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ORIGINAL ARTICLE

Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables

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Abstract FTIR analysis for five selected green leafy vegetables (GLVs) viz., *Hibiscus cannabinus* L., (kenaf), *H. sabdariffa* L., (roselle), *Basella alba* L., (vine spinach), *B. rubra* L. (malabar spinach) and *Rumex vesicarius* L., (sorrel) confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching. The chlorophyll content was higher in native leaves of *B. alba* (2.96 g/kg) than defatted samples (1.11 g/kg). Total phenolic content (TPC) in *H. sabdariffa* native methanol extractives is more (17.6 g/kg) than defatted leaves (9.67 g/kg). Native *B. rubra* methanol extractives exhibited highest total flavonoid content (TFC) (21.59 g/kg), while that of *R. vesicarius* was lowest (3.21 g/kg). In general, antioxidant activities showed a significant reduction in retention of antioxidants in both native and defatted GLVs samples of ethanol and methanol extractives. Methanol extractives showed significantly stronger antioxidant activity probably due to greater solubility of phenolics and destruction of cellular components.

Keywords Leafy vegetables · FTIR · Antioxidant properties · Flavonoids · Phenolics

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Introduction

Plants are a good source of various functionally active secondary metabolites and a good source of essential nutrients (Khan et al. 2011; Khan et al. 2012). Recent studies have demonstrated the nutritional and nutraceutical efficacy of different plant tissues proving their commercial value (Sri Harsha et al. 2013; Khan and Giridhar 2014; Khan et al. 2014). Green leafy vegetables (GLVs) – rich source of carotenoids (a precursor of Vitamin A) – are available throughout the year in India and grown commonly for human consumption (Khader and Rama 2003). In addition, GLVs are cost effective and sustainable source of water soluble vitamins, chlorophyll, minerals and other vital nutrients. GLVs are largely considered as the cornerstones of both traditional and primary health care systems in alleviating common diseases (Negi and Roy 2000). Both native and defatted leafy vegetables have significant use as food additives. Oils of native leafy vegetables possess an important role as a source of energy, to maintain the integrity of the cell membrane, protect adipose tissues during mechanical injuries and give toothsome for eating. Vegetable fats and oils function as precursors of prostaglandins that have both vasoconstrictor, vasodilator function and serves as precursors of thromboxane that alleviate blood clotting in humans (Ononoghu 2002). Defatting of leafy vegetables before subjecting them for post-harvest preservation may be helpful in preventing rancid flavor that occurs due to autoxidation of lipids. Studies have demonstrated the variations in chemical constituents of native and defatted leafy vegetables and their bioavailability (Iheanacho and Udebuani 2009).

In the southern part of India, *Hibiscus cannabinus* (kenaf), *H. sabdariffa* (roselle), *Basella alba* (vine spinach), *B. rubra* (Malabar spinach) and *Rumex vesicarius* (sorrel) are commonly

used as GLVs. *H. cannabinus* (green stem) and *H. sabdariffa* (red petiole and stem) belongs to the family Malvaceae. Kenaf and roselle have various health benefits such as diuretic effects, antiscorbutic, emollient and sedative, etc (Ramakrishna et al. 2008). *B. alba* with green twine and leaves and *B. rubra* with red twine and red leaves (Basellaceae) are used for their medicinal properties in Unani-ancient Indian medical system and in Chinese traditional medicine to treat constipation, as a diuretic, as toxicide, an anti-inflammatory and to enhance fertility in women (Murakami et al. 2001; Mensah et al. 2008). *Rumex vesicarius* (Polygonaceae) has many important medicinal uses such as, analgesic, antiulcer, hepatoprotective agent and is useful in scabies, leucoderma, toothache, asthma, heart troubles, tumours and scurvy (Mostafa et al. 2011). All these GLVs used in making pickles; cooking and soups are the rich sources of iron and folic acid (Saran and Lyness 2004).

