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Determination of functional constituents (Phytochemicals, micronutrients), anti-nutrients and antioxidant activity in commonly grown genotypes of *Basella* L.

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Abstract
Basella L. is a very important and commonly used leafy vegetable in tropical and sub-tropical region. It is a poor man's spinach for traditional cuisine and micronutrient source. Naturally, it has three common types i.e. green, red and intermediate in colours, of them green followed by red are most preferred among the consumers. The present study observed significant ($p<0.05$) variation between three genotypes of *Basella* L.. The leaf of red type 'CIARI Shan' had highest ascorbic acid (138.0±1.7 mg/100g), and anthocyanin (280.0±2.0 mg/100g), green type 'CARI Poi Selection' was rich in chlorophyll (135.0±7.6 mg/100g) while flavonoids was highest in leaf of their intermediate type (459.0±3.2 mg/100g). The RP-HPLC analysis of leaf samples identified noticeable difference in three types for major carotenoids (Lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene), phenolics (Caffeic acid, chlorogenic acid, ellagic acid, sinapic acid and naringin) and anthocyanin (Petunidin, cyandin, petunidin, pelargonidin, peonidin and malvidin). Strong positive correlation was observed between antioxidant activity and carotenoids (r=0.70, $p<0.05$ for DPPH; r=0.64, $p<0.05$ for ABTS), chlorophyll (r=0.78, $p<0.05$ for DPPH; r=0.84, $p<0.05$ for ABTS) and ascorbic acid (r=0.31, $p<0.05$ for DPPH; r=0.36, $p<0.05$ for ABTS). The information highlights nutritive potential of green and red types of *Basella* L. or its further promotion in nutritionally challenged communities in tropical and sub-tropical regions.

Keywords: Phytochemicals, anthocyanin, micronutrients, underutilized leafy vegetable, tropical islands

Introduction
Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major sources of oxidative stress in cells which cause damage to proteins, lipids, and DNA. They are causative factors for cardiovascular diseases, atherosclerosis, cancer, diabetes mellitus, neuro-degeneration and ageing (Azizova, 2002) [3]. The exogenous antioxidants are crucial for maintaining *in built* redox homeostasis of body (Bouayed and Bohn, 2010) [7]. The phytochemical like anthocyanin, carotenoids, phenolics, flavonoids and ascorbic acid (Rahman, 2007) [19] are the strong agents for the antioxidant hypothesis which state that antioxidants can prevent oxidative damages and increased intakes from the diet will also reduce the risks of chronic diseases (Stanner *et al*., 2004) [29]. Green leafy vegetables are rich in antioxidants and dietary minerals (Singh *et al*., 2011) [26] and relatively inexpensive and widely consumed foods with ethno-medicinal perceptions (Vishwakarma and Dubey, 2011; Thongam *et al*. 2016; Singh *et al*. 2018) [33, 31, 24].

Indian spinach (*Basella* spp.; Basellaceae) is one of the leafy vegetables which are very popular and commonly consumed particularly in tropical regions. It is highly productive leafy vegetables and known as Poi, Ceylon spinach, Malabar spinach, Indian spinach and poor man's spinach. Indian spinach or *Basella* has two commonly grown species namely *Basella alba* L (green leaf and stem, white flowers) and *B. rubra* L. (green purplish leaves, red vein and stem, purplish white flowers). There is also an intermediate type which is seen at farmers' field and vegetable market. *Basella* L. Leaves are most commonly consumed as boiled, fried or mix vegetable items however, its tender stem part is also being consumed (Singh *et al*. 2018) [24]. *Basella* leaves (100g dry) contain 9.01g crude fibre, 7.8g protein, 3.7g ash, 79 mg potassium, 24 mg phosphorus and provide 270.6 calories (Vishwakarma and Dubey, 2011) [33]. The leaves and stem are mucilaginous and consumed as vegetable, soup, roasted, fried or even in some traditional health practices (Adhikari *et al*., 2012). Haskel *et al*. (2004) [2, 13]

invested that intake of Indian spinach increased β-carotene and iron level in blood serum in human. It highlights the potential this leafy vegetable in fight against micronutrient malnutrition prevalent in children and women in tropical region. Besides, a number of health perception were also reported for *Basella* as laxative, alleviate labour pain, blood purifier, blood enhancer, improve vision ointment for ulcers, irritant, bruise, ringworm, acne freckle, anti-pruritus and burn and to treat diarrhoea, inflammation, and as rubefacient, diuretic and febrifuge (Adhikari *et al*., 2012) [13]. However, occurrence of two dominant types (green and red) and one intermediate coloured *Basella* invites attention to investigate the promising type for promotion as source of nutrition.

