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ORIGINAL ARTICLE

High resolution LC-MS characterization of phenolic compounds and the evaluation of antioxidant properties of a tropical purple radish genotype

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Abstract This study reports qualitative profiling of the phenolic compounds in an indigenously developed purple radish genotype VRRAD-151 using ultra performance liquid chromatography with quadrupole time of flight mass spectrometry. The root and leaf samples were harvested at the horticultural maturity stage of the genotype. Roots were divided into the periderm, and xylem, and the leaf samples were divided into petiole, and lamina, and these were separately extracted with methanol before the LC-MS analysis. A total of 66 compounds, including 23 flavonols, 1 dihydroflavonols, 4 flavonones, 4 flavones, 28 anthocyanins, 2 isoflavonoids, 3 phenolic acids, and 1 hydroxybenzaldehyde were putatively identified based on high resolution accurate mass analysis with the data processing through UNIFI®, which is a comprehensive compound identification software solution. An in-house developed database comprising the secondary metabolites of polyphenols was used for the screening purpose, and each phenolic compound was identified based on the detection of the precursor ion, and at least one characteristic fragment ion, each with less than 5 ppm of mass error. Anthocyanins were the most abundant type of phenolics exhibiting 59% in leaf petiole, 80% in root periderm, and 90% in root xylem. The relative concentration of anthocyanins was lower (11%) in the leaf lamina. Cyanidins were the most predominant anthocyanins accounting for 54, 100, 90 and 65%, in leaf lamina, leaf petiole, root periderm and root xylem, respectively.

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Eight anthocyanins and 25 flavonols (except kaempferol-3-*O*-*p*-coumaryl-shophoroside-7-*O*-glucoside) are tentatively new identifications and reported for the first time in radish. Flavonols were found to be the predominant group of phenolic compounds in the leaf lamina, and interestingly, the gradient of antioxidant activity followed the (relative) concentration gradient of flavonols in the samples. The relative antioxidant activity of various fractions when compared with each other, followed the trend: leaf lamina > root periderm > leaf petiole ≈ root xylem. Based on the results it can be reflected that this genotype can be utilized as a functional food for management of various human and animal diseases. Since the detected anthocyanins were mostly present in acylated forms, this genotype can function as a potential source of stable natural colorants.

1. Introduction

Radish is an important salad crop grown and consumed throughout the world for its fleshy edible roots which is either eaten as a crunchy salad, or consumed after cooking or preservation by salting, pickling, canning and drying. Botanically, radish belongs to the order Brassicales (Cruciales), family Brassicaceae (Cruciferae), genus *Raphanus* and species *sativus*. It has numerous categories- varying in color, size, shape and flavour of root; period of maturity; leaf morphology and color; and vernalization requirement. There are quick-growing spring type radishes (25–30 days), and also, slow-growing summer and winter radishes (40–55 days). The uses of colored radish varieties are gaining popularity because of their color characteristics, as well as potential antioxidant activities.

In recent years, studies on plant phenolics have gained enormous popularity due to their potential health benefits. Usually, these are by-products of the phenyl propanoid pathway and play crucial roles in the plant defense mechanism, maintaining stress homeostasis and pollination (Cheynier et al., 2013). In humans, these compounds are reported to be significant for mediating the secondary antioxidant defense mechanism, after ingestion (Rice-Evan et al., 1997). Besides, a wide range of beneficial properties of plant phenolics include anti-inflammatory, anti-allergic (Rathee et al., 2009), hepatoprotective, anti-atherosclerotic, anti-thrombotic, anti-viral (Nijveldt et al., 2001), anti-bacterial (Cushnie and Lamb, 2011), and anti carcinogenic (Ren et al., 2003) activities.

Since there is an increasing global demand of natural food colorants, plant breeders are highly interested to develop various hued cultivated crops. Radish is a rich source of phenolic compounds, including anthocyanins. Some of the phenolic compounds, for example acylated anthocyanins, have good color properties with high stability in various processing conditions, including temperature, light, and change in pH. Among the various anthocyanins, pelargonidin (acylated pelar gonidin-3-sophoroside-5-glucoside and its derivatives) is predominant in the red genotypes, while cyanidin (acylated cyani din-3-sophoroside-5-glucoside and its derivatives) is commonly found in the purple-colored root of radish (Giusti and Wrolstad, 1996; Tatsuzawa et al., 2010). These anthocyanins are known for their antioxidant properties, and therefore, can be used as active ingredients of functional foods for combating various life style syndromes, such as diabetes, and hyperlipidemia (Matsufuji et al., 2007). Due to the optimum hue and tinctorial strength, these anthocyanins are proven effective as natural colorants.

The color potential and multiple health benefits have encouraged the plant breeders to develop pigment-rich radish genotypes through selective breeding approaches (Lin et al., 2011a; Jing et al., 2012). 'VRRAD-151', a purple colored radish genotype, has been developed at the ICAR (Indian Council of Agricultural Research) - Indian Institute of Vegetable Research (IIVR), located in Varanasi, Uttar Pradesh, India. Unlike the earlier cultivars, its roots are iciclical in shape with an intense purple exterior as well as interior xylem. Its leaf petiole and veins are purplish, as well (Singh et al., 2016).

In recent times, researchers have used advanced technologies such as gas chromatography mass spectrometry (GC–MS), and high performance liquid chromatography (HPLC) for the metabolic profiling of radish (Park et al., 2016). Previous researchers have exclusively focused on radish root, although a very few investigations have been done on the phenolic profiling of its leaves (Beevi et al., 2010; Goyeneche et al., 2015). Some studies have also reported HPLC based identification of anthocyanins in red radish (Matsufuji et al., 2007; Liu et al., 2008). Elsewhere, use of liquid chromatography mass spectrometry (LC-MS) for characterizing anthocyanins in the root of purple radish (Lin et al., 2011a) is also available in literature.

Although its purple pigmentation suggests the presence of anthocyanins, the phenolic profiling of the root and leaf of this particular genotype has not been reported yet. Also, what has never been reported so far is the characterization of its non-pigmented phenolic compounds. Despite its leaves being consumed largely as a leafy vegetable in the Indian subcontinent, there is no detailed study performed on the phenolic composition of this purple radish leaf utilizing LC-MS, which undeniably establishes the necessity of this study.

Usually, antioxidant activity is determined to understand possible health benefits of phenolics including anthocyanins. Previously, researchers have reported pelargonidin in red radish using LC-MS (Giusti and Wrolstad, 1996; Jing et al., 2012; Papetti et al., 2014). A few attempts have also been made to characterize anthocyanins in purple radish. Using LC-PDA-ESI/MS, Lin et al. (2011a) had identified 60 cyanidins in the root of purple Bordeaux radish. Among the identified anthocyanins, there were 38 acylated cyanidin-3-sophoroside-5-diglu coside, and 10 acylated cyanidin-3-sophoroside-5-malonylglu coside. Similar to its root, the phenolic composition of the sprout of purple radish has also been well studied. By using HPLC-PDA-MS, Matera et al. (2012) had identified 70 cyanidins in sango radish sprouts. Later, the same group had also reported that anthocyanins bearing sinapic acid are more effective than those bearing the ferulic moiety, which were isolated from the sprouts of *R. sativus* cv. Sango (Matera et al., 2015).

A few reports are also available on the antioxidant activity of purple radish. For example, Hanlon and Barnes (2011) reported antioxidant activity of the sprout and root of the purple variety.