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the metabolic composition of leaves at a given time (Bobby et al. 2012). It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra (Surewicz et al. 1993). FTIR is employed to elucidate the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of respective chemical functional groups (Bobby et al. 2012). FTIR has been used to identify the complicated structures of plant secondary metabolites and in the characterization of bacterial, fungal and plant species (Hori and Sugiyama 2003; Yang and Yen 2002). Advocating FTIR method to determine the spectral variations of targeted metabolites of GLVs would have implications in their post-harvest processing. However, no such reports are available for these GLVs and to the best of our knowledge; this is the first report on the FTIR method to find the variations and antioxidant activities of native and defatted foliage of selected leafy vegetables.

The aim of the present study is to identify the variations in FTIR spectra (of the selected GLVs viz., kenaf, roselle, vine spinach, Malabar spinach and sorrel), retention of chlorophyll, TPC, TFC and antioxidant properties of native and defatted leaves of these plants and also to find out the extraction efficiency of different solvents namely, methanol and ethanol on antioxidant properties.

Materials and methods

Collection and processing of plant materials

Hibiscus cannabinus L., *H. sabdariffa* L., *Basella alba* L., *B. rubra* L. and *Rumex vesicarius* L. leaves (GLVs) were collected from two months old plants maintained at greenhouse facility in CFTRI campus, Mysore. The leaves were cleaned with water, blotted on filter paper to remove water and dried in an oven set at 50 °C for 6 h. The specimen of GLVs plants were deposited at Herbarium Center of the University of Mysore. The dry leaf material was pulverized to a fine powder with motor and pestle and stored in amber colour air tight containers at room temperature until use.

Defatting of GLVs

GLVs were defatted with hexane at 40 °C for 8 h in a soxhlet extractor following soxhlet extraction method Ba 3–38 (AOCS 2003).

FTIR analysis of native and defatted GLVs

The FTIR spectra of native and defatted GLVs was recorded in FTIR instrument (Model/Make: IFS 25, Bruker, Germany), with PC based software controlled instrument operation and data processing. A small amount of powdered leaf samples were made into pellets using KBr for FTIR analysis and a thin film was prepared by applying pressure. The data of infrared transmittance was collected over a wave number ranged from 4000 cm^{-1} to 500 cm^{-1}. All the samples were analyzed in triplicates with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the sample.

Determination of chlorophyll content

The native and defatted GLVs samples were extracted with 80 % aqueous acetone (1:10 *w/v*) at room temperature under subdued light and centrifuged at 7000 rpm for 10 min. The absorbance of the clear supernatant was measured at 661.5 nm, 663 nm and 645 nm in a double beam spectrophotometer (UV-1800 A, Shimadzu Corporation, Kyoto, Japan). The concentration of chlorophyll a (chl-a), chlorophyll b (chl-b) and total chlorophyll (chl-t) were calculated using the following equations and the difference in chlorophyll contents quantified by two different methods was analyzed.

According to Arnon 1949.

Chl a (μg/ml) = 12.72 A_{663} - 2.59 A_{645}.

Chl b (μg/ml) = 22.9 A_{645} – 4.67 A_{663}.

Chl t (μg/ml) = 20.31 A_{645} + 8.05 A_{663}.

According to Lichtenthaler 1996.

Chl a (μg/ml) = 11.24 $A_{661.5}$–2.04 A_{645}.

Chl b (μg/ml) = 20.13 A_{645} – 4.19 $A_{661.5}$.

Chl t (μg/ml) = 7.05 $A_{661.5}$ + 18.09 A_{645}.

In vitro antioxidant assays

Antioxidant extraction

The extraction efficiency of different solvents namely methanol and ethanol were evaluated in all native and defatted GLVs samples. Antioxidant components were extracted using 2.5 g

of leaf powder with 50 ml of solvent by using mortar and pestle and kept in a gyrorotary shaker at 100 rpm for 30 min and centrifuged at 10,000 rpm for 10 min. The pellet was collected and re-extracted as above, and both the supernatants were pooled and stored in amber tubes (Kumar et al. 2015).