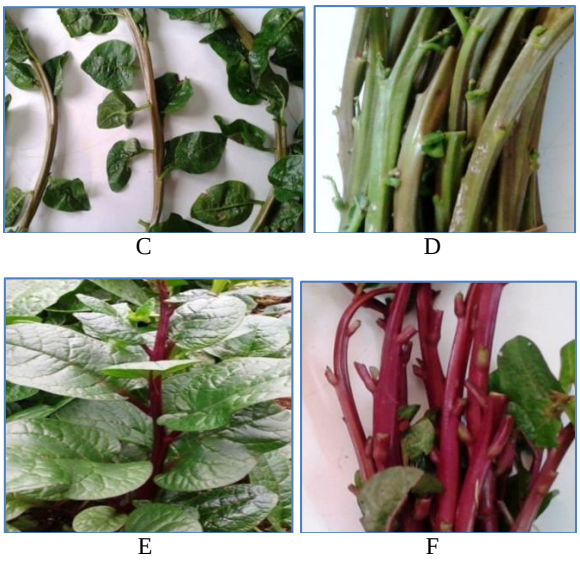
The quality and quantity of light affect the physiological activities of plants and influence synthesis process of secondary metabolites (Bian *et al*., 2014) [6]. Some of them like carotenoids, anthocyanin and flavonoids are directly associated with light through light-interception process or their own synthesis (Iigusa *et al*., 2005; Besseau *et al*., 2007) [14, 4]. These phytochemicals contribute in functional properties of the plant extracts (Rahman, 2007) [19]. Further, colour of edible plant tissues also play important role in phytochemical constitution and degree of functionality against *in vitro* free radical scavenging activity (Tinrat, 2016) [32]. Although, *Basella* green is most preferred followed by red while intermediate type is least preferred among the consumers (Singh and Singh, 2012; Singh *et al*. 2018) [25, 24], however detailed information on micronutrients and phytochemical constituents in these different coloured variants was required to upscale the cultivation and promote in grow-an-eat type nutrition programme. Hence, the study was aimed to analyse micronutrients and phytochemicals in two most commonly consumed parts i.e. leaf and tender stem of all three natural coloured types of *Basella* using spectrophotometric and high performance liquid chromatography (HPLC).

Materials and Methods

Plant materials

Three genotypes of *Basella* L. namely *B. alba* 'CARI Poi Selection' (green leaf & stem type), *B. rubra* 'CIARI Shan' (green-light red leaves, red veins and stem type) and intermediate genotype (appears to be a natural hybrid of green and red type with green leaves, light purple veins and stem) were selected for present study (Figure 1a-f). The CARI Poi Selection and 'CIARI Shan' has been identified by Institute Variety Release Committee of ICAR- CIARI, Port Blair for commercial cultivation in tropical island condition (Gautam *et al*. 2016) [12]. Fresh young edible stage leaves and tender stem parts of ten random plants in each of three genotypes were collected in ice-box in morning hours (9.00- 10.00 AM) from Indigenous Vegetables Germplasm block at ICAR (Indian Agricultural Research Council)-Central Island Agricultural Research Institute (CIARI), Port Blair. The genotypes were grown with standard crop practices. The collected parts were washed with Millipore water and cut into small pieces in dark room condition.

A B



C D E F

Fig 1: *Basella* L. CIARI Poi Selection leaf & stem (a-b), Intermediate type leaf & stem (c-d) 'CIARI Shan' leaf & stem (e-f)

Sample preparation

Samples (2g) were repeatedly ground four times with methanol solvent (10 ml each time) till devoid of colour. Sample mixture (40 ml) was centrifuged at 8000 rpm for 10 min and filtered through Whatman No. 1 filter paper. Acetone was used as a solvent for sample preparation for carotenoids and chlorophyll while methanol: formic acid: water (70:2:28) for anthocyanin estimation. Solvent evaporated from sample by rotary evaporator at 40-45˚C and samples again dissolved in a known volume of methanol and 1ml used for spectrophotometric analysis. All the chemicals used in study were from Merk, Sigma-Aldrich and Hi-Media. The samples were kept at -20ºC for further analysis.

Total phenolics was analysed by spectrophotometer (Elico SL-164, Elico Ltd., Hyderabad, India) at 700 nm using Folin–Ciocalteau reagent (10%, v/v) (AOAC, 1995) [1] with some modifications (Singh and Singh, 2013) [27] and expressed as mg gallic acid/100g of fresh weight. Total tannin was estimated by Folin–Danis reagent and results expressed as mg tannic acid/100g FW. Standard methods were employed for estimation of flavonoids, ascorbic acid, chlorophyll and carotenoids (Sadasivam and Manickam, 1996) [22]. The pH differential method was used for estimation of anthocyanin (mg /100g using cynidine-3- glucoside) (Fuleki and Francis, 1968) [11].

