J.Food Pharm.Sci. 6.3 (2018) 35-43

Journal of Food and Pharmaceutical Sciences
jfoodpharmsci
Available online at jfoodphamsci.com

Research Article

Chemical Characterisation, Organic Acids by HPLC, Fatty Acids by GC-GCMS and Antioxidant Activity of Commonly Consumed Leafy Vegetables in India

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ARTICLE INFO

Received 16/01/2016
Received in revised form 11/06/2018
Accepted 25/12/2018
Available online 01/01/2019

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ABSTRACT

Chemical, organic acids, fatty acid composition and antioxidant activity of commonly consumed leafy vegetables such as *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis* were investigated. High performance liquid chromatography (HPLC) was used to quantify organic acids in leaf powders. Protein and fibre contents of *Alternanthera sessilis* leaf powder were the highest (31.2, 12.5% respectively) and lowest in *Hibiscus cannabinus* leaf powder (20.86 and 3.94%) among the leaf powders analysed. Oxalic acid was found to be the dominant acid in all the leafy vegetable powders and it was maximum in *Alternanthera sesilis* (10733 mg/100g) and *Basella rubra* (23331 mg/100g) powders. Gas and mass chromatography (GC-MS) analysis of leaf powder lipids were rich in palmitic (14.6 – 24.2%), linolenic (25.6 -56.9%), linoleic (15.4-21.1%) and oleic (2.6 - 13.7%) acids. Hibiscus leaf powder exhibited maximum inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical with 95% inhibition at 2.8 mg/ml concentration and assay 92% of ABTS (2, 2-Azinobis-3-ethyl Benzothiazoline-6-Sulfonic acid) diammonium salt) at 0.6mg/ml concentration.

Keywords: *Hibiscus cannabinus; Rumex vesicarius; Basella rubra; Alternanthera sessilis;* chemical composition; organic acids; fatty acid; antioxidant activity;

1. Introduction

Leafy vegetables are rich source of bioactive compounds such as beta carotene, ascorbic acid, polyphenols, dietary fibre and minerals such as iron, calcium and phosphorous. Leafy vegetables are most abundant sources of protein, vitamins and minerals (Shukla et al., 2006). Leafy vegetables such as *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis,* amaranth, fenugreek, palak and spinach has attained commercial status and its cultivation is wide spread in India. Because of their low production cost and high yield, Leafy vegetables are considered to be one of the cheapest vegetables in the market and also highly perishable in nature. The essential oil of Hibiscus cannabinus was reported to rich in phytol (36%) examined by GC-MS. The oil was phytotoxic to lettuce and bentgrass and had antifungal activity (Kobaisy et al., 2001). The anticancer activity of *Hibiscus* leaf polyphenolic extract in melanoma cells was reported by Chiu et al. 2015.The aqueous and methanolic extract of *Hibiscus cannabinus* (Malvaceae) showed anti-inflammatory activity on carrageenan-induced rat paw edema. The extracts showed significant inhibition of rat paw edema in dose-dependent manner. The maximum percent inhibition in paw edema was

found in MHCL at dose of 400 mg/kg was 52.00% (Saba Shaikh and Joshi 2016).

The present investigation on the leafy vegetable was carried out to determine the chemical composition, organic acids, fatty acids and antioxidants activity of locally popular *Hibiscus cannabinus* (HC), *Rumex vesicarius* (RV), *Basella rubra* (BR) and *Alternanthera sesislis* (AS) to highlight their nutritional importance and also help the common public to choose the leafy vegetable according to its organic acid composition and their sensitivity to each of the acid.

2. Materials and Methods

Materials Freshly harvested *Hibiscus cannabinus* (15 kg), *Rumex vesicarius* (12 kg), *Basella rubra* (10 kg) and *Alternanthera sessilis* (14 kg) were collected in batches from different vendors on different days from a Rhythu Bazar at Hyderabad, Telangana, India. The material was immediately processed after procurement. Reagents and solvents used in the study were of analytical and laboratory grade respectively and procured from Sd Fine-Chem Ltd. (Mumbai, India). Chemicals used in antioxidant assays were purchased from Sigma Aldrich, Philadelphia, USA.