Determination of total phenolic content

Total phenolic content (TPC) of crude extractives of respective GLVs were determined by using Folin-Ciocalteau's method (Sadasivam and Manickam 2008). The extractives (0.2 ml) were taken in a test tube and the volume in all the tubes was made to 3 ml with distilled water followed by addition of 0.5 ml of Folin-Ciocalteau's reagent and incubated for 3 min. Subsequently, 2 ml of 20 % Na_2CO_3 solution (*w*/*v*) was added to each tube, vortexed and placed in boiling water bath for 1 min. The contents were cooled, and the absorbance was measured at 650 nm. The amount of phenolics present in the samples was expressed as Gallic acid equivalent (GAE) in g/kg of leaf material.

Determination of total flavonoid content

To 1 ml of diluted GLVs extract, 1 ml of 2 % (*w*/*v*) methanolic solution of $AlCl_3$ was added and mixed well followed by incubation at room temperature for 15 min (Djeridane et al. 2006). The absorbance of the reaction mixture was read at 430 nm with a double beam spectrophotometer (UV-1800 A, Shimadzu Corporation, Kyoto, Japan). The TFC was expressed as Rutin equivalent (RE) in g/kg of leaf material.

Determination of total antioxidant activity

An aliquot of 0.3 ml GLV extractives (2 mg/ml) was combined with 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated at 95 °C for 90 min. The absorbance of the solution was measured at 695 nm using a double beam spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan) against blank after cooling to room temperature (Prieto et al. 1999). The total antioxidant activity was expressed as the number of gram equivalent of ascorbic acid (AA).

DPPH radical scavenging activity

Different aliquots of GLV extractives (0.2–4.0 mg/ml) were taken in different test tubes. Accordingly, DPPH (50 μM in methanol) was added to make up the total volume to 2 ml. The contents of the test tubes were thoroughly mixed, and the reaction time of 15 min was allowed in dark at room temperature. The absorbance was measured at 517 nm with methanol as blank (Khan et al. 2012).

From the absorbance, % inhibition or % scavenging activity is calculated using the formula,

$$\text{DPPH scavenging activity} = (\text{OD control} - \text{OD sample}) / \text{OD control} * 100$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power assay was performed according to the method of Oyaizu (1986). Different dilutions of GLVs extractives (1 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1 % *w*/*v*) and the mixture was incubated for 30 min at 50 °C. To this, 2.5 ml of 10 % trichloroacetic acid (TCA) was added and centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was taken and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride. The procedure was carried out in triplicate and absorbance was measured at 700 nm. Increase in concentration of the extractives shows higher absorbance which in turn indicates good reducing capacity.

Statistical analysis

All the results were presented in the form of mean ± S.D. of three replicates. Data were subjected to one-way ANOVA followed by post hoc Duncan's Multiple Range Test (DMRT) using SPSS 17 (SPSS Inc., Chicago, IL, USA) for determining significance at $p < 0.05$.

Results and discussion

FTIR analysis of native and defatted GLVs

In the present study, the FTIR spectroscopy was used to identify the functional groups based on the peak values in the IR present in native and defatted samples of GLVs. The native and defatted GLVs powders were subjected to FTIR analysis and the functional groups of the components were separated based on its peaks. The results obtained indicated the presence of following functional groups viz., free alcohol; inter- and intra-molecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching (Table 1). The total fat content in the selected GLVs were found to be high in roselle leaves (1.6 %) and least in sorrel (*R. vesicarius*) leaves (0.73 %).

Determination of chlorophyll content

The chlorophyll content in native and defatted GLVs is shown in Table 2. Among native GLVs chlorophyll content in *B. alba* was high (2.96 g/kg dry weight (DW)) while that of *R. vesicarius* was least (2.12 g/kg DW). Similarly in defatted

Table 1 FTIR peak assignment table of all selected GLVs compared with standard chart