Anti-nutrients

Phytate content was anlaysed using method described by Hassan *et al*. (2011) [13] and Singh *et al*. (2015) [28]. For this, 4 g sample soaked in 100 ml of 2% HCl (v/v) for 3 h and filtered. 25 ml of the filtrate was mixed with 5 ml of 0.3% Ammonium thiocyanate (aq) and 53.5 ml of Millipore water. The mixture was titrated against standard ferric chloride (aq) solution containing 0.00195 g Fe/ml until a brownish yellow colour persisted for 5 min. Blank was treated in a similar manner. Phytin-Phosphorus (1 ml Fe = 1.19 mg Phytin-Phosphorus) 3 was determined and the phytate content calculated by multiplying the value of Phytin-Phosphorus by 3.55.

Nitrate content was determined using spectrophotometric method as per the procedure described by Hassan *et al*. (2011) [13]. In brief, 5 g sample was added with 150 ml millipore water and kept in water bath at 45 °C for 1 h after that the

mixture was cooled and centrifuged at 5000 rpm for 15 min and supernatant was taken into test tube. 0.2 ml extract was added with 0.8 ml of 5% (w/v) salicylic acid-sulphuric acid reagent and mixed thoroughly. After 20 min of incubation, the pH was raised to above 12 using 2 M NaOH solution. The mixture was cooled to room temperature and absorbance was taken at 410 nm using spectrophotometer. Concentration of nitrate in the samples was extrapolated using calibration curve (made by use of KNO_3) and nitrate content in the sample was calculated by using the formula: nitrate (mg/100 g) = [(titre $value_{ppm}$ - solution $volume_{ml}$)/(aliquot $volume_{ml}$ × sample $weight_g$) × 100]. Saponin content was determined as per AOAC method (1995) [1]. Oxalate content in *Basella* samples was determined by titration method described by Day and Underwood (1986). The content was titrated against standard 0.05 M $KMnO_4$ aqueous and oxalic acid was calculated from 1 ml 0.05 M $KMnO_4$ = 0.00225 g anhydrous oxalic acid equation. The titration method as described by Day, and Underwood, 1986 was followed. For this, 1g of sample in 100ml conical flask was added with 75ml 3M H_2SO_4 and stirred for 1hr with a magnetic stirrer. This was filtered using a Whatman No. 1 filter paper. 25ml filtrate was taken and titrated while hot against 0.05M KMnO4 solution until a faint pink colour persisted for at least 30 sec. The oxalate content was then calculated by taking 1ml of 0.05m KMnO4 as equivalent to 2.2mg oxalate (Chinma and Igyor, 2007) [8].

Antioxidant activity

The electron-donating capacity of methanol extract of samples was determined using DPPH (Wang *et al*., 2006) [34] and ABTS (Re *et al*., 1999) [21] methods. Samples prepared as per the procedure described for phytochemicals in sub-section 2.2. In DPPH method, 0.1 ml of sample extract added to 3 ml of 0.001M DPPH methanol solution and incubated for 30 min. Absorbance took at 517 nm in UV–visible spectrophotometer for sample, blank (1 ml of DPPH· and 5 ml of 80% methanol) and rutin and ascorbic acid standards. In ABTS method, $ABTS^{\bullet+}$ solution was prepared by mixing equal quantities of 7 mM ABTS solution with 2.4 mM potassium persulfate and keeping for 12 h at room temperature in dark. Solution diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 unit at 734 nm. The sample extract (1 ml) added to 1 ml of ABTS solution and absorbance took after 7 min at 734 nm. Absorbance was corrected at each time point using ABTS blank and trolox standard. Both DPPH and ABTS activities calculated by formula: Antioxidant activity (%) = $[(A_o - A_e) / A_o] \times 100$; where, A_o for absorbance without extract and A_e was absorbance with extract.

HPLC analysis of phytochemicals

Carotenoids

The carotenoids were extracted from leaf portion of three genotypes of India spinach using the method followed by Olives-Barba *et al*. (2006) [18] with minor modifications. In brief, 2g sample ground in 10 ml absolute methanol, centrifuged at 5000 rpm for 20 min and process repeated till devoid the colour Extract filtered through Whatman No. 1 filter paper and than through 0.45 µm membrane filter. 20µl sample injected in RP-HPLC (Dionex India Pvt. Ltd., Mumbai) and analysed under isocratic condition. Mobile phase had methanol (solvent A) and acetonitrile (solvent B) in 90:10 ratio at a flow rate of 1.0 ml / min. Column temperature was 22°C and absorbance read at 450 nm. Peaks for carotenoids confirmed by retention time of their respective standards.