2.1. *Preparation of leafy powders*

The leafy vegetables were washed with running water, stalks removed manually and treated with 5 ppm o 0.1% sodium hypochlorite for 30 min. The water was drained and the leaves were dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55 ± 2 ℃ for 8 h to 12 h. The dried materials were ground to powder using a high speed mixer (M/s. Sumeet, Nasik, India), passed through BS 72 (220 µ) mesh. The powders were packed in metallized polyester polyethylene (MPE) laminate pouches and stored at room temperature 29° C for investigation of physico-chemical composition and antioxidant activity. Fatty acids were quantified using gas chromatography, gas chromatography-massspectrometric (GC, GC-MS) analysis and organic acids using high performance liquid chromatography (HPLC).

2.2. *Chemical characterisation*

Physico-chemical composition such as moisture, ash, fat, protein and fiber, total acidity of leaf powders were carried out using standard methods (Ranganna 1986; Pellett and Young 1980). The percent carbohydrate content was calculated by difference method as follows :

% Carbohydrate = [100 – % (moisture + total ash + protein + fiber + fat)].

2.3. *Estimation of total polyphenols*

Quantification of total polyphenol content (TPC) in leaf powders was measured by using a method reported by Sadasivam and Manickam (1997). The powder (1 g of each) was dispersed in 50 ml of 85% ethanol at room temperature (RT) using a magnetic stirrer. An aliquot of the extract (0.5 ml) was mixed with 0.5 ml Folin–Ciocalteau reagent (Sd Fine Chem Mumabai, India) and 5 ml water. The contents were vortexed for 2 min and allowed to settle at RT for 5 min. Satuaretd solution of sodium carbonate (1 ml) was added to the contents, and the volume was made up to 10 ml with distilled water. All the contents were mixed thoroughly by vortexing for 2 min. The contents were allowed to stand at RT for 60 min. The colour developed was measured at 675 nm and total polyphenol content was calculated from a standard gallic acid calibration curve (19-76 µg/ml) and expressed as mg of gallic acid equivalent (GAE) /100 g sample. The total polyphenol content was caliculated as mentioned below:

$$\text{Polyphenols, mg/100 g} = \frac{\text{polyphenols in the aliquot (µg)} \times \text{total volume of solution}}{\text{volume of aliquot taken} \times \text{weight of the sample} \times 1000} \times 100$$

2.4. *Determination of antioxidant activity*

DPPH radical scavenging activity, ABTS assay and ferric ion reducing power was used to determine the antioxidant activity of leaf powders in the range 0.4-20 mg (Nanjo et al., 1996; Re et al., 1999; Yildrim et al., 2001) and compared with that of Trolox at 5-30µg/ml. Each sample of 1 g was dispersed in 50 ml methanol independently and the extraction was carried out using a magnetic stirrer at room temperature (RT) for 20 min. The extracts were stored separately at – 4°C for investigation of antioxidant activity.

2.5. *DPPH radical scavenging activity*

Antioxidant activity of the methanol extract was determined on the basis of their scavenging acidity of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. The leaf extract of 1-5 mg was dispersed in 1 ml of methanol and methanolic solution, (4 ml) of DPPH (0.004% solution) was added. The contents were incubated at RT for 30 min and the colour absorbance was read at 517 nm. The percent inhibition was calculated as follows:

$$\text{Inhibition, \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6. *Ferric ions (Fe3+) reducing power*

The ferric reducing power of the leaf extract was determined by dispersing 1-5 mg in a mixture of 1 ml

methanol, 2.5 ml of phosphate buffer (pH 6.6) in different test tubes. Potassium ferricyanide (2.5 ml) 1% solution was added and the contents were incubated for 20 min at 50 °C. After incubation 2.5ml of 10% tri-chloroacetic acid was added and centrifuged at 8000 rpm for 10 min. The aliquot 2.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was read at 700 nm and expressed as absorbance per mg sample.