Functional groups	HC	HCD	HS	HSD	BA	BAD	BR	BRD	RV	RVD
Unknown	3740.9	3750.2	3750.3	3750.6	3741.5	3750.3	3740.1	3740.4	3750.1	3741.0
Unknown	3708.0	3707.6	3708.0	3707.6	3707.6	3707.6	–	–	3708.0	3707.6
Free alcohol O-H Stretching	3672.0	3675.6	3672.0	3671.6	3675.6	3671.6	3671.6	3671.6	3672.0	3671.6
Intermolecular bonded alcohol O-H Stretching	3381.3	3383.2	3408.0	3408.8	3339.2	3380.5	3337.0	3366.5	3378.8	3408.2
Intramolecular bonded alcohol O-H Stretching	2925.4	2932.8	2934.5	2929.9	2926.5	2931.2	2925.7	2930.6	2960.0 2926.2	2930.5
C-H stretching alkane	2852.0	–	2856.0	–	2851.5	–	2851.5	–	–	–
C-H bending aromatic compounds overtone	1733.3	1783.4 1727.4	1792.0 1734.8	1783.4 1736.5	1731.4	1731.4	1731.4	–	–	1728.0
C = N stretching imine/oxime or C = O stretching conjugated ketone or alkenes	1659.3 1625.8	1659.2 1607.3	1659.1 1635.1	1659.2 1611.3 1635.0	1659.4 1635.4	1659.4 1626.3	1659.3 1626.4	1659.1 1605.8	1660.3 1635.6	1660.3 1636.0
O-H bending phenol	1372.0 1316.0	1375.3	1368.0	1367.3 1319.3	1371.3 1317.5	1375.3 1320.9	1384.9 1315.3	1315.3	1373.7 1322.9	1372.3 1324.3
C-N stretching amine	1233.3 1152.0 1057.2 1028.0	1232.7 1151.3 1057.4	1230.0 1156.0 1096.0 1062.0	1230.7 1155.3 1099.3 1063.6	1242.5 1153.7 1052.5	1243.5 1151.3 1076.6	1239.3 1153.8 1025.4	1246.8 1154.1 1079.8 1027.1	1243.2 1156.0 1104.0 1065.4	1237.8 1153.9 1083.3 1023.4

HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± SD of three replicates ($n = 3$)

Table 2 Chlorophyll content (g kg^{-1} DW) of the selected GLVs native and defatted leaf samples

	Lichtenthaler			Arnon		
Sample name	chl.a	chl b	Total chl	chl.a	chl b	Total chl
HCD	0.48 ± 0.05[a]	0.11 ± 0.02 [a]	0.58 ± 0.08 [a]	0.56 ± 0.08 [a]	0.11 ± 0.01 [a]	0.67 ± 0.08 [a]
HSD	0.43 ± 0.04 [a]	0.15 ± 0.02 [a]	0.58 ± 0.05 [a]	0.51 ± 0.06 [a]	0.16 ± 0.02 [b]	0.67 ± 0.07 [a]
BAD	0.66 ± 0.05 [b]	0.34 ± 0.03 [b]	1.00 ± 0.08 [b]	0.73 ± 0.09 [b]	0.39 ± 0.03 [c]	1.11 ± 0.11 [b]
BRD	0.69 ± 0.07 [b]	0.19 ± 0.02 [a]	0.88 ± 0.08 [b]	0.80 ± 0.07 [b]	0.21 ± 0.03 [b]	1.00 ± 0.09 [b]
RVD	0.40 ± 0.04 [a]	0.10 ± 0.02 [a]	0.50 ± 0.05 [a]	0.47 ± 0.03 [a]	0.11 ± 0.01 [a]	0.57 ± 0.04 [a]
HC	1.94 ± 0.14 [c]	0.33 ± 0.04 [b]	2.28 ± 0.17 [c]	2.36 ± 0.18 [c]	0.31 ± 0.03 [d]	2.67 ± 0.21 [d]
HS	1.86 ± 0.11 [c]	0.31 ± 0.06 [b]	2.17 ± 0.13 [c]	2.19 ± 0.13 [c]	0.31 ± 0.03 [d]	2.50 ± 0.16 [d]
BA	1.92 ± 0.19 [c]	0.72 ± 0.07 [c]	2.64 ± 0.26 [d]	2.15 ± 0.18 [c]	0.80 ± 0.05 [e]	2.96 ± 0.24 [e]
BR	1.38 ± 0.12 [d]	0.77 ± 0.09 [c]	2.15 ± 0.15 [e]	1.71 ± 0.12 [d]	0.80 ± 0.06 [e]	2.52 ± 0.19 [d]
RV	1.57 ± 0.12 [d]	0.28 ± 0.03 [b]	1.84 ± 0.15 [e]	1.84 ± 0.11 [d]	0.28 ± 0.05 [d]	2.12 ± 0.16 [c]

HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± SD of three replicates ($n = 3$). Significance was tested by Duncan Multiple Range Test at p 0.05, and values with different superscript in each column were found be significant difference from each other

GLVs also, chlorophyll content in *B. alba* was high (1.11 g/kg DW) and the same was least in *R. vesicarius* (0.57 g/kg DW). The method of analysis also plays a pivotal role. In the present study, there was a 10–15 % variation in chlorophyll contents quantified by two methods such as Arnon and Lichtenthaler (Arnon 1949; Lichtenthaler 1996). Generally, post-harvest treatments and processing conditions influence chlorophyll retention as demonstrated in *Moringa oleifera* (Saini et al. 2014) and *Hibiscus sabdariffa* (Kumar et al. 2015). In our study, the variation in chlorophyll content of native and defatted samples looks more like the difference is mainly due to chlorophyll a, since the values of chlorophyll b between two methods was almost similar. In general, while using the optical methods such as spectrophotometry for analysis of chlorophyll, the presence of other pigments (as chlorophyll b, c, and the respective degradation products) is the main interferers in chlorophyll-a determination (Dos Santos et al. 2003). There was a reduction of 74 % and 73 % of total chlorophyll content in defatted leaves of *H. cannabinus* and *H. sabdariffa* compared to native samples when analyzed by Lichtenthaler method. Similarly 62 % and 59 % reduction in total chlorophyll content was observed in *B. alba* and *B. rubra* defatted leaves. In case of *R. vesicarius* it was 73 % reduction compared to native samples. Overall, the 74 % reduction in total chlorophyll content was maximum in *H. cannabinus* defatted leaves compared to native leaves.

In vitro antioxidant assays

Determination of total phenolics content (TPC)

Phenols are aromatic secondary plant metabolites in plants associated with colour, sensory qualities, nutritional and antioxidant properties of foods. The antioxidant property of phenolics is due to redox properties (Kaur and Kapoor 2002). There was a varying amount of TPC in different solvent extractions of both native and defatted samples (Fig. 1). Methanol extractives of native and defatted *H. sabdariffa* showed highest TPC content (17.61 and 9.67 g/kg GAE DW) whereas native *H. sabdariffa* and defatted *B. alba* ethanol extractives showed maximum and minimum TPC respectively (10.82 and 0.74 g/kg GAE DW). There was 50 % reduction in TPC content from GLVs of native and defatted leaf samples. Earlier reports embodies that hot water extract of *Phyllanthus amarus* leaves showed higher TPC than organic solvents. Methanol extractives of fresh samples will be higher than that of processed, because methanol can denature polyphenol oxidases in the plant cell wall degradation, so it can extract

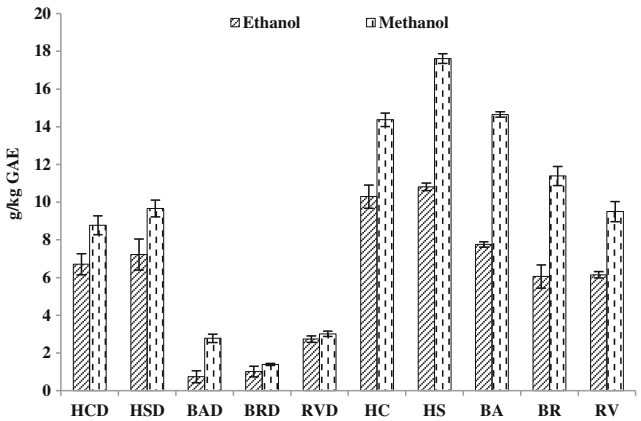

Fig. 1 Total phenolic content (g kg^{-1} GAE) of native and defatted leaf samples. HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± S.D. of three replicates

