentioned in the DPPH assay. Hydrogen peroxide solution (20 mM) was prepared in phosphate-buffered saline (PBS) (pH 7.4). 0.2 ml of extract was added to 2.0 ml of H_2O_2 solution in PBS. The absorbance of H_2O_2 was measured at 230 nm, after 10 minutes against a blank solution that contained PBS without H_2O_2. Ascorbic acid was used standard as a compound. The percentage of H_2O_2 scavenging by the plant extracts was calculated as,

$$\% \text{ scavenged hydrogen peroxide} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where:

A_0- Absorbance of control.

A_1- Absorbance in the presence of plant extract.

Phosphomolybdenum reduction

The total antioxidant activity of the finger millet grains was estimated using the phosphor-molybdenum assay (Prieto *et al*., 1999). The powdered samples were extracted in 1% HCl-methanol and 0.2 ml was used. To that 3.0 ml of Phosphomolybdenum reagent was added and the final volume was adjusted using DW 3 ml of Phosphomoly bdenum reagent alone serves as blank. The reaction mixture was incubated at 97°C for 90 minutes. After cooling the absorbance was measured at 695 nm using a UV/Vis spectrophotometer against the blank. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard ascorbic acid.

Ferric reducing antioxidant potential (FRAP)

The ability of finger millet extracts to chelate ferrous ions was measured according to a prescribed method (Dinis *et al*., 1994). Briefly, 0.4 ml of extracts in distilled water was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by adding 5 mM Ferrozine (0.2 ml) and the total volume was adjusted to 4 ml with distilled water. The mixture was vigorously shaken and left at room temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm. For the control distilled water was used instead of the extract. The antioxidant power of the extract was compared with standard antioxidant Ascorbic acid. The results were expressed as micromoles of ascorbic acid equivalents per gram of sample.

Identification of phenolic compounds by LC-ESI-QTOF/MS

Phenolic compounds are characterized using the prescribed method (Gu *et al*., 2019) and were performed by the Agilent LC1200 series (Agilent Technologies, USA) equipped with an Agilent 6540 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, CA, USA). Reverse-phase HPLC column Zorbax Eclipse plus C_{18} with dimensions of 2.1*50 mm and pore size of 1.8 µM was used for the study. The column was maintained at a temperature of 40°C. The mobile phase consisted of 0.1% formic acid in acetonitrile. The flow rate was maintained at 0.35 ml/min. The total run for separation of samples was 15 min. 1 µl of the sample was injected into the column. Gradient elution was done to the identification of various components in the solution. Agilent 6540UHD Q-TOF-MS was used for the analysis of various phenolic compounds in the sample. The instrument had a mass range of 50-2000. Electrospray ionization with positive and negative ion modes was used for the identification of the compounds. The gas flow was maintained at 8l/min and the gas temperature at 295°C. The data analysis was done using Mass hunter workstation software V. B.05.01. The database used for analysis was METLIN.

Statistical analysis

All antioxidant assays were performed in triplicate and the data were analysed for one-way ANOVA by using Graph Pad Prism software version 7. All the values were expressed as Mean±S.D. Pearson's correlation coefficient was done by bivariate correlation analysis. Three correlation levels were defined as strong (r-value= 0.600-1.000), moderate (r-value = 0.400-0.599) and weak (r-value= 0.000-0.399).

RESULTS AND DISCUSSION

Antioxidant studies

Numerous *in-vitro* assays can be used to assess the antioxidant potential of various plant extracts. Each of these tests focuses on a different aspect of antioxidant activity, such as the ability to scavenge free radicals or inhibit lipid peroxidation. However, due to their complex composition, a single method is not recommended for evaluating the antioxidant activities of various plant products. To obtain relevant data, the antioxidant effects of plant products must be evaluated by combining two or more different in vitro assays (Arfa *et al*., 2015; Khyade *et al*., 2017). In this regard, three different assays were used to investigate the antioxidant potential of landraces.

Fig 1 shows the concentration-dependent hydrogen peroxide decomposition activity of methanolic extracts. Hydrogen peroxide is a weak oxidising agent that can directly inactivate a few enzymes by oxidising essential thiol (-SH) groups. Hydrogen peroxide can rapidly cross cell membranes; once inside the cell, H_2O_2 is likely to react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals, which may be the source of many of its toxic effects (Gupta and Sharma, 2010: Halliwell and Gutteridge, 2015; Khyade *et al*., 2017). As a result, it is biologically advantageous for cells to control the amount of hydrogen peroxide that accumulates. From the results, it appeared that H_2O_2 scavenging activity is remarkably higher as compared to DPPH scavenging assay (Kazi *et al*., 2022). The highest H_2O_2 scavenging activity was shown by T1, T2, T3, T5, T8, T10 and T29 (90.12%, 82%, 79.8%, 82.9%, 79% and 85.78% respectively); however, the control sample T had significantly lower activity (55.87%), indicating selected finger millet landraces possess good radical scavenging activity over control, (Fig 1). Because of their high phenolic and flavonoid content, the above landraces have the highest H_2O_2 scavenging activity (Kazi *et al*., 2021). Additinally, the H_2O_2 radical scavenging potential of the methanolic extracts of finger millet landraces indicates quite similar results as compared to those in DPPH reactions (Kazi *et al*., 2021).