2.7. *ABTS radical scavenging activity*

2, 2-Azinobis-3-ethyl Benzothiazoline-6-Sulfonic acid (ABTS) diammonium salt solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate and incubated in the dark at RT for 16 h. The mixture was diluted with 80% (v/v) water to obtain an absorbance of 0.700 at 734 nm. ABTS solution (3 ml) was mixed with 1-5 mg/ml powder vigorously. The control was prepared using water instead of leaf powder extract and its absorbance was recorded at 734 nm after 10 mn. The percent inhibition of the samples was calculated using following expression.

$$\text{Inhibition, \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.8. Determination of organic acids by HPLC

Quantification of organic acids in leafy powders was measured by using a method reported in literature (Silva et al. 2002). The powder (1 g of each) was dispersed in 50 ml of 0.0025 N H_2SO_4 at room temperature (RT) using a magnetic stirrer for 30 min at a pH 2. The contents were filtered and filterate was diluted to obtaine a concentration of 1mg/ml. An aliquot of 20 µl was injected in RP'HPLC using C18 column (250 × 4.6 mm). Quantification of organic acids in powder samples were measured by injecting standard organic acids at 214 nm. The individual organic acid content was caliculated using following expression:

$$\text{Organic acids, mg/100 g} = \frac{\text{Organic acid in the aliquot (µg)} \times \text{total volume of extract}}{\text{volume of aliquot taken} \times \text{weight of the sample} \times 1000} \times 100$$

2.9. *Estimation of β-carotene, lycopene and chlorophyll*

Quantification of pigments such as β-carotene, lycopene and chlorophyll in the powder samples were carried out by extracting 1 g sample in a 50 ml solvent mixture, (acetone:hexane, 4:6) using a magnetic stirrer at room temperature for 30 min (Barros et al., 2011). The contents were filtered and passed through anhydrous sodium sulphate and the absorbance of the filtrate containing the pigment components were measured using a UV-Visible spectrophotometer at 452, 505, 645 and 663 nm. The quantities of pigments were calculated using the following expressions and reported as mg per 100g leaf powder.

β-carotene (mg/100ml) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304\ A_{505} + 0.452 \times A_{453}$

Lycopene (mg/100ml) = $- 0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304\ A_{505} + 0.452 \times A_{45}$

Chlorophyll (mg/100ml)

$$a = 0.999 \times A_{663} - 0.0989 \times A_{645}$$

$$b - 0.328 \times A_{663} + 1.77 \times A_{645}$$

2.10. Determination of fatty acid composition of leaf oils by GC and GC-MS

Leafy powders (50 g) was extracted with hexane solvent at RT. The powder to solvent ratio of 1:3 was maintained and the extraction was carried out using a magnetic stirrer. The hexane solvent was decanted every 3 h, dried over anhydrous sodium sulphate and fat was recovered by distilling off the solvent in a rotary vacuum evaporator at 50 °C. The fat was weighed and stored in glass vials at RT for fatty acid composition.

The fatty acid methyl esters (FAME) of fat was prepared by using mixture of sulphuric acid in methanol (2%, v/v) and were analysed by Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC-MS) as per the column oven programming reported by Syed et al. (2008). An Agilent 6850 series gas chromatograph equipped with an FID detector was used. A DB-225 capillary column (30 m × 0.25 mm i.d) was employed for resoving the fatty acid methyl esters. A HP-5 MS capillary column (30 m × 0.25 mm i.d.) connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; *m/z* 50– 550GC-MS analyses was utilized for mass spectral determinations. The source temperature of 230 °C and a quadruple temperature 150 °C were set as temperature parameters. Structural interpretations were carried out using mass spectral fragmentation patterns and comparing with the retention times of authentic compounds. The fatty acid composition was expressed as weight percent of the fat.

2.11. *Statistical analysis*

Chemical composition, organic acid composition and antioxidant activity were carried out in triplicate and mean values with standard deviation (SD) were computed by using MS excel, 2007. The seed fatty acid composition was analysed in duplicate.

3. Results

3.1. *Chemical composition and yield of leafy vegetables*

Fresh *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis* on dehydration in a tray drier yielded 13.26, 13.48, 10.12 and 16.20% respectively. The photographs of fresh and dehydrated leaf powders are presented in Fig. 1. Chemical composition of powders on fresh weight basis are presented in Table 1. The powders possessed good quantities of protein between the range 20.86 – 31.19% and fibre 3.94 – 12.51%. Total acidity of *Hibiscus cannabinus, Rumex vesicarius* were found to be high at 16.62 and 6.91% respectively**.** The crude fat was higher in *Hibiscus cannabinus* (10.6%) when compared to other leaf powders (2.6-3.7%). The results are comparable with the repoted values by Parvathi and Kumar (2002). Moringa leaf powder was found to contain 30.29% protein and 6.5 % fat (Moyo et al., 2011).