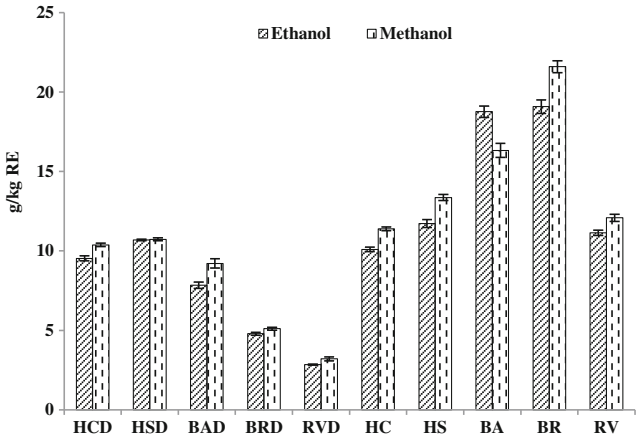

Fig. 2 Total flavonoid content (g kg^{-1}RE) of native and defatted leaf samples. HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± S.D. of three replicates

more TPC than water. But processed plant material extracted with methanol will have lower TPC and antioxidant properties than water (Lim and Murtijaya 2007).

Determination of total flavonoid content (TFC)

Flavonoids demonstrate a wide range of biochemical and pharmacological effects such as anti-oxidation, anti-inflammation, anti-platelet, anti-thrombotic action and anti-allergic effects (Nijveldt et al. 2001). The data clearly outlines (Fig. 2) that among the native GLVs, methanol extractives of *B. rubra* possessed maximum flavonoid content (21.6 g/kg RE DW) and *H. cannabinus* lowest (11.4 g/kg RE DW). Among defatted GLVs, TFC was more in ethanol extractives of *H. sabdariffa* (10.73 g/kg RE DW) and lower in *R. vesicarius* (2.84 g/kg RE DW). In *Basella* spp. there was a drastic (76 %) decrease in flavonoid content, compared to other two GLVs.

Determination of total antioxidant assay

This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex with a maximal absorbance at 695 nm. The native and defatted GLVs exhibited various degrees of antioxidant activity (Fig. 3). The defatted *H. sabdariffa* methanol extractives showed highest TPC (41.06 g/kg AAE) and methanol extractives of *B. rubra* contains least (14.65 g/kg AAE). In native leaves *H. cannabinus* methanol extractives exhibited highest (50.2 g/kg AAE) TPC, while *R. vesicarius* least (29.04 g/kg AAE). The variations in antioxidant activity may be due to phenolics, flavonoids and ascorbic acid contents. Similarly, the extractive type is also important for antioxidant activity as shown for methanol extractives of *Sauropus androgynous* (Subhasree et al. 2009). The correlation between phenolic compounds reflects antioxidant activity (Miller et al. 1993). Especially the post-harvest processing of GLVs influence their antioxidant activity as demonstrated in spinach (Artes-Hernandez et al. 2008) wherein, there was a decrease in total antioxidant activity of UV-C radiation treated minimally processed spinach leaves of about 70–75 % stored at 8 °C and 50 % at 5 °C.

DPPH radical scavenging activity

The DPPH radical scavenging activity is most widely used method to determine the primary antioxidant activity i.e., free radical scavenging activities of antioxidant compounds in

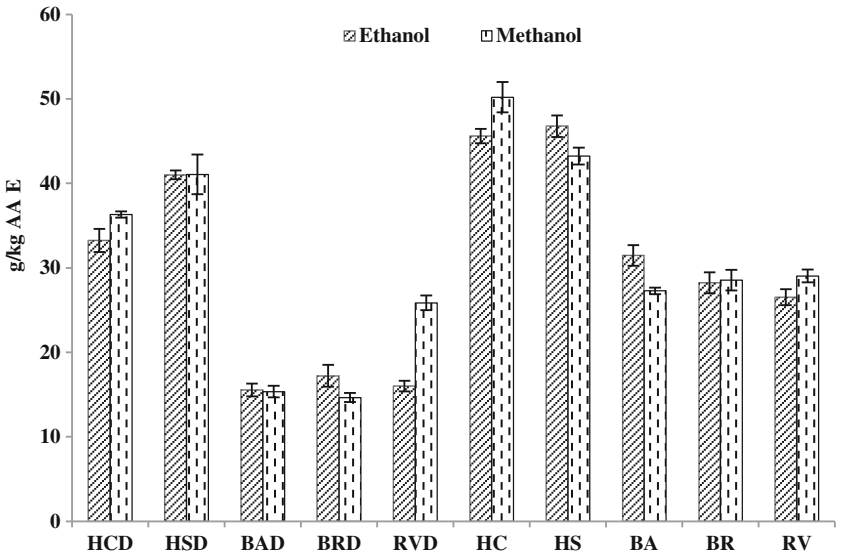

Fig. 3 Total antioxidant assay in crude ethanol and methanol extractives of native and defatted leaf samples. HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± S.D. of three replicates