The ferric reducing antioxidant power (FRAP) is a commonly used metric to assess an antioxidant's ability to donate an electron. The presence of a reductant (antioxidant) in the extract causes the Fe^{3+}/ferricyanide complex to be reduced to the ferrous form (Megdiche-Ksouri *et al*., 2015; Khyade *et al*., 2020). In the different finger millet landraces evaluated, the FRAP activity ranged from 1.02-1.76 mM of Fe

Table 1: Landraces collected from different sites.

Landrace saccession number	Village	Tehsil	District	Latitude	Longitude	Altitude (m)
T	Dapoli-1	Dapoli	Ratnagiri	17°460′4″N	73°11′28″E	176
T1	Vangni	Peth	Nashik	20°16′13″N	73°29′10″E	700
T2	Hattipada-white	Peth	Nashik	20°15′52″N	73°29′25″E	603
T3	Rajbari	Peth	Nashik	20°18′03″N	73°26′29″E	558
T5	Kengpada	Peth	Nashik	20°17′27″N	73°30′20″E	545
T8	Haattipada-Red	Peth	Nashik	20°15′55″N	73°29′52″E	607
T10	Pratabgad	Surgana	Nashik	20°32′23″N	73°34′44″E	459
T14	Manjdeja	Peth	Nashik	20°16′53″N	73°30′55″E	647
T15	Jale	Peth	Nashik	20°17′11″N	73°30′17″E	572
T19	Kavthe	Sakri	Dhule	20°59′53″N	74°16′42″E	342
T29	Karanajli-4	Peth	Nashik	20°15′01″N	73°35′06″E	503

(II)/gm. The highest reducing power was shown by T2, T15 and T8 as 1.98, 1.94 and 1.89 mM of Fe (II)/gm respectively; while lower values were recorded in T19 (0.89 mM of Fe (II)/gm) and T1 (1.02 mM of Fe (II)/gm), (Fig 2).

This assay is based on the extract reducing Mo (VI) to Mo (V) and the subsequent formation of the green phosphate complex at acid P_H (Behera, 2018). The total antioxidant activity of the methanolic extract was calculated as ascorbic acid equivalents (AAE) per gram. Based on the results, T2, T5, T8 and T10 all had a high reducing potential (158.71, 143.2, 149.5 and 152.65 equivalents of ascorbic acid in g/gm of a sample, respectively). Extracts with reducing capacities ranging from 73.86 to 158.71 equivalents of ascorbic acid µg/gm of extract. The control sample, on the other hand, had a much lower reducing capacity of 47.69 equivalents of ascorbic acid in µg/gm of extract of sampling (Fig 3).

Identification of the major phenolic compounds in ten landraces

The LC-ESI-QTOF/MS has been proved to be an effective tool for tentatively identifying and characterizing phenolic compounds in several plants (Hossain *et al*., 2010; Subbiah *et al*., 2020; Zhu *et al*., 2022). The phenolic compounds were identified and characterized based on their *m/z* value from MS spectra in both positive and negative ionization modes. As shown in Table 2, identified compounds were listed along with their molecular formula, retention times, ionization modes, molecular weight and mass error. Seventeen different phenolic compounds were characterized in all the ten selected landraces of finger millet, including 10 phenolic acids and 7 flavonoids (Table 2).

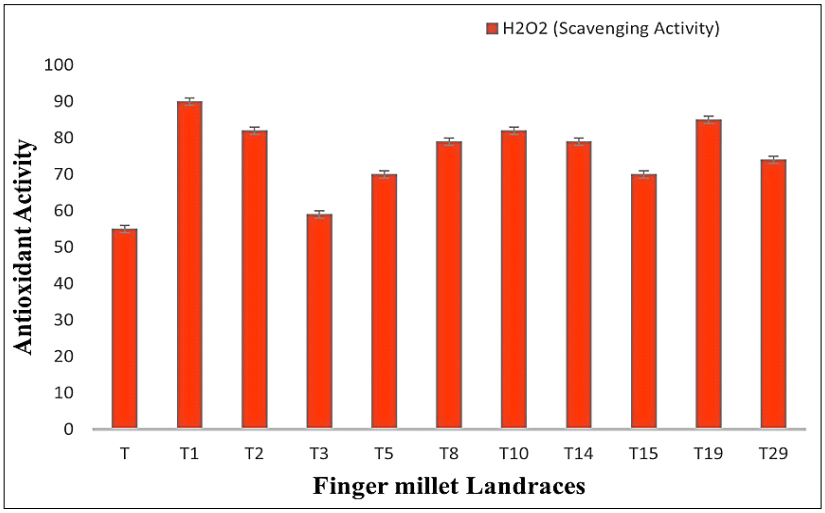


Fig 1: H_2O_2 antioxidant activity in 10 finger millet landraces.