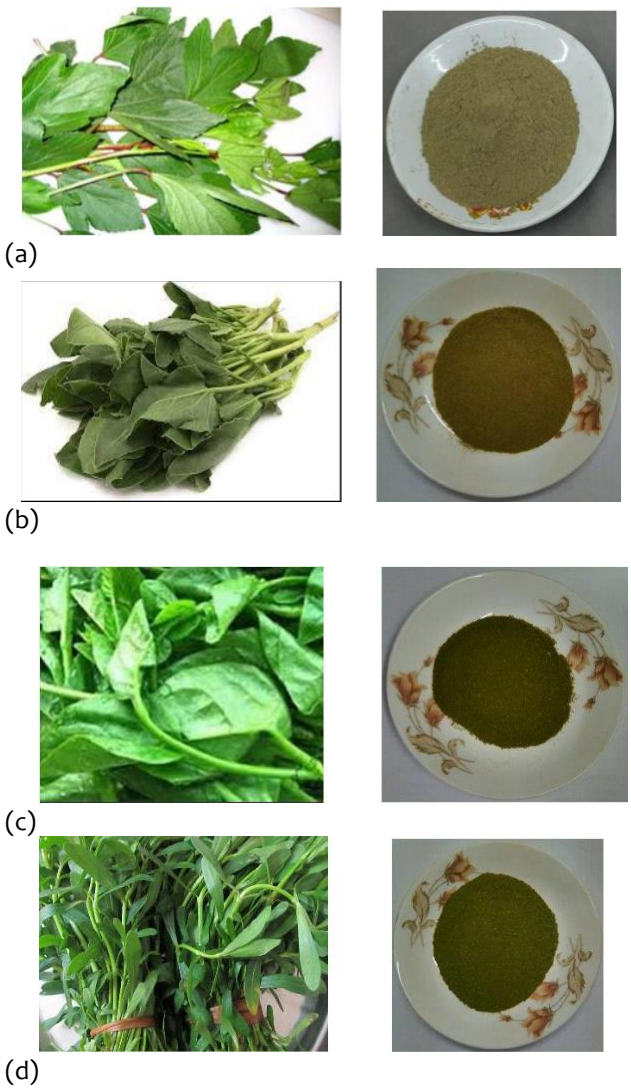

Fig. 1. Photographs of fresh and powderd leafy vegetables (a) Gongura (*Hibiscus cannabinus* L.) (b) *Rumex vesicarius* (c) *Basella rubra* and (d) *Alternanthera sessilis*

Table 1 Physico-chemical composition of leafy powders on fresh weight basis

Parameter, %	HC	RV	BR	AS
Moisture	2.32 ± 0.18	7.00 ± 0.111	7.90 ± 0.18	8.75 ± 0.58
Total ash	7.22 ± 0.27	11.27 ± 0.73	15.66 ± 1.94	10.94 ± 0.20
Crude fat	10.62 ± 0.43	3.70 ± 0.40	3.35 ± 0.18	2.66 ± 0.28
Crude protein	20.86 ± 0.93	27.05 ± 1.08	29.96 ± 1.04	31.19 ±1. 08
Crude fiber	3.94 ± 0.36	7.84 ± 0.39	5.63 ± 0.29	12.51 ± 1.24
Total titratable acidity	16.62 ± 0.46	6.91 ± 0.46	2.07 ± 0.46	1.47 ± 0.46
Total polyphenol content	2861 ± 4.35	1264 ± 9.54	1012 ± 14.57	1638 ± 7.21
β-Carotene	61.96 ± 0.24	110.99 ± 0.24	60.25 ± 0.24	76.29 ± 0.24
Lycopene	114.16 ± 0.60	143.45 ± 0.60	218.47 ± 0.60	182.87 ± 0.60
Chlorophyll a	289.25 ± 7.02	305.09 ± 7.02	299.41 ± 7.02	222.93 ± 7.02
b	29.68 ± 2.46	32.68 ± 2.46	158.98 ± 2.46	100.82 ± 2.46
Hunter colour L*	60.31 ± 0.24	51.17 ± 0.12	53.69 ± 0.006	55.41 ± 0.33
a*	2.49 ± 0.06	-0.62 ± 0.015	-0.38 ± 0.01	-3.74 ± 0.17
b*	23.1 ± 0.96	17.38 ± 0.38	14.60 ± 0.45	19.85 ± 2.49