In this experiment, the leaf and root of VRRAD-151 were screened for the phenolic compounds using a high resolution quadrupole-time of flight (QToF) LC-MS, and 28 anthocyanins were identified. Of these, at least 8 are reported for the first time in radish. Besides, 23 flavonols are also reported for the first time in radish. The compounds were identified based on high resolution accurate mass analysis with the data processing through UNIFI®, which is a unique compound identification software solution. Additionally, the antioxidant activities were studied using four *in vitro* methods for understanding the possible health benefits of this genotype.

2. Materials and methods

2.1. Plant material

The purple radish (*Raphanus sativus* L.) genotype (VRRAD-151) was grown in accordance with good agricultural practices in the farm of ICAR-IIVR, Varanasi. Ten radish plants were randomly harvested at the horticultural maturity stage in February 2016, and then divided into separate lots of roots and leaves. Following harvesting, the plant samples were divided into two parts: one was for the *in vitro* studies, and the remaining was used for the LC-MS characterization at the National Referral Laboratory, ICAR-National Research Centre for Grapes.

2.2. Chemicals and apparatus

Methanol (LC-MS grade) and formic acid (88%) were supplied by J.T. Baker (NJ, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) (Pub Chem CID: 2735032), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] (Pub Chem CID: 5360881), TPTZ (2,4,6-Tripyridyl-s-triazine) (Pub Chem CID 77258), neocuproine (Pub Chem CID: 65237), trolox (Pub Chem CID: 40634), quercetin (>95% purity) and pelargonidin-3-O-glucoside (>95% purity) were purchased from Sigma-Aldrich, Bengaluru, India. The instruments used in sample preparation included a mixer and grinder (Bajaj India Pvt. Ltd., Mumbai), homogenizer (Heidolph 900, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), analytical and precision balance (Vibra, Adair Dutt, Mumbai, India), vortex mixer (Scientific Industries Inc., New York, USA), refrigerated centrifuge (Kubota, Tokyo, Japan), microcentrifuge (Biofuge, Kendro Laboratory Products GmbH, Hanau, Germany), and ultrasonic bath (Oscar Electronics, Mumbai, India). A water purification system (Sartorius, Gottingen, Germany) was used to generate HPLC-grade water for utilization in sample preparation as well as for LC mobile phase preparation.

2.3. Characterization of phenolic compounds

2.3.1. Plant sample preparation

The root samples were divided into the periderm and xylem, and the leaf samples were divided into petiole and lamina. For extraction, each sample was thoroughly homogenized under fresh condition. The homogenized sample (5 g) was drawn in a 50 mL amber-colored polypropylene centrifuge tube with a quality control standard (Forchlorfenuron, 1 mg/L), and immediately extracted with methanol (20 mL, 1% formic acid) by vortexing at 2000 rpm for 2 min in the dark, followed by centrifugation at 10000 rpm for 10 min at 10 °C. The supernatant was diluted with water (1:2, v/v), filtered through 0.2 μm PTFE membrane, and finally injected (5 μL) into the LC-MS.

2.3.2. LC-MS [UPLC-(ESI)-QToFMS] conditions

The analysis was performed on an Acquity Ultra Performance Liquid Chromatograph (UPLC), coupled to a QToF-MS (Synapt G2 HDMS, Waters Corporation, Manchester, UK). The QToF-MS was operated with electrospray ionization (ESI) at the mass resolution of 20000, and controlled by MassLynx 4.1 software. which simultaneously provided quick switching from low energy scan at 4 V (full scan MS) to high energy scan (10–60 V ramping) during a single LC run. The low-CE experiments provided information about the intact molecular ion (e.g. M^+, $[M+H]^+$), while the high-CE scan generates fragment ion information. The source parameters were set as follows: capillary 3 kV, sampling cone 30 V, extraction cone 5 V, source temperature 120 °C, desolvation temperature 500 °C, desolvation gas flow 1000 L/h, and cone gas flow 50 L/h. The mass spectrometer was calibrated with 0.5 mM of sodium formate. The lock spray and the reference mass leucine enkephalin (m/z 556.2771 in positive and 554.2670 in negative polarity) was used for the mass correction with a flow rate of 10 μL/minute at the concentration of 2 μg/mL at an interval of 20 s. At 35 °C, the chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.8 μm, Waters India Pvt. Ltd., Bengaluru). The mobile phase consists of A: methanol:water (10:90, v/v) and B: methanol:water (90:10, v/v) with 0.1% formic acid in both the phases. A gradient program was used with 0.4 mL/min flow rate, with 0–0.5 min/90% A, 4.5 min/50% A, 4.5–8 min/50–2%A, 8–11 min/2% A, 11–11.5 min/2–90% A, 12–15 min/90% A. The total ion chromatograms (TICs) for the acquired samples i.e. leaf lamina, leaf petiole, root xylem, and root periderm are given in the Supplementary Fig. S1.

2.3.3. Data analysis

The LC-MS data files (n = 6) were processed by the UNIFI software (version 1.7, Waters Corporation) with a screening solution workflow which helped in automated data processing to reporting of the positive identifications by comparison with a database of polyphenols and their derivatives. This database was developed for more than 1200 compounds by collecting the compound specific information (chemical structure, molecular formula, molecular mass) from various web-based resources (e.g. Chemspider) and published research papers. Based on the database information, the compounds were identified with mass errors of <5 ppm for the precursor as well as one or more product ion. The UNIFI software has a unique database feature which allows the users to quickly add any new compounds and create a customized library of compounds that includes detailed structural information (Deng et al., 2016). These product ions generated through collision induced dissociation were matched against the theoretical fragmenta-

tion pattern. The identified compounds were relatively quantified against quercetin and pelargonidin-3-*O*-glucoside, which were used as the external reference standards at 2 μg/mL concentration for the non-anthocyanins and anthocyanin derivatives, respectively. From these relative concentrations, the percentage distribution of various classes of phenolic compounds in the root and leaf fractions was estimated.

2.4. Determination of antioxidant activity

2.4.1. Sample preparation

For the evaluation of antioxidant activity, root periderm, root xylem, leaf petiole and leaf lamina were separately homogenized into pulp, and extracted with ethanol (80%) by stirring for 30 min, and centrifuged for 15 min at 13,000 rpm at 4 °C. The supernatant was collected in a test tube, and the residue was re-extracted twice with 80% ethanol, and then centrifuged. All the supernatants were then collected and pooled together. Before further analysis, the extracts were preserved at −20 °C.

Four *in vitro* methods were used for the analysis of antioxidant activities. The metal reducing potential of radish extracts was evaluated using the ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) methods, while the free radical scavenging assay was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and trolox equivalent antioxidant capacity (TEAC) assays.

2.4.2. FRAP

For the FRAP method (Benzie and Strain, 1996), the reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ [2,4,6-Tripyridyl-s-triazine] in 40 mmol HCl and 20 mmol $FeCl_3$ in the ratio 10:1:1 (v/v/v). An amount of 3 mL of the FRAP reagent was mixed with 100 μL of aliquot in a test tube and vortexed in an incubator at 37 °C for 30 min in a water bath. Reduction of ferric tripyridyltriazine to the ferrous complex formed an intense blue color which was measured through a UV–Visible spectrophotometer (Shimadzu UV 1601) at 593 nm at the end of the 4 min. Results were expressed in terms of μmol Trolox Equivalent (TE)/g fresh weight (fw).