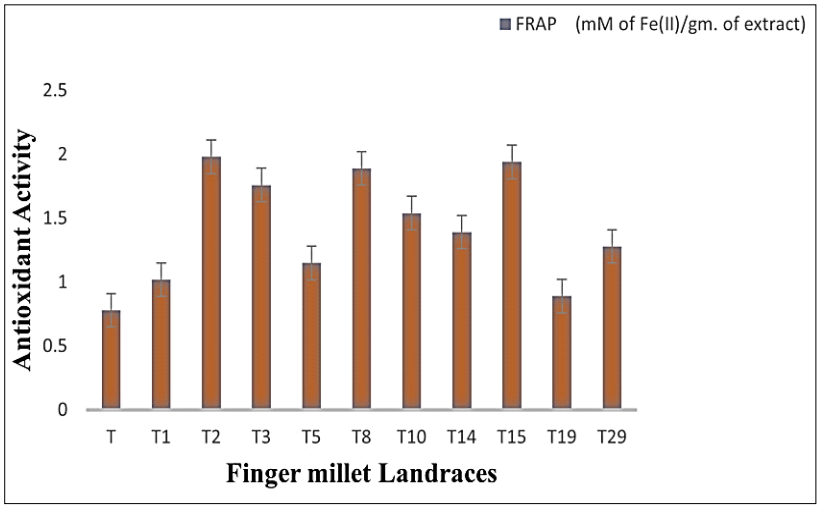


Fig 2: FRAP antioxidant activity in 10 Finger millet landraces.

Table 2: Qualitative characterization of phenolic compounds in selected Landraces of finger millet by LC-ESI-Q-TOF-MS.

Compounds	Molecular formula	RT (Min)	*m/z*	Molecular weight	Error (ppm)	Samples (Landraces)
1. Hydroxybenzoic acid						
Gallic acid (3,4,5-trihydroxybenzoic acid)	$C_7H_6O_5$	0.92	169.0137	170.021	-3.16	T1,T10,T15,
Genistic acid	$C_7H_6O_4$	1.94	153.0189	154.0262	-2.52	T1,T2,T3,T5,T8,T10,T14,T15,T19,T29
p-hydroxy benzoic acid (4-hydroxybenzoic acid)	$C_7H_6O_3$	3.85	137.0242	138.0315	-1.66	T1,T2,T3,T5,T8,T10,T14,T15,T19,T29
Vanillic acid (4-hydroxy-3-methoxybenzoic acid)	$C_8H_8O_4$	5.7	167.0347	168.0419	-2.01	T1,T2,T3,T5,T8,T10,T14,T15,T19,T29
Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	$C_9H_{10}O_5$	5.74	179.0347	198.0526	-1.01	T1,T5,T8,T10,T14,T19,T29
2. Hydroxy cinnamic acid						
Ferulic acid (4-hydroxy-3 methoxycinnamic acid)	$C_{10}H_{10}O_4$	5.15	221.0453	194.0571	-4.3	T1,T2,T3,T5,T8,T10,T14,T19,T29
Caffeic acid	$C_9H_8O_4$	5.74	179.0347	180.0416	-3.48	T1,T5,T8,T14,T15,T19,T29
p-coumaric acid (Trans-4-hydroxycinnamic acid)	$C_9H_8O_3$	4.96	205.0501	164.0466	-4.4	T1,T2,T2,T5,T8,T10,T15,T19,T29
Sinapinic acid	$C_{11}H_{12}O_5$	5	205.0505	224.0682	-1.39	T1,T3,T5,T8,T10,T15,T19
Trans-cinnamic acid (3 phenylacrylic acid)	$C_9H_8O_2$	6.03	147.0443	148.0511	-8.87	T2,T8,T10,T15,T19,T29
3. Flavonoids						
Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy -4H-1-Benzopyran-4-one)	$C_{15}H_{10}O_7$	6.85	301.0345	302.0418	-2.66	T1,T2,T5,T19,T29
Catechin	$C_{15}H_{14}O_6$	5.35	289.0718	290.0789	-0.41	T1,T2,T3,T5,T8,T10,T15,T19,T29
Iso -orientin	$C_{21}H_{20}O_{11}$	5.56	447.0925	448.0996	-2.09	T1,T2,T5,T8,10,T15,T29
IsoVitexin	$C_{21}H_{20}O_{10}$	5.8	431.0986	432.1058	0.43	T1,T2,T5,T10,T19,T29
Lucenin	$C_{27}H_{30}O_{16}$	5.9	591.1344	610.1524	-1.64	T1,T2.T8,T19
Tricin	$C_{17}H_{14}O_7$	7.25	329.0646	330.0707	-9.9	T5,T8,T10,T15,T29
Vitexin	$C_{21}H_{29}O_5$	10.1	361.2027	361.2014	-0.24	T1,T2,T8,T19,T29