[a]Values are average of triplicate analysis with ± SD n=3; L*: Lightness; a*: Redness; b*: Yellowness; HC: *Hibiscus cannabinus,*RV: *Rumex vesicarius,* BR: *Basella rubra;* AS: *Alternanthera sessilis*

3.2. *Active components and colour value*

Active components and colour value of leafy powders of *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis were presented* in Table 1. Leafy powders were found to be rich sources of bio active compounds. *Hibiscus cannabinus* possessed higher amounts of total polyphenols (2861 mg/100 g) than other leafy powders (1012-1638 mg/100g). Polyphenols such as rutin, kaempferol, quercetin, etc., are some important plant flavonoids known for their anti-inflammatory, anti-allergic, antithrombitic, hepatoprotective, antispasmodic and anticancer properties reported in the literature (Bruneton, 1999). Carotenoid pigments such as β-Carotene was found to be higher in *Rumex vesicarius* leaf (110.9 mg/100 g), where as lycopene was found to be higher in *Basella rubra* and *Alternanthera sesilis* leaf powders. *Basella rubra* was found to be rich in the chlorophyll pigments. Moyo et al. (2011) reported that moringa leaf powder was rich in polyphenols (2020 mg/100 g) and β-carotene (18.5 mg/100g). Natural antioxidants are found in various parts of plants such as leaves, vegetables, seeds, roots and bark (Mathew and Abraham 2006; Chanda and Dave 2009). Antioxidants especially phenolics and flavonoids from tea, wine, fruits, vegetables and spices are already being exploited commercially either as antioxidant additives or as nutritional supplements (Schular, 1990). The leafy powders were rich in green colour to naked eye after drining process, which was further by the Hunter colour lab units L* and b* range between 51 - 60 and 14 – 23.

3.3 *Organic acid composition*

Oxalic acid was found to be the dominant acid in all the leafy vegetable powders and it was maximum in *Alternanthera sesilis* (10733) and *Basella rubra* (23331 mg/100g) powders (Table 2). Acetic (4786, 14779 mg/100 g)and citric acids (4992, 3231 mg/100g) were found to be major in *Alternanthera sesilis* (10733 mg/100g) and *Basella rubra* leaves respectively. The oxalic acid content in fresh turnip greens was reported in the range of 138.40 and 83.89 mg/100 g (Carmona et al., 2014). Oxalic acid in spinach and amaranth leaves on fresh basis was reported as 420 mg/100 g and 40–50 mg/100 g respectively (Wu et al., 1999; Uusiku et al. 2010). Good quantities of ascorbic acid were observed in Hibiscus and Alternanthera sesilis whereas fumaric acid was a minor acid in all the leaf powders. Citric acid acetic acids were not observed in Hibiscus leaf powder. It was reported that vegetarians who consume large quantities of vegetable rich in oxalic acid may be affected by lower calcium absorption and the maximum risk is for women (Noonan et al., 1999).

Table 2 Organic acids of leafy powders by HPLC analysis [a]

Type of acid mg/100 g	*HC*	*RV*	*BR*	*AS*
Oxalic acids	*5175*	*10733*	*23331*	*4400*
Ascorbic acid	*1865*	*108*	*332*	*1336*
Citric acid	*-*	*29*	*4992*	*3231*
Acetic acid	*-*	*89*	*4786*	*14779*
Fumaric acid	*2*	*12*	*48*	*41*

[a]Values are average of duplicate analyses n= 2; HC: *Hibiscus cannabinus,*RV: *Rumex vesicarius*; BR: *Basella rubra;* AS: *Alternanthera sessilis*.