2.4.3. CUPRAC

In this method (Apak et al., 2008), an aliquot (100 μL) was mixed with 1 mL each of $CuCl_2$ (1.0×10^{-2} mol L^{-1}), neocuproine alcoholic solution (7.5×10^{-3} mol L^{-1}), ammonium acetate (1 mol L^{-1}, pH 7.0) buffer solution and 1 mL of water to make the final volume to 4.1 mL. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. A standard curve was prepared using different concentrations of Trolox (100–2000 μM). The results were expressed as μmol TE/g fw.

2.4.4. DPPH

The DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical (Brand-Williams et al., 1995). An aliquot (100 μL) of the radish extract was added to 3.9 mL DPPH in methanol (95%) and shaken vigorously. Any change in the absorbance of the sample extract was measured at 515 nm for 30 min until the absorbance reached a steady state. The percentage inhibition of DPPH was calculated by the following formula:

$$\%\ \text{Inhibition} = \frac{A0 - A}{A0} \times 100$$

where A0 is the initial absorbance, and A is the final absorbance of the sample extract measured at 515 nm. Methanol (95%) was used as the blank. The calibration curve between % inhibition and concentration of Trolox (100–2000 μM) was then established. Results were expressed as μmol TE/g fw.

2.4.5. TEAC

The TEAC method that was proposed by Re et al. (1999) was used. The $ABTS^{\bullet+}$ free radical (7 mmol) was prepared by mixing 7 mmol ABTS with 2.45 mmol of potassium persulphate, which acted as an oxidizing agent. An aliquot (10 μL) of the extract was added to 90 μL of $ABTS^{\cdot+}$ solution and the absorbance was measured at 734 nm (at 30 °C), exactly 10 min after initial mixing. The percentage inhibition of $ABTS^{\cdot+}$ was calculated in the same way as for the DPPH method. The radical-scavenging activity of the test samples was expressed as trolox equivalent antioxidant capacity (TEAC μmol TE/g fw).

2.5. Statistical analysis

Results are presented as the mean ± standard deviation of three technical replications. The statistical analyses were performed with SAS software. One-way analysis of variance (ANOVA) by Duncan's test was used to compare the mean values. Differences were considered to be significant at $\alpha = 0.05$.

3. Results and discussion

3.1. Identification of phenolic compounds

The MS^E raw data when processed using the UNIFI software workflow putatively identified the chemical components (Table 1) based on the compound database. A total of 66 compounds, including 23 flavonols, 1 dihydroflavonols, 4 flavonones, 4 flavones, 28 anthocyanins, 2 isoflavonoids, 3 phenolic acids, and 1 hydroxybenzaldehyde is reported.

The recoveries of the reference standards used for relative quantifications, viz. quercetin and pelargonidin-3-*O*-glucoside in each of the component matrices at 2 and 5 mg/kg were more than 80% with precision RSD of less than 20% when analysed in ten replicates. This establishes the satisfactory quantitative performance of the extraction method used in this study.

3.1.1. Flavonol

The putatively identified flavonols included 12 kaempferol glycosides, 6 quercetin glycosides, 2 isorhamnetin glycosides, 1 spinacetin glycoside, and 1 methyl galangin. The identifications were based on certain criteria. For example, kaempherol-3-*O*-rhamnoside (Table 1, Sr. 1) was identified based on the protonated molecular ion *m*/*z* 433.1137 (mass error 2.11 ppm) with elemental composition $C_{21}H_{20}O_{10}$ and two characteristic fragment ions (*m*/*z* 153.0177 and *m*/*z* 287.0548) (Fig. 1a). Kaempherol-3-*O*-rhamnoside (Table 1, Sr. 1 and 2) appeared at two different retention times with

Table 1 Identification of phenolic compounds by high resolution LC-MS.

Sr. No.	Compound name	Molecular formula	Expected mass (m/z)	Observed mass (m/z)	Mass error (ppm)	RT (min)	Fragment ions (relative intensity,%)	Source
Flavonol $[M+H]^+$								
1.	Kaempferol-3-*O*-rhamnoside[I]	$C_{21}H_{20}O_{10}$	432.10560	433.11380	2.11	6.66	287.05514 (100%), 433.11375 (12%)	L_L
2.	Kaempferol-3-*O*-rhamnoside[II]	$C_{21}H_{20}O_{10}$	432.10560	433.11310	0.32	6.38	287.05485 (100%), 303.04989 (25%), 433.11305 (12%)	L_L
3.	Kaempferol-3-*O*-glucoside	$C_{21}H_{20}O_{11}$	448.10060	449.10870	1.95	7.11	303.04992 (100%), 449.10802 (15%), 176.07012 (15%), 161.04747 (12%)	L_P
4.	Kaempferol-3-*O*-rutinoside	$C_{27}H_{30}O_{15}$	594.15850	595.16530	−0.83	6.45	303.04960 (100%), 449.10795 (25%), 290.06171 (5%), 153.01716 (5%)	L_P
5.	Kaempferol-3-*O*-xylosyl-rutinoside	$C_{33}H_{40}O_{19}$	740.21640	741.22450	1.13	6.54	325.09179 (100%), 433.11358 (80%), 303.04993 (75%), 181.04954 (25%), 595.16453 (15%)	L_L
6.	Kaempferol-3-*O*-glucosyl-rhamnosyl-glucoside	$C_{33}H_{40}O_{20}$	756.21130	757.21900	0.56	6.28	287.05501(100%), 433.11292 (11%), 595.16575 (3%)	L_L
7.	Kaempferol-3-*O*-*p*-coumaroyl-diglucoside	$C_{36}H_{36}O_{18}$	756.19020	757.19960	2.79	6.5	163.03879 (100%),325.9237 (18%), 307.08137 (10%)	L_L
8.	Kaempferol-3-*O*-*p*-coumaroyl-sophoroside-7-*O*-glucoside	$C_{42}H_{46}O_{23}$	918.24300	919.25280	2.81	6.34	163.03897 (100%), 325.09198 (22%), 919.25307 (10%)	L_L, R_X R_P
9.	Kaempferol-3-*O*-caffeoyl-sophoroside-7-*O*-glucoside	$C_{42}H_{46}O_{24}$	934.23790	935.24680	1.70	6.02	176.01946 (100%), 535.10915 (50%), 112.02135 (45%), 935.24658 (35%)	R_X
10.	Kaempferol-3-*O*-feruloyl-sophoroside-7-O-glucoside	$C_{43}H_{48}O_{24}$	948.25360	949.26240	1.69	6.39	919.25139 (100%), 949.26011 (95%), 449.10791 (55%), 595.14516 (25%), 317.06722 (20%)	R_X, R_P
11.	Kaempferol-3-*O*-*p*-coumaroyl-sophorotrioside-7-*O*-glucoside	$C_{48}H_{56}O_{28}$	1080.29580	1081.30680	3.4	6.33	163.03871 (100%), 325.09198 (22%), 1081.30631 (20%), 919.25307 (10%)	L_L
12.	Kaempferol-3-*O*-feruloyl-sophorotrioside-7-*O*-glucoside	$C_{49}H_{58}O_{29}$	1110.30640	1111.31450	0.76	6.15	303.04983 (100%), 163.03852 (25%), 449.10837 (20%)	L_L
13.	Kaempferol-3-*O*-*p*-coumaroyl-sinapoyl-sophorotrioside-7-*O*-malonyl-glucoside	$C_{56}H_{58}O_{30}$	1210.30130	1211.31250	3.26	6.68	433.11375 (100%), 1211.31363 (30%), 535.10887 (10%),1181.30380 (7%)	L_L, L_PR_X, R_P
14.	Quercetin-3-*O*-rhamnoside	$C_{21}H_{20}O_{11}$	448.10060	449.10870	2.00	7.05	303.05038 (100%), 134.09633 (25%), 153.01753 (10%), 449.10978 (10%)	L_L
15.	Quercetin-3-*O*-rhamnosyl-galactoside	$C_{27}H_{30}O_{16}$	610.15340	611.16170	1.72	6.05	303.05005 (100%), 449.10836 (15%), 611. 16138 (10%)	L_L
16.	Quercetin-3-*O*-rutinoside	$C_{27}H_{30}O_{16}$	610.15340	611.15990	−1.19	6.12	303.04929 (100%), 449.10781 (25%), 611.15929 (10%)	L_P
17.	Quercetin-3-*O*-glucosyl-rhamnosyl-galactoside	$C_{33}H_{40}O_{21}$	772.20620	773.21540	2.50	5.08	287.05532 (100%), 449.10901 (25%), 773.21531 (25%), 611.16180 (20%),	R_P
18.	Quercetin-3-*O*-*p*-coumaroyl-sophoroside-7-*O*-glucoside	$C_{42}H_{46}O_{24}$	934.23790	935.24700	1.9	6.18	163.03852 (100%), 935.24619 (20%), 773.19374 (10%), 465.10407 (5%)	L_L, L_P R_P
19.	Quercetin-3-*O*-*p*-coumaroyl-sophorotrioside-7-*O*-glucoside	$C_{48}H_{56}O_{29}$	1096.29070	1097.30090	2.68	6.02	163.03852 (100%), 1097.30114 (28%) 935.24619 (25%), 325.09196 (25%) 487.08659 (15%), 773.19878 (10%)	L_L
20.	Isorhamnetin-3-*O*-*p*-coumaroyl-sophorotrioside-7-*O*-glucoside	$C_{43}H_{48}O_{24}$	948.25360	949.26080	−0.07	6.35	147.04323 (100%), 119.04898 (50%), 758.20607 (35%), 905.23416 (15%)	L_P
21.	Isorhamnetin-3-*O*-*p*-coumaroyl-caffeoyl-sophorotrioside-7-*O*-malonyl-glucoside	$C_{55}H_{56}O_{30}$	1196.28560	1197.29730	3.65	6.46	1197.29767 (100%), 949.25334 (40%), 697.16224 (35%), 177.05399 (10%)	R_P
22.	Spinacetin-3-*O*-(2″-*p*-coumaroyl-glucosyl)(1 −6)-[apiosyl(1 −2)]-glucoside	$C_{43}H_{48}O_{24}$	948.25360	949.26250	1.71	5.77	147.04402 (100%), 919.25332 (75%), 757.19960 (70%), 488.10881 (70%), 449.10880 (45%), 328.07900 (45%)	R_P
23.	Methylgalangin	$C_{15}H_{10}O_6$	286.04770	287.05510	0.36	6.65	287.05514 (100%),153.01783 (10%)	L_L