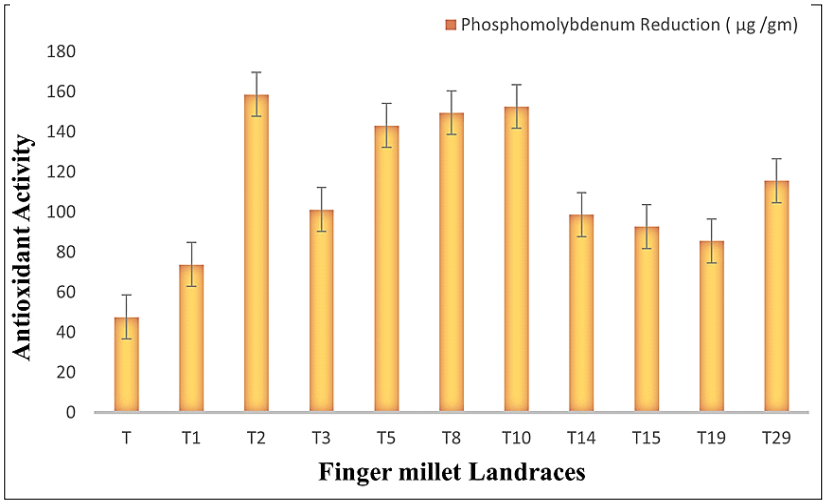


Fig 3: Phosphomolybdenum reduction antioxidant activity in 10 Finger millet landraces.

Flavonoids

Flavonoids are a diverse group of phenolic compounds that are present in many dietary plant foods. It has attracted interest due to their antioxidant, anti-inflammatory effects and their ability to modulate certain enzymatic functions (Panche *et al*., 2016; Al-Khayri *et al*., 2022). In this experiment, seven flavonoids were identified from the selected landraces quercetin, catechin, Iso-orientin, Iso-vitexin, lucenin, tricin and vitexin.

Phenolic acids

Phenolic acids are a subclass of phenolic compounds with a carboxyl group. Phenolic acids mainly comprise hydroxybenzoic acids and hydroxycinnamic acids and they have been extensively studied for their antioxidant, antimicrobial and anti-inflammatory effects (Kumar and Goel, 2019). In this study, two subclasses of phenolic acid were identified, which include hydroxybenzoic acids (5) and hydroxycinnamic acids (5).

Among the different types of hydroxybenzoic acid, hydroxycinnamic acid and flavonoid, the least frequent one is gallic acid; while p-hydroxybenzoic acid and catechin were identified in all the accessions. Ferulic acid, a common hydroxycinnamic acid derivative, was the predominant phenolic compound found in all finger millet landraces except T15. Obtained results revealed that T1, T19 and T29 have the highest fractionations over T2, T3 and T14. The landraces T3 and T14 do not show the presence of any flavonoid constituents. Among flavonoids, quercetin and catechin were the most predominant followed by iso-orientin. T2 and T8 landraces reveal the difference in the phenolic compounds; T2 and T8 exhibit 12 and 14 phenolic compounds respectively. The landraces T2 is specific for vanillic acid, trans-cinnamic, isovitexin and lucenin, while T8 is for genistic acid, syringic acid, sinapinic acid and tricin (Fig 1 and 2). Obtained results revealed that T1, T2, T5, T8, T10, T19 and T29 exhibit maximum types of phenolic compounds.

CONCLUSION

This study analysed the phenolic composition and antioxidant profile of ten finger millet landraces grown and consumed in Maharashtra, India. Phytochemicals and antioxidant activity were significantly higher in T1, T2, T5, T8, T10 and T19 finger millet landraces. The most abundant phenolic component was ferulic acid, a common hydroxycinnamic acid derivative found in all finger millet landraces except T15. In contrast, the phenolic compounds of T2 and T8 landraces differ. Taking this into account, T2 and T8 contain 12 and 14 phenolic compounds, respectively. The studied landraces can be considered health-promoting tools for local populations and their consumption should be encouraged to combat nutrient deficiencies and noncommunicable diseases. They also appear to be a valuable source of various phenolic compounds, with potential as a functional food as well as for nutraceutical applications. Furthermore, based on antiradical activities, T2, T5, T8, T10 and T29 could be potential cultivars as a climate resilient crop for sustainable agriculture.

Conflict of interest

All authors declare that they have no conflicts of interest.

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