Phenolics

The phenolic content in leaf samples was analysed by injecting 20 µl sample in RP-HPLC. The flow rate was 0.8ml/min and mobile phase was a binary solvent system consisting of solvent A (dilute acetic acid; 0.9%; pH-2.7) and solvent B (100% acetonitrile) and gradient was 9% (0- 5 min), 11% (5-15 min), 18% (15-22 min), 23% (22-38 min), 90% (38-43 min), 80% (43-44 min), 80% (44-45 min), 5% (45-60 min) at 38°C and 280 nm. Column temperature was 38°C and absorbance read at 280 nm and compounds identified using retention time of the standards.

Anthocyanin

Anthocyanin compounds in leaf part were quantified by injecting 20 µl sample in RP-HPLC and flow rate kept at 0.8ml/min. Mobile phase was a binary solvent system consisting of solvent A (100% acetonitrile) and solvent B (5% acetic acid and 10 % acetonitrile) and gradient was 100% (0-5 min), 80% (5-20 min), 60% (20-25 min), 100% (25-30 min) and column temperature was 25°C and absorbance read at 530 nm. HPLC grade standards were used for identification of individual compound. Previously reported retention time of anthocyanins was also used as reference.

Micronutrient analysis

For micronutrient analysis, the samples were converted to ash by combusting plant materials in silica crucibles in a muffle furnace at 600 °C for 6 hours (AOAC, 1995) [1]. 2 ml HCl added to sample and volume made to 50 ml using Millipore water. Mixture filtered through Whatman No.1 filter paper and took readings using respective PHOTON Lamps for Mg, Fe, Ca, Co, Cu, Mn and Zn in Atomic Absorption Spectrophotometer (AAS; Shimandzu AA 6200). Micronutrient content was determined by the calibration curve and following equation: micronutrient (mg/100g dry weight, DW) = $(C \times df \times 100) / 10^6$, where, C was absorbance and df is dilution factor.

Statistical analysis

All the data were recorded in triplicate and analyzed for ANOVA and Pearson's correlation coefficient using OPSTAT software (http://hau.ernet.in/opstat). Standard deviation from triplicate was analyzed by Microsoft Excel software.

Results and Discussion

Micronutrients analysis revealed the *Basella rubra* 'CIARI Shan' leaves as rich source of Fe (8.4±0.8 mg/100 g) and Mn (9.0±1.6 mg/100g) while Mg and Ca content were the highest in *Basella alba* 'CARI Poi Selection' (71.8 ± 1.7 mg/100 g; 152.5± 2.5 mg/100 g, respectively). Leaf portion of 'CARI Poi Selection' and 'Intermediate type' had higher Cu than 'CIARI Shan' and 'Intermediate type' leaves were rich in Ca (205.5±2.5mg/100g) and Zn (5.9±0.7mg/100g). Stem portion was also rich in Ca, Mg and Fe (Table 1). The findings for micronutrients in 'CARI Poi Selection' were in agreement with reports of Vishvakarma and Dubey (2011) [33] and information on 'Intermediate type' and CIARI Shan' highlights their nutritional significance. Here, it is important to mention that Cu, Fe, Zn and Mn contribute as cofactors in antioxidant activity of plant extracts. Such as, copper is an essential cofactor for critical enzymes such as cytochrome C oxidase and copper-zinc superoxide dismutase (Evan and

Helliwell, 2001) [9], Fe for catalase enzymes haemoglobin and myoglobin, Mn for Mn-SOD enzyme and Zn for over 30 mammalian proteins (Rahman, 2007) [19]. Thus, intake of 125 g of Indian spinach can contribute around 25-30 % of recommended dietary allowance (RDA) of Ca and Fe suggesting Indian spinach as potential source in reducing micronutrient deficiency in human body.