(*continued on next page*)

Table 1 (*continued*)

Sr. No.	Compound name	Molecular formula	Expected mass (*m*/*z*)	Observed mass (*m*/*z*)	Mass error (ppm)	RT (min)	Fragment ions (relative intensity,%)	Source
Dihydroflavonol[M + H]$^+$								
24.	Dihydro-kaempherol-3-*O*-rutinoside	$C_{21}H_{22}O_{10}$	434.12130	435.12680 $[M+H]^+$	−4.07	7.14	435.12568 (100%), 130.06474 (95%), 147.04406 (35%), 207.06474 (20%)	L_L
Anthocyanin [M]$^+$								
25.	Cyanidin-3-*O*-rhamnoside	$C_{21}H_{21}O_{10}$	433.11350	433.11280	−0.38	6.72	287.05484 (100%), 433.11260 (10%)	L_P
26.	Cyanidin-3-*O*-glucoside	$C_{21}H_{21}O_{11}$	449.10840	449.10710	−1.54	6.12	303.004929 (100%), 449.10781 (25%)	L_P
27.	Cyanidin-3-*O*-sophoroside-5-*O*-glucoside	$C_{33}H_{41}O_{21}$	773.21400	773.21170	−2.28	6.01	176.01896 (100%), 112.02087 (35%), 611.15940 (20%)	L_P
28.	Cyanidin-3-*O*-sophoroside-5-*O*-malonylglucoside	$C_{36}H_{43}O_{24}$	859.21440	859.21420	0.38	5.23	287.05438 (100%), 188.07031 (35%), 535.10884 (30%), 859.21487 (30%)	L_P, R_P
29.	Cyanidin-3-*O*-xylosyl-*p*-coumaroyl-glucosyl-galactoside	$C_{41}H_{45}O_{22}$	889.24020	889.24290	3.64	6.49	163.03879 (100%), 307.08137 (8%), 889.24315 (7%)	L_L
30.	Cyanidin-3-*O*-xylosyl-feruloyl-glucosyl-galactoside	$C_{42}H_{47}O_{23}$	919.25080	919.25290	2.85	6.45	177.05419 (100%), 339.10730 (55%), 919.25026 (28%), 535.10823 (20%)	L_L
31.	Cyanidin-3-*O*-*p*-coumaroyl-sophoroside-5-*O*-malonyl-glucoside	$C_{45}H_{49}O_{26}$	1005.25120	1005.25310	2.47	6.29	287.05475 (100%), 535.10876(30%), 1005.25252 (10%), 787.20843(5%)	R_P, L_P
32.	Cyanidin-3-*O*-caffeoyl-sophoroside-5-*O*-malonyl-glucoside	$C_{45}H_{49}O_{27}$	1021.24610	1021.24910	3.42	6.19	287.05519 (100%), 1021.24931 (90%), 535.10967 (45%), 773.19421 (15%)	R_P R_X, L_P
33.	Cyanidin-3-*O*-feruloyl-sophoroside-5-*O*-malonyl-glucoside	$C_{46}H_{51}O_{27}$	1035.26180	1035.26430	2.98	6.08	287.05530 (100%), 535.10992 (35%), 1035.26514 (25%), 787.20862 (10%),	R_P, R_X L_P
34.	Cyanidin-3-*O*-[2-O-(xylosyl)-6-O-(*p*-O-(glucosyl)-*p*-coumaroyl-glucoside]5-*O*-glucoside	$C_{47}H_{55}O_{27}$	1051.29310	1051.29520	2.57	6.34	163.03897 (100%), 325.09179 (20%), 919.25026 (9%), 1051.29252 (8%)	L_L
35.	Cyanidin-3-*O*-caffeoyl-*p*-coumaroyl-sophoroside-5-*O*-glucoside	$C_{51}H_{53}O_{26}$	1081.28250	1081.28520	3.02	6.38	919.25167 (100%), 757.19879 (50%), 449.10843 (50%)	R_P
36.	Cyanidin-3-*O*-*p*-coumaroyl-feruloyl-sophoroside-5-*O*-glucoside	$C_{52}H_{55}O_{26}$	1095.29820	1095.30030	2.43	6.54	287.05499 (100%), 535.10912 (50%), 757.19855 (10%)	R_P
37.	Cyanidin-3-*O*-caffeoyl-feruloyl-sophoroside-5-*O*-glucoside	$C_{52}H_{55}O_{27}$	1111.29310	1111.29650	3.53	6.31	1111.29634 (100%), 935.22857 (60%), 575.21013 (50%),398.07644 (35%)	R_P
38.	Cyanidin-3-*O*-di-*p*-coumaroyl-sophoroside-5-*O*-malonylglucoside	$C_{54}H_{55}O_{28}$	1151.28800	1151.29010	2.33	6.77	287.05474 (100%), 535.10871 (95%), 1151.29009 (25%)	R_P, R_X
39.	Cyanidin-3-*O*-*p*-coumaroyl-triglucoside-5-*O*-malonyl-glucoside	$C_{51}H_{59}O_{31}$	1167.30400	1167.30420	0.65	6.44	1167.30536 (100%), 949.25334 (50%), 697.16224 (35%), 177.05399 (15%)	R_P R_X, L_P
40.	Cyanidin-3-*O*-*p*-coumaroyl-feruloyl-sophoroside-5-*O*-malonyl-glucoside	$C_{55}H_{57}O_{29}$	1181.29860	1181.30080	2.35	6.75	1181.30075 (100%), 287.05474 (90%), 535.10871 (80%), 1151.29009 (25%)	R_PR_X
41.	Cyanidin-3-*O*-*p*-coumaroyl-caffeoyl-sophoroside-5-*O*-succinoyl-glucoside	$C_{55}H_{57}O_{29}$	1181.29860	1181.30130	2.80	6.70	287.05484 (100%), 535.10929 (35%), 963.25672 (10%), 433.11260 (5%)	L_P
42.	Cyanidin-3-*O*-caffeoyl-triglucoside-5-*O*-malonyl-glucoside	$C_{51}H_{59}O_{32}$	1183.29890	1183.30280	3.68	6.16	773.19421 (100%), 1183.30082 (35%), 999.24058 (15%), 697.16069 (15%)	R_P, L_P
43.	Cyanidin-3-*O*-caffeoyl-feruloyl-sophoroside-5-*O*-malonyl-glucoside	$C_{55}H_{57}O_{30}$	1197.29350	1197.29550	2.12	6.65	1167.28524 (100%), 1197.29642 (27%), 919.23076 (10%), 271.06002 (10%)	R_PR_X
44.	Cyanidin-3-*O*-*p*-coumaroyl-feruloyl-sophoroside-5-*O*-malonyl-sophoroside	$C_{61}H_{67}O_{34}$	1343.35140	1343.35130	0.37	6.70	1343.35224 (100%), 697.16079 (50%), 177.05395 (40%), 1125.27785 (25%)	R_P, R_X, L_P
45.	Cyanidin-3-*O*-caffeoyl-feruloyl-sophoroside-5-*O*-	$C_{61}H_{67}O_{35}$	1359.34630	1359.34640	0.51	6.39	1167.30364 (100%), 697.16095 (90%), 177.05400 (60%),	L_P