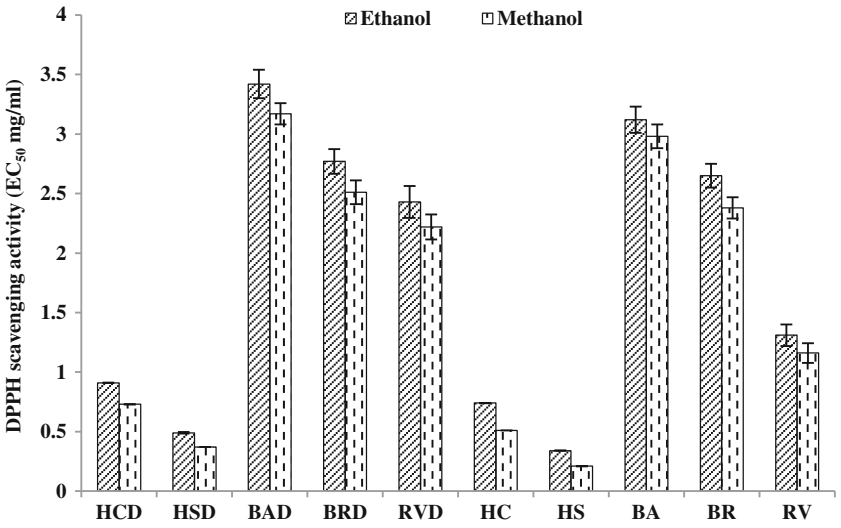

Fig. 4 DPPH free radical scavenging activity of ethanol and methanol extractives of native and defatted leaf samples. HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± S.D. of three replicates

plants and fruit extracts (Sri Harsha et al. 2013). The assay is based on the ability of DPPH; a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance

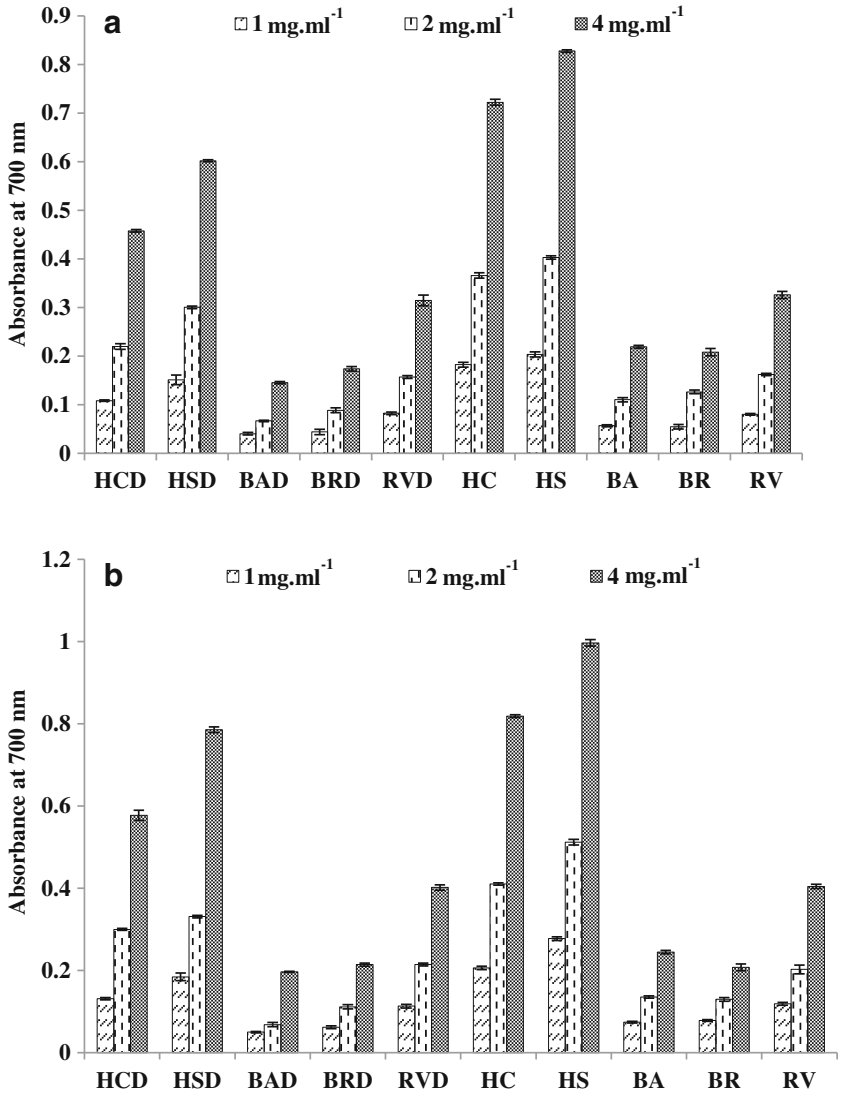

Fig. 5 FRAP in crude ethanol (a) and methanol (b) extractives of native and defatted leaf samples. HCD, *Hibiscus cannabinus* defatted; HSD, *H. sabdariffa* defatted; BAD, *Basella alba* defatted; BRD, *B. rubra* defatted; RVD, *Rumex vesicarius* defatted, HC, *H. cannabinus* native; HS, *H. sabdariffa* native; BA, *B. alba* native; BR, *B. rubra* native; RV, *R. vesicarius* native. Values are mean ± S.D. of three replicates

at 517 nm. DPPH accepts an electron donated by an antioxidant compound, which results in the drop of absorbance values which we can quantitatively measure by recording change in absorbance. In this study, ascorbic acid is used as standard antioxidant. The DPPH free radical scavenging activity of different GLVs native and defatted samples extractives were shown in Fig. 4. When compared to defatted samples, native extractives of GLVs exhibited highest radical scavenging activity. Similar observations were reported in *Sauropus androgynous* (Subhasree et al. 2009), but the free radical scavenging activity potential of respective extracts are dose dependent as showed in *Bambusa vulgaris* (Goyal et al. 2010).

Ferric reducing antioxidant power (FRAP) assay

The reducing power activity of native and defatted GLVs in different solvent extractives is shown in Fig. 5. The purpose of the test of ferrous ion chelating activity was to determine the capacity of dried leaf extractives of GLVs to bind the ferrous ion catalyzing oxidation. In this assay, the leaf extractives showed chelating activity by capturing ferrous ions before ferrozine. The extent of reduction is explained in terms of absorbance values at 700 nm for the increasing concentration ranging from 1 to 4 mg/ml. All the extractives exhibited a concentration dependent increase in reducing activity. In the present study, all the native GLVs extractives exhibited higher levels of reducing power compared to defatted samples. *H. sabdariffa* and *H. cannabinus* exhibited higher absorbance values compared to others. A similar observation was reported for other leafy vegetables (Gupta and Prakash 2009). The efficient ferric reducing power of leaf extractives in this study is due to their TPC, TFC and TAA.

Conclusions

During post-harvest processing of GLVs, off-flavours and odorous state normally happen as a result of lipid autoxidation which eventually leads to rancidity. The same can be eliminated by defatting of GLVs as opitimised in the present study which contributes to extended shelf life. In the present study, the results of the five selected GLVs of native and defatted leaf samples by FTIR analysis confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching. The observations made it evident that the manifestations of bands in the IR region results primarily from the chlorophyll molecule masking the other molecules. Similarly, the method of analysis also plays a pivotal role in chlorophyll determination. In the present study, there was a 10–15 % variation in chlorophyll contents quantified by two methods such as Lichtenthaler and Arnon. Methanol extractives of both native and defatted GLVs showed maximum TPC, TFC and antioxidant potentials.

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Declaration of Interest Authors have no conflict of interest to declare.

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