3.4. *Fatty acid composition of total lipid*

The leaf powders of *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis* on soxhlet extraction using hexane yielded 10.62, 3.70, 3.35 and 2.66% fat respectively. The gas chromatograms of the leaf fats are presented in Fig. 2. The fatty acid composition of fats are reported in Table 3. Polyunsaturated fatty acids were highest (75.2%) in *Hibiscus cannabinus* and lowest (46.7%) in *Alternanthera Sesilis* where as the SFA were found to be highest (47%) in *Alternanthera Sesilis* and lowest (20.9%) in *Hibiscus cannabinus*. Monounsaturated fatty acids were in the range of 3.9 - 15.5% in the leafy vegetable oils with a maximum in *Basella rubra*. Linolenic acid was the major polyunsaturated fatty acid in all the leaf powders and it was maximum (56.9%) in *Hibiscus cannabinus.* Moringa leaf fat was reported to possess α-linolenic acid to an extent of 44. 6% (Moyo et al., 2011). Similar observations on the polyunsaturated fatty acid content of 36.7% comprising linoleic cid (28.83%) and eicosadienoic acid (4.98%) from the total lipid of jatropha. (Akbar et al., 2009). Derewiaka et al. (2014) reported the presence of oleic acid 23.9% in pine nuts and 17.8% in walnuts.

High intake of diets enriched with MUFA protected against atherosclerosis (Moreno and Mitjavila 2003). The ratio of polyunsaturated to saturated fatty

acids (PUFA/SFA) was found to be 0.99 and the ratio of polyunsaturated to monounsaturated fatty acids (PUFA/MUFA) was 1.64 in the total lipid. High PUFAs/SFA and PUFAs/MUFAs ratio increases the level of very low density lipoprotein in plasma but reduces the effect of dietary cholesterol in elevating the triglycerides level in liver (Chang et al., 2004). PUFA + MUFA/SFA ratio was found to be 1.60. The effects PUFA + MUFA/SFA on plasma and liver lipid concentrations in rats were reported earlier (Chang and Huang, 1998. Apart from these fatty acids other fatty acids such as 24:0 (1.8-14.3%) and 22:0 (1.6- 4.2%) were observed in all the leaf fats except *Hibiscus cannabinus*, whereas 20:0 (0.9-2.2%) was observed in all the leaf fats. The air-dried flowers of *Hibiscus sabdariffa* was subjected to hydrodistillation yielded of the essential oil 0.13% on a dry weight basis and fat which is rich in palmitic acid 64.3% and linoleic acid 22.7% (Ebije et al. 2014).

Fatty acid	Retention time (min.)	HC	RV	BR	AS
Palmitic acid (16:0)	10.86	15.60	23.25	22.70	24.20
Stearic acid (18:0)	13.78	3.90	3.30	4.40	3.70
20:0	18.69	1.40	0.90	2.30	0.60
22:0	20.52	-	1.60	2.20	4.20
24:0	26.14	-	1.80	2.00	14.30
Saturated		20.90	30.40	33.60	47.00
Palmitoleic acid (16:1)	11.07	1.22	2.10	1.80	2.30
Oleic acid (18:1)	14.01	2.68	5.40	13.70	4.00
Monounsaturated		3.90	7.50	15.50	6.30
Linoleic acid (18:2)	14.40	18.30	15.40	21.10	21.10
Linolenic acid (18:3)	15.27	56.90	46.70		
Polyunsaturated	-	75.20	62.10	50.90	46.70

[a]Values are mean of duplicate analyses n=2; HC: *Hibiscus cannabinus*,RV: *Rumex vesicarius,* BR: *Basella rubra;* AS: *Alternanthera sessilis*.