Table 1 (*continued*)

Sr. No.	Compound name	Molecular formula	Expected mass (*m*/*z*)	Observed mass (*m*/*z*)	Mass error (ppm)	RT (min)	Fragment ions (relative intensity,%)	Source
	malonyl-sophoroside						1359.34653 (40%), 949.25330 (35%)	
46.	Cyanidin-3-*O*-*p*-coumaroyl-sinapoyl-sophoroside-5-*O*-malonyl-sophoroside	$C_{62}H_{69}O_{35}$	1373.36190	1373.36030	−0.79	6.09	287.05435 (100%), 535.10808 (50%), 1373.36187 (35%), 1183.30041 (25%),)	L_P
47.	Delphinidin-3-*O*-rutinoside	$C_{27}H_{31}O_{16}$	611.16120	611.16250	5.12	3.02	287.0553 (100%), 449.1090 (11%), 611.16120(13%)	R_P
48.	Pelargonidin-3-*O*-sambubioside	$C_{26}H_{29}O_{14}$	565.15573	565.15610	1.64	6.37	287.0548 (100%), 303.0498 (31%), 433.1130 (12%)	L_L
49.	Pelargonidin-3-*O*-*p*-coumaroyl-diglucoside-5-*O*-glucoside	$C_{42}H_{47}O_{22}$	903.25589	903.25818	3.14	6.63	287.0551 (100%), 433.1137 (11%)	L_L
50.	Pelargonidin-3-*O*-feruloyl-diglucoside-5-*O*-glucoside	$C_{43}H_{49}O_{23}$	933.26646	933.26830	2.56	6.59	177.0543(100%), 339.1077(51%), 207.0647(22%), 697.1623 (17%)	L_L
51.	Pelargonidin-3-*O*-caffeoyl-diglucoside-5-*O*-malonyl-glucoside	$C_{45}H_{49}O_{26}$	1005.25121	1005.25295	2.28	6.51	287.0548 (100%), 535.1088 (39%), 1005.2512 (34%)	R_X
52.	Pelargonidin-3-*O*-caffeoyl-caffeoyl-diglucoside-5-*O*-malonyl-glucoside	$C_{54}H_{55}O_{29}$	1167.28290	1167.28497	2.25	6.65	1167.2852 (100%), 919.2307 (1 5 5), 271.0602 (10%)	R_P
Flavonone [M + H]								
53.	6-Prenyl-naringenin	$C_{20}H_{20}O_5$	340.13110	341.13860	0.64	7.10	137.05881 (100%), 323.12741 (80%), 271.09540 (60%), 175.07553 (45%)	R_P
54.	Naringenin-7-*O*-glucuronide	$C_{21}H_{20}O_{11}$	448.10060	449.10760	−0.61	6.46	303.04960 (100%), 449.10795 (25%), 257.04455 (5%), 153.01716 (5%)	L_P
55.	Naringenin-4′-*O*-glucuronide	$C_{21}H_{20}O_{11}$	448.10060	449.10810	0.49	6.15	303.04983 (100%), 163.03852 (30%), 449.10837 (15%)	L_L
56.	Naringenin-5-*O*-glucuronide	$C_{21}H_{20}O_{11}$	448.10060	449.10820	0.74	6.4	303.04989 (100%), 433.11305 (45%), 449.10832 (15%)	L_L
Flavone [M + H]								
57.	Luteolin-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	448.10060	449.10820	0.73	6.05	303.05005 (100%), 449.10836 (18%)	L_L
58.	Apigenin-7-*O*-rutinoside	$C_{27}H_{30}O_{14}$	578.16360	579.17270	3.3	6.66	287.05515 (100%), 433.11375 (12%)	L_L
59.	Apigenin-7-*O*-neohesperidoside	$C_{27}H_{30}O_{14}$	578.16360	579.17170	1.48	6.72	287.05484 (100%), 433.11260 (5%), 491.11912 (5%)	L_P
60.	Chrysoeriol-7-*O*-apiosyl-glucoside	$C_{27}H_{30}O_{15}$	594.15850	595.16670	1.57	6.39	287.05504 (100%), 433.11363 (12%), 595.16695 (5%)	L_L
Isoflavonoids $[M + H]^+$								
61.	6,7,3′,4′-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	286.04770	287.05500	−0.18	7.08	287.05476 (100%), 153.01753 (9%)	L_L
62.	Genistin	$C_{21}H_{20}O_{10}$	432.10560	433.11340	1.21	6.28	287.05504 (100%), 433.11363 (10%)	L_L
Phenolic acid $[M + H]^+$								
63.	*m*-Coumaric acid	$C_9H_8O_3$	164.04730	165.05400	−4.00	1.19	119.04873 (100%), 147.04345 (30%), 95.04852 (30%), 165.05337 (20%)	L_L
64.	1,2-dihydroxyferuloyl-gentiobiose	$C_{32}H_{38}O_{19}$	726.20070	727.20830	0.44	6.27	287.05504 (100%), 433.11363 (11%), 595.16695 (5%)	L_L
65.	Dihydro-caffeoyl-3-*O*-glucuronide	$C_{15}H_{18}O_{10}$	358.09000	358.09010	1.75	6.28	358.09022 (100%), 130.06471 (20%),	R_P
Other phenolic compounds $[M + H]^+$								
66.	4-Hydroxybenzaldehyde	$C_7H_6O_2$	122.03680	123.04350	−4.89	1.21	123.04328 (100%), 119.04867 (85%), 147.04342 (40%), 165.05361 (35%)	L_P

RT = Retention time, LL = Leaf lamina, LP = Leaf petiole, RP = Root periderm RX = Root xylem.

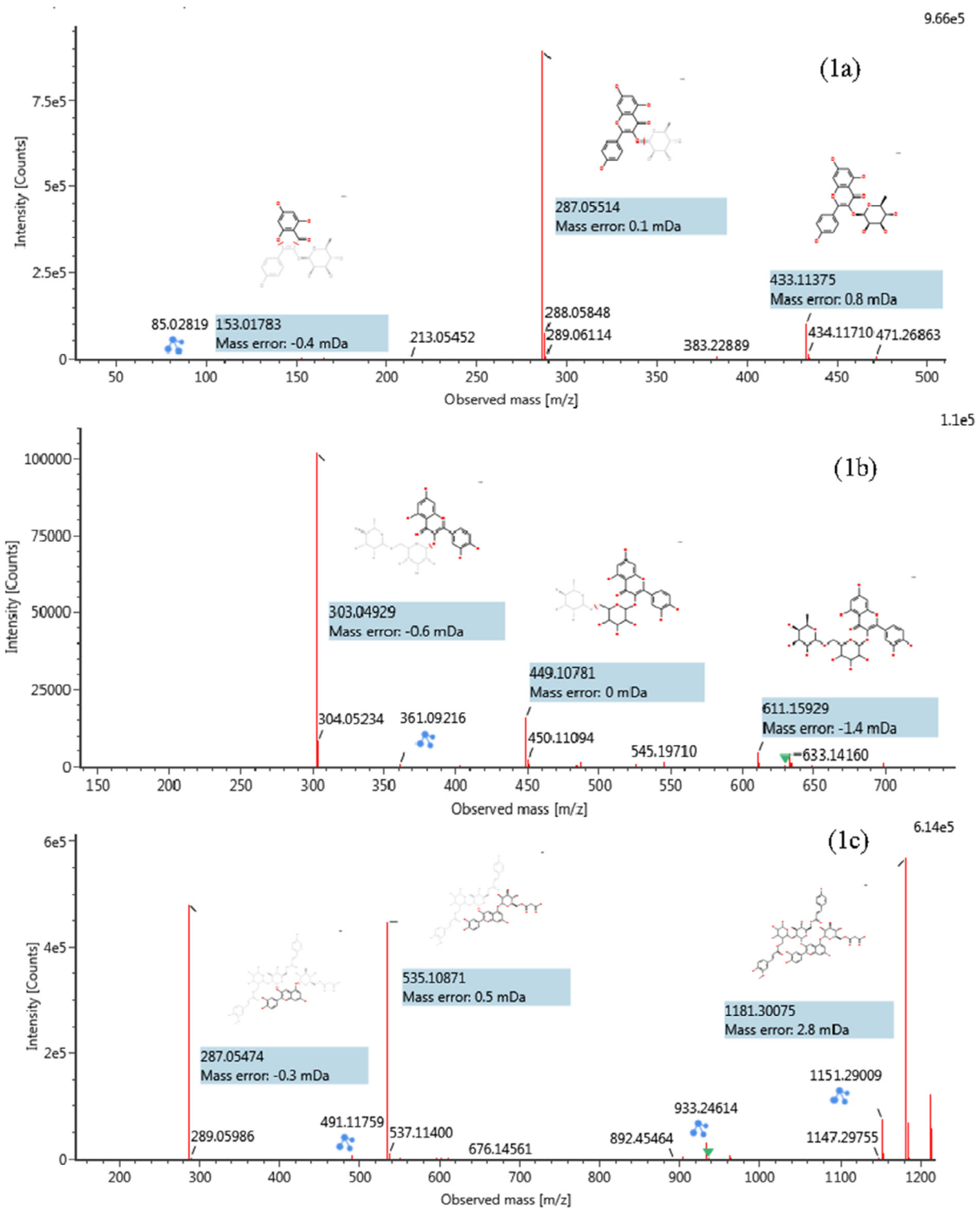

Fig. 1 Product ion mass spectra for (1a) Kaempferol-3-*O*-rhamnoside, (1b) Quercetin-3-*O*-rhamnosyl-galactoside and (1c) Cyanidin-3-*O*-(*p*-coumaroyl-feruloyl)-sophoroside-5-(malonyl) glucoside.

baseline separation which indicated that this compound probably appeared in two isomeric forms. Quercetin-3-*O*-rhamnosyl-galactoside (Table 1 sr. 15) was identified based on the protonated molecular ion *m*/*z* 611.1592 (mass error -1.72 ppm), elemental composition $C_{27}H_{30}O_{16}$ and characteristic fragment ions with *m*/*z* 449.10781 and 303.04929 (Fig. 1b). In a similar manner, the other compounds (Table 1) were identified. Among the flavonols, 11 were non-acylated glycosides and 12 were acylated glycosides. The acylated flavonols could be identified based on the increase in the mass of the corresponding precursor ions. They were either acylated singly (10 compounds), or triply (2 compounds) with hydroxycinnamic

acid (*p*-coumaric acid, caffeic acid, ferulic acid, or sinapic acid) at the 3-position of the C ring or aliphatic acid (malonic acid) at the 7-position of the A-ring. The non-acylated flavonols were mostly identified in the leaf fraction; however, all the acylated flavonols were detected in the leaf, as well as in the root fractions.

Except kaempferol-3-*O*-*p*-coumaryl-shophoroside-7-*O*-glucoside (compound 8, Table 1) none of the other flavonols was earlier reported either in radish root or leaf (Kamil and Kalina, 1977; Papetti et al., 2014). Among the identified flavonols, 7 similar compounds were earlier reported in mustard, broccoli and Chinese cabbage (Lin and Harnly, 2010), and 13 similar flavonols were also recorded in red mustard greens (Lin et al., 2011b).

3.1.2. Anthocyanins

Similar to flavonols, the anthocyanins were putatively identified based on their precursor and one or more product ions, each with < 5 ppm of mass errors. For example, Cyanidin-3-*O*-(*p*-coumaroyl-feruloyl)-sophoroside-5-(malonyl) glucoside was identified based on the observed accurate mass (m/z 1181.2986) with 2.35 ppm of mass error and its identity was further confirmed by the product ions with m/z of 287.0547, 535.1087, and 1151.29009 (Fig. 1c). The twenty-eight putatively identified anthocyanins included 22 cyanidin glycosides, 5 pelargonidin glycosides, and 1 delphinidin glycoside. Among these, 6 were non-acylated and 22 were acylated glycosides. The non-acylated anthocyanins were mostly identified in the leaf fraction, whereas all the acylated anthocyanins were detected both in the leaf and root fractions. The predominant structural backbones of the acylated anthocyanins were either anthocyanin-3-*O*-diglucoside-5-*O*-glucoside, or anthocyanin-3-*O*-triglucoside-5-*O*-glucoside, or anthocyanin-3-*O*-diglucoside-5-*O*-diglucoside. These are specific to the family Brassicaceae (Lin et al., 2011a). In addition, 2 mono-acylated galactosides were also putatively identified (compounds 29, 30, Table 1).