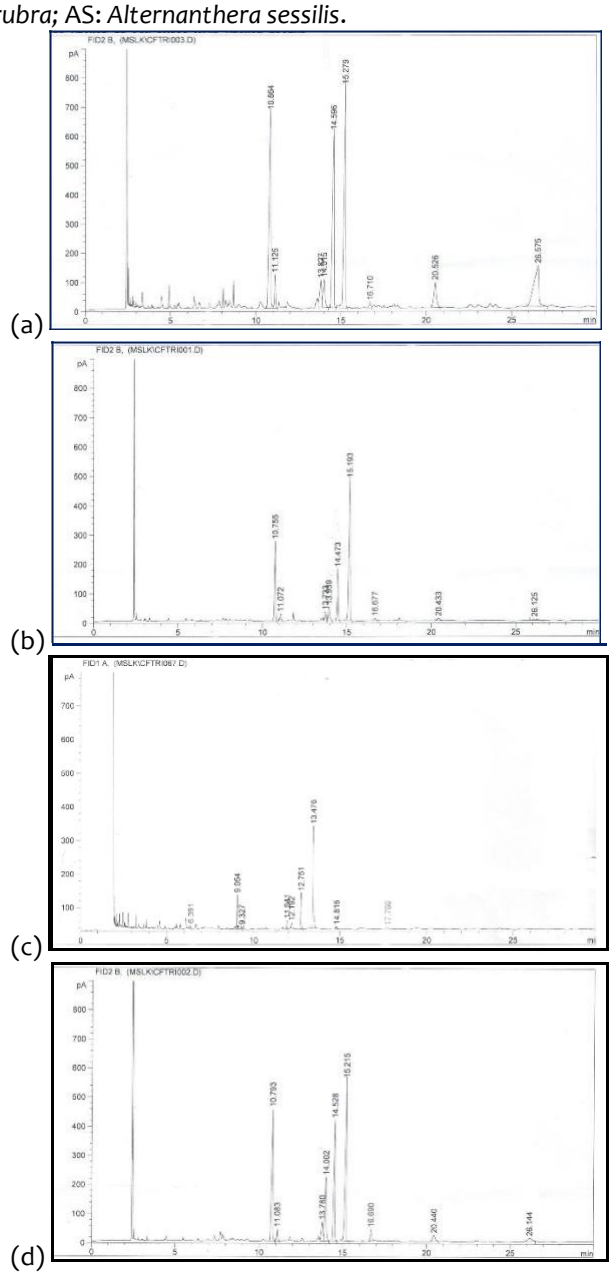

Fig. 2. Chromatograms of GC-GCMS of leafy powders total lipid (a) Gongura (*Hibiscus cannabinus*) (b) *Rumex vesicarius* (c) *Basella rubra* and (d) *Alternanthera sessilis*.

3.5 *Antioxidant activity of methanol extracts*

Data on DPPH radical scavenging activity ABTS assay and ferric ion reducing power of leaf powder extracts were presented in Fig. 3. In all the methods the activity of extracts was dose dependent. Hibiscus lef

powder exhibited maximum inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical with 95% inhibition at 2.8 mg/ml and other leaf powder namely Rumex, Basella and Alternanthera leaf powders showed 95, 90 and 85% inhibition respectively at 10 mg/ml concentration.