Hydroxycinnamic acids (*p*-coumaric/caffeic/ferrulic/sinapic acid) were attached with the C3 of the flavynium ring and aliphatic acids (either malonic or succinic acid) were attached to the C5 of the flavylium ring. The acylated anthocyanins included 6 mono-acylated, 12 di-acylated, and 9 tri-acylated compounds, some of which were also reported earlier in the root of purple radish varieties: 'Benikanmi' (Tatsuzawa et al., 2010), 'Bordeaux' (Lin et al., 2011a), and the radish sprout (Baenas et al., 2015). Similarly, some of these compounds were also earlier reported in the leafy vegetables belonging to the Brassicaceae family, e.g., red mustard greens (Lin et al., 2011b).

Anthocyanins that are reported for the first time includes the compounds 29, 30, 31, 34, 35, 36, 38, and 41 (Table 1). In this experiment, along with cyanidins, a few pelargonidins and one delphinidin were also identified. The findings are in agreement with Baenas et al. (2015), who reported the presence of pelargonidin, delphinidin and peonidin along with cyanidin in radish sprout. The presence of these compounds indicates possible mixing of the genes from pink and purple radish. Since radish is highly cross-pollinated in nature with a complex nature of pigment inheritance, the possibilities of occurrence of all of these pigments in the purple genotype are justifiable.

3.1.3. Other compounds

In addition to flavonols and anthocyanins, a dihydroflavonol, 4 flavones, 2 isoflavonoids, 3 phenolic acid, and a hydroxybenzaldehyde were putatively identified based on matching with the compound database through UNIFI. Except 6-prenylnaringenin and dihydro-caffeoyl-3-*O*-glucouronide, all the other compounds were detected only in the leaf samples. Among these compounds, except coumaric acid, which was earlier reported by Goyeneche et al. (2015), none has any mention in the literature. However, the occurence of dihydroflavonol (Marles et al., 2003), and 1,2-dihdroxy-feruloyl-gentibiose were reported in other crops of the Brassicaceae family (Lin et al., 2011b). Many of the compounds reported here are known for their potential health benefits, including antioxidant, anti-diabetic, anti-hypertensive, anti-inflammatory, and anti-cancer activities. (Tapas et al., 2008).

3.2. Relative distribution of phenolic compounds

The relative distribution of the major classes of phenolic compounds putatively identified in the root xylem, root periderm, leaf petiole, and leaf lamina is presented in Fig. 2. Based on the peak area response of quercetin (for non-anthocyanins) and pelargonidin-3-*O*-glucoside (for anthocyanins), the flavonols representing around 56% of the total phenolics, was the most predominant group in leaf lamina. The predominant flavonols in leaf lamina included quercetin-3-*O*-rhamnosyl-galactoside and kaemferol-3-*O*-rhamnoside, accounting for around 35% and 34% of the total flavonols, respectively. Other than the leaf lamina, flavonol was also the second most abundant group, exhibiting 25% in leaf petiole, 18% in root periderm, and 8.5% in root xylem. In leaf lamina, about 21% flavonols were in the acylated form against 42% in leaf petiole. In the root samples, however, the extent of acylation was up to 88% in root periderm and 100% in root xylem.

It was also noted that anthocyanin was the most abundant phenolics in leaf petiole and root, exhibiting 59% in leaf petiole, 80% in root periderm, and 90% in root xylem. The relative concentration of anthocyanins was lower (11%) in the leaf lamina. Cyanidins were the most predominant anthocyanins accounting for 54, 100, 90 and 65%, in leaf lamina, leaf petiole, root periderm and root xylem, respectively. Throughout the samples, the relative concentration of acylated anthocyanins was significantly higher than the corresponding non-acylated forms viz., 74% in leaf lamina, 90% in leaf petiole, 97% in root periderm, and 100% in root xylem. Di-acylated anthocyanins were dominant in root xylem, root periderm and leaf petiole accounting for 88%, 52% and 67% of total anthocyanins, respectively. However, mono-acylated anthocyanins were more abundant in the leaf lamina accounting for around 70% of the total anthocyanins. These results are in agreement with the findings of Jing et al. (2012) who also reported a higher proportion of di-acylated anthocyanins in various cultivars of Chinese red radish. This indicates its significance and potential applications as natural colorants since these compounds are expected to remain stable on exposure to heat, light and other environmental conditions, as opposed to their non-acylated forms (Matsufuji et al., 2007). These acylated compounds could also effectively inhibit enzymes, such as alpha glucosidase, thereby mitigating the chances of diabetes (Matsui et al., 2001). In root periderm, the predominant

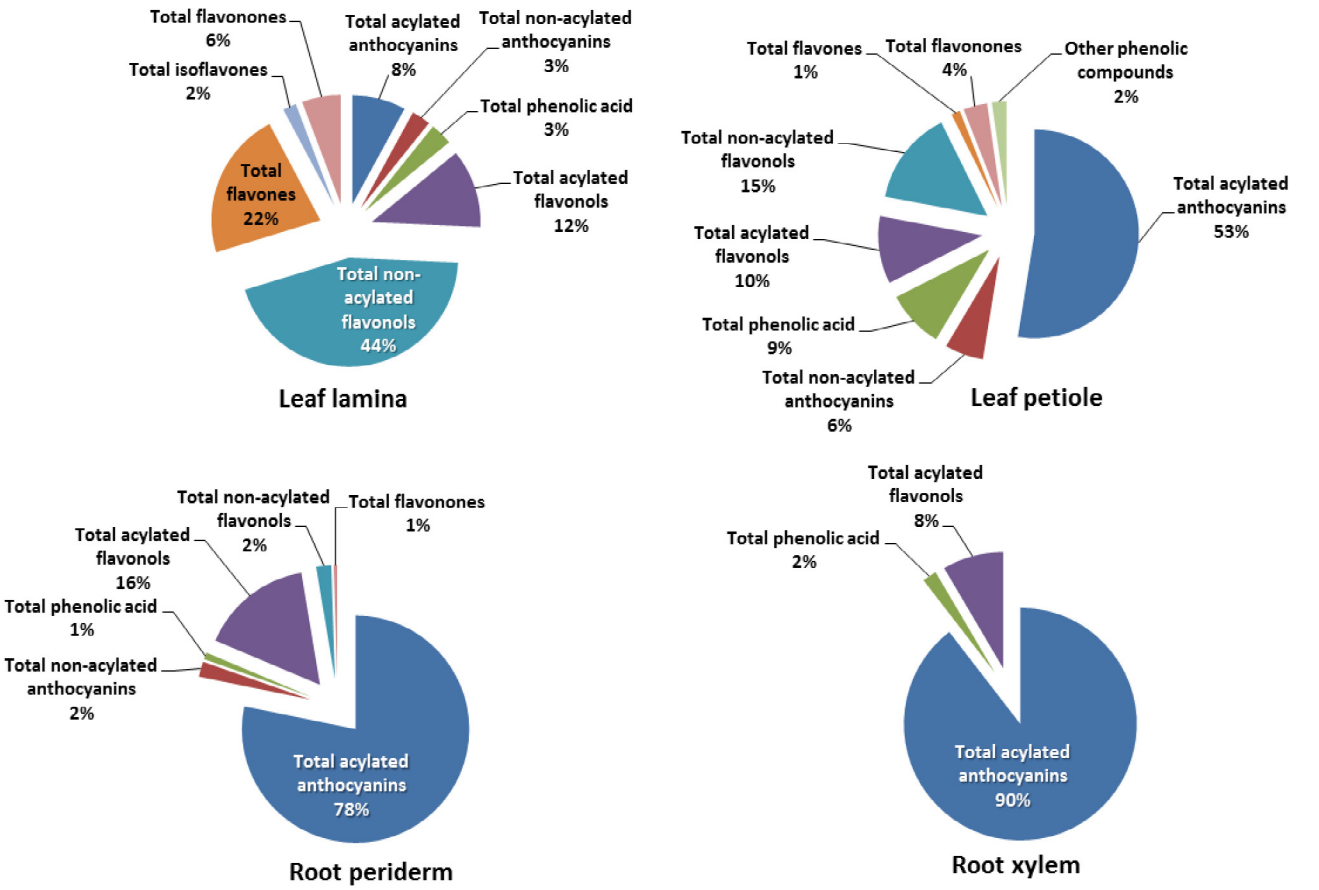

Fig. 2 Relative distribution of identified phenolic compounds in radish leaf and root.

anthocyanins included Cyanidin-3-*O*-caffeoyl-sophoroside-5-*O*-malonyl-glucoside and Cyanidin-3-*O*-*p*-coumaroyl-feruloyl-sophoroside-5-*O*-malonyl-glucoside.

The relative concentrations of the other groups of phenolic compounds were relatively low, except the flavones, which were the second predominant group in leaf lamina and accounted for 22% of the total phenolics. The predominant flavones included Apigenin-7-*O*-rutinoside and Chrysoeriol-7-*O*-apiosyl-glucoside. Genistin was the most predominant isoflavone in leaf lamina, whereas, *m*-Coumaric acid was the most abundant phenolic acid in leaf petiole.

3.3. Assessment of antioxidant potentiality

As there is no single versatile method that can assess the 'total antioxidant capacity' of food accurately and quantitatively (Özyürek et al., 2011; Koley et al., 2014), two *in vitro* electron transfer (ET) based assays (FRAP and CUPRAC) were utilized. Additionally, two other *in vitro* methods that are based on the hydrogen atom transfer (HAT) and ET (TEAC, and DPPH) were also used.

In the FRAP assay, the antioxidant activity of various fractions followed the following trend, leaf lamina > root periderm > leaf petiole ≈ root xylem. A similar trend was observed in all the other assays (Table 2). Interestingly, the gradient of antioxidant activity followed the (relative) concentration gradient of flavonols in the samples. Thus, flavonols may be a predominant contributor to the antioxidant potentiality of radish extract. In-house study of this experiment has indicated that the antioxidant activity of purple root (Table 2) is 4–6 times higher than the white colored radish (in-house unpublished data), which suggests that the purple root is healthier than the white root. Fairly recent, Goyeneche et al. (2015) reported antioxidant activities of radish using DPPH, FRAP, and ORAC assays. They noted that the antioxidant potentiality of leaves, measured by DPPH and FRAP methods, were higher (1.3 and 1.17 times, respectively) than its root. High antioxidant capacity in its leaves, therefore, indicated a high content of phenolics, which is in agreement with the current results.

It is worth observing that the overall values of the CUPRAC were higher than the FRAP, which was also reported earlier in other fruits (Koley et al., 2016), and vegetables (Koley et al., 2014). Since both the CUPRAC and FRAP assays are based on the same reaction mechanism and measure the ability of the naturally occurring antioxidant compounds to transfer one electron to reduce copper and iron ions, respectively, it was decided to compare the results achieved through both the methods. In the CUPRAC method, the antioxidant potency of the flavonoids is roughly proportional to the total number of -OH groups per molecule and is positively affected by the presence of an *o*-dihydroxy moiety in the B-ring (Apak et al., 2008). Interestingly, quercetin fulfills these requirements completely and was also detected in both leaves and roots of radish.

Table 2 Antioxidant potentiality of radish leaves and roots.

Sample	FRAP	CUPRAC	DPPH	TEAC
Leaf lamina	29.7 ± 3.9[a]	45.2 ± 6.0[a]	20.86 ± 2.6[a]	25.7 ± 2.2[a]
Leaf petiole	5.2 ± 0.5[c]	8.1 ± 0.8[c]	3.50 ± 0.04[c]	4.8 ± 0.3[c]
Root periderm	20.4 ± 2.0[b]	28.1 ± 3.6[b]	13.04 ± 1.3[b]	17.8 ± 1.0[b]
Root xylem	3.5 ± 0.5[c]	7.8 ± 0.9[c]	2.24 ± 0.1[c]	2.3 ± 0.3[c]

Note: Values are the mean of three replicates ± standard error. Different letters in the same column represent statistically different results ($p < 0.05$). Activity in each method was expressed in the common unit of μmol Trolox Equivalent/g fresh weight (fw).

Similar to the FRAP and CUPRAC, the overall values of the TEAC assay were higher than the DPPH values (Koley et al., 2014). A higher TEAC value could be due to matrix interferences from colored compounds (anthocyanins) in the extracts. According to Arnao (2000), if a sample contains compounds having intense colors, the absorbance gets reduced, and thus, the antioxidant activity would always be less, even when working with minimal sample volumes (5–10 μL). Since radish anthocyanins maximally absorbed at 510–530 nm, their colors interfered with the DPPH chromogen, which has an absorption maximum at 515 nm, and this resulted in a relatively low measured activity.

Overall, the CUPRAC value was the highest among the methods used. This could be attributed to the rapid reaction kinetics, which was faster than the other three methods. As a result, several flavonoids and hydroxycinnamic acids in CUPRAC completed the reaction within the protocol period of the assay (Apak et al., 2008). Moreover, the CUPRAC method measures the antioxidant capacity nearly at the physiological pH (i.e., pH 7), and hence, offers distinguishing advantages over the FRAP assay, which works at an acidic pH.

4. Conclusion

This study reports the profile of the phenolic compounds in the purple radish genotype VRRAD-151 and confirms that the LC-MS-based profiling is a powerful technique for the phenolic characterization. A total of 66 phenolic compounds, such as flavonols, dihydroflavonols, anthocyanins, flavonones, flavones, isoflavonoids, phenolic acids, and hydroxybenzaldehydes were putatively identified. Anthocyanins detected were mostly acylated in nature, and hence this genotype can function as a potential source of stable natural colorants which are considered safe to the consumers with potential health benefits. The *in vitro* antioxidant activity assays revealed that the purple radish leaf is richer in antioxidants than its root. Based on the observed results in conjunction with the existing literature, it is anticipated that this purple radish genotype might be used for mitigating human and animal diseases. This genotype could further be used in the genetic improvement programs to breed varieties with high nutraceutical properties. This will further trigger extensive research for better understanding of the impact of phenolic compounds on the human health.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.arabjc.2017.11.007.

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