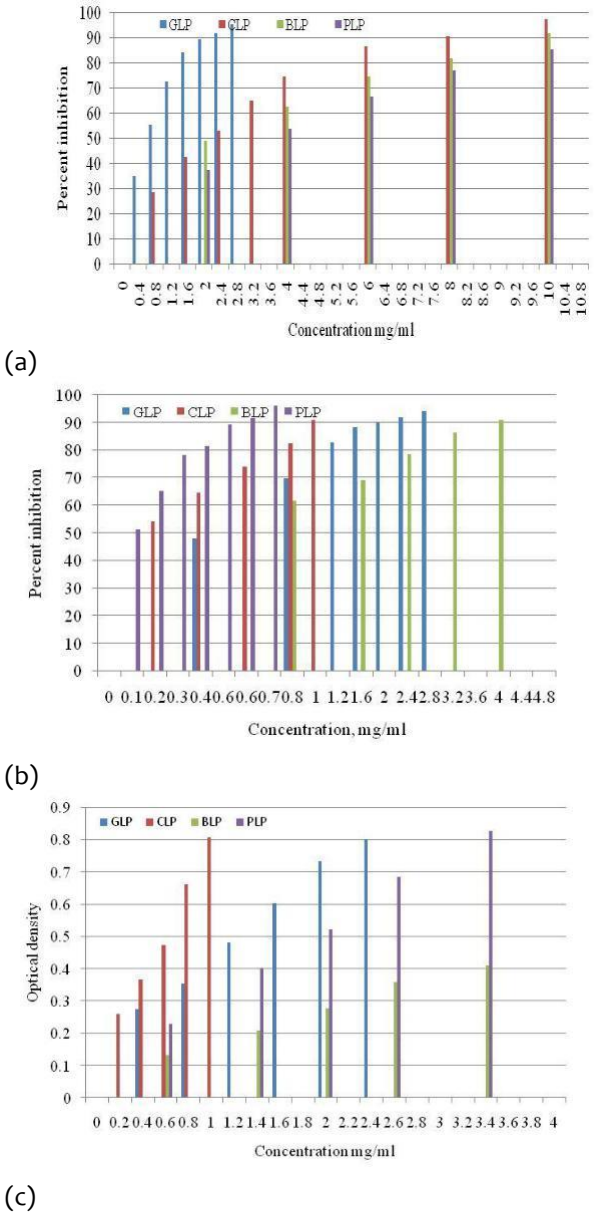

Fig. 3. Antioxidant activity (a) DPPH (b) ABTS and (c) FRP of methanol extract from leafy powders of *Hibiscus cannabinus, Rumex vesicarius, Basella rubra* and *Alternanthera sessilis;* Values are average of triplicate analysis with ± SD (0.004-1.56)

The activity of *Hibiscus cannabinus* was more pronounced in the DPPH assays and *Alternanthera sessilis* followed by *Rumex vesicarius* in ABTS assay. IC_{50} value of DPPH radical scavenging activity of leafy vegetable extracts were 0.7, 2.2, 2.1 and 3.5 mg/ml respectively for *Hibiscus cannabinus*, *Rumex vesicarius*, *Basella rubra* and *Alternanthera sessilis* leaf extracts. However, in case of ABTS assay, IC_{50} values were 0.05, 0.1, 0.4 and 0.4 mg/ml respectively for *Rumex vesicarius* , *Alternanthera sessilis*, *Hibiscus cannabinus* and *Basella rubra* leaf extracts. In ferric ion reducing power, the increase in optical density (0.25-0.8) was much higher for chukkakura for the concentration range of 0.2 to 1.0 mg/ml, whereas the concentration of 2.4 and 3.4 mg/ml showed an optical density of 0.8 for *Hibiscus cannabinus* and ponnaganti leaf extracts resectively. In case *Basella rubra*, the activity was observed to be minimum with a optical density of 0.4 at a concentration of 3.4 mg/ml.

The variation in total polyphenol content on changes in % inhibition in leafy powders might be one of the responsible factors, apart from the other chemical constituents possessing antioxidant activity. The DPPH activity of ethanolic extract and aqueous extract of stevia (20 - 200 µg/ml) increased from 36.93 - 68.76% and 40 - 72.37% in a dose dependent manner and the total phenolic contents were measured as 6.15 and 5.67%. (Shukla et al., 2009; Shukla et al., 2012). The stevia leaf extract with higher total phenol (131 µg) content showed greater antioxidant activity than stevia callus extract with a total phenolic content of 44 µg/ml (Kim et al., 2011).The higher DPPH radical scavenging activity (77.7%) was reported when 250 µg/ml methanolic extract of stevia leaf was used (Ahmad et al., 2010). A similar trend was observed in the cases of ABTS activity and total antioxidant activity of *Momordica cymbalaria* with in IC_{50} value of 13 µg/ml (Prashanth et al., 2013). Phytochemicals of plant origin have been found to possess antioxidant or free radical scavenging activity which find application pharmaceuitical formulations in oxidative stress associated disorders (Lee et al., 2000). The polyphenols of mushroom, tomato and orange were found to responsible for antioxidant activity (Elmastas et al., 2007; Guil-Guerrero and Rebolloso-Fuentes 2009; Klimczak et al., 2007).

4. Conclusion

This study provides the first chemical characterization of commonly consumed leafy vegetables of Hyderabad region in India. This study shows that the selected leafy vegetable powders are rich sources of protein, fibre, polyphenols, β-carotene and lycopene. Oxalic acid was the major acid followed by citric, acetic and ascorbic acids. People allergic to oxalic acid and low calcium absorption can avoid consumption of food prepared with such leafy vegetables. These leafy powders are

also rich source of poly unsaturated fatty acids, particularly linolenic acid (18:3), linoleic acid (18:2) and oleic acid (18:1). The leaf powders possessed considerably high antioxidant activity. Highly periciable and short shelf-life leafy vegetables can be dehydrated and store for long time at room temperature for further culinary preparations. Hence, these leafy vegetable powders could be exploited as a good source of vitamin A as food supplements to overcome vitamin A defficiency.

5. Acknowledgment:

Authors thank the Director, Council of Scientific and Industrial Research-Central Food Technological Research Institute, (CSIR-CFTRI), Mysore for permitting to carry out the research work under the major Laboratory Project No. MLP-0157 (2014-15).

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