The results for phytochemical contents in three genotypes of *Basella* revealed significant (p<0.05) differences among the genotypes and also between the leaf and stem parts (Table 1). Tannin was most important constituent followed by carotenoids, flavonoids and anthocyanin in *Basella*. The leaf part was significantly rich in ascorbic acid content than stem part and it was highest in leaves of red type 'CIARI Shan' (138.0±1.7 mg/100g) while lowest in red type (9.8±0.3 mg/100g). The leaves of CARI Poi Selection was rich in chlorophyll (41.6±0.9 mg/100g) followed by 'Intermediate type' (21.6±1.8 mg/100g) while it was lowest in 'Intermediate type' (51.0±3.6 mg/100g). Chlorophyll a had higher proportion in total chlorophyll in all three types of Indian spinach. Anthocyanin was highest in leaves of red type (280.0±2.0 mg/100g) and lowest in CARI Poi Selection (160.0±3.0 mg/100g) which reflected by colour pattern of three types. Stem of red (410.0±3.0 mg/100g) and 'Intermediate type' (320.0±2.65 mg/100g) had significantly (p<0.05) higher anthocyanin than their leaves. Flavonoid content was high in leaf (459.0±3.2 mg/100g) than stem portion (307.0±2.0 mg/100g) in 'Intermediate type' which was also higher than parts of CARI Poi Selection and red types. Anthocyanin had CARI Poi Selection >'Intermediate type' >CIARI Shan order while reverse order was observed for chlorophyll, carotenoids, phenolics and tannins. Chlorophyll acts as antioxidant (Ferruzzi *et al*., 2006) [10] and also contributes in synthesis of carotenoids, phenolics and tannins and these phytochemicals are antioxidants therefore, health benefits of Indian spinach could be attributed to high content such compounds. The variation among three different coloured genotypes could be due to their difference in genotypic levels as they represent different botanical species (Besseau *et al*., 2007; Raju *et al*., 2007) [4, 20].

Phytate, nitrate and oxalate contents were significantly (p<0.05) high in leaf part than stem part of all three genotypes (Table 1). 'CARI Poi Selection' leaves had higher oxalate (40.0±2.2 mg/100g) and phytate (293.8±2.4 mg/100g) while nitrate was highest in 'Intermediate type' leaves (75.0±2.6 mg/100g). Nitrate content in *Basella* genotypes are in agreement with reports of Sanchez-Echaniz *et al*. (2001) [23]. High content of nitrate and phytate in leaves might be due to their role in photosynthetic tissues and activities. Oxalate storage in leaf portion was in conformity with reports of Noonan and Savage (Noonan and Savage, 1999) [17]. Phytate and oxalate interfere with bioavailability of micronutrients and enzymes (Evans and Helliwell, 2001) [9] and excess nitrate causes methemoglobinemia (Sanchez-Echaniz *et al*., 2001) [23] but their values were low in Indian spinach than their risk levels. It is consumed after cooking which destroy cell matrix and leach out anti-nutritional compounds (Singh *et al*., 2013; 2015) [27, 28].

Table 1: Phytochemicals (mg/100g FW) and micronutrient (mg/100g DW) contents in leaf and stem of Indian spinach types

Phytochemicals	Leaves			Stem			C.D.
	CARI Poi Selection	Intermediate type	CIARI Shan	CARI Poi Selection	Intermediate type	CIARI Shan	(at 5%)
Anthocyanin (mg/100 g)	160.0±3.0	186.7±2.3	280.0±2.0	120.0±5.0	320.0±2.6	410.0±1.0	5.8
phenols (mg/100 g)	175.3±2.1	150.7±3.6	142.0±1.7	61.0±2.6	67.0±1.7	70.0±1.7	3.7
Carotenoids (mg/100 g)	437.3±5.1	208.7±4.9	179.2±10.3	261.3±22.7	205.6±11.8	155.8±5.6	22.5
Tannins (mg/100 g)	790.0±6.9	782.0±1.7	685.0±5.6	320.0±1.7	385.0±2.6	412.0±2.6	6.0
Ascorbic acid (mg/100 g)	113.6±0.4	100.0±1.5	138.4±1.7	63.2±1.2	60.8±1.1	66.4±0.1	0.4
Flavonoid (mg/100 g)	407.6±4.5	459.0±3.2	453.6±4.0	275.0±2.6	307.0±2.0	268.0±1.0	2.7
Chlorophyll (mg/100g)	41.6±0.9	21.6±1.8	9.8±0.3	12.9±2.6	11.567±0.9	7.067±0.7	2.8
Phytate (mg/100 g)	293.8±2.0	274.0±3.1	287.0±2.6	97.0±2.0	85.3±1.9	72.7±1.5	0.1
Nitrate (mg/100 g)	69.0±3.6	75.0±2.6	45.0±1.7	41.0±1.0	43.1±0.9	36.0±1.0	2.6
Oxalate (mg/100 g)	40.0 ± 2.2	37.6±0.2	29.3±0.1	36.4±1.4	29.7±1.2	23.6±1.1	0.3
Micronutrients (mg/100g DW)							
Co	13.8±2.1	13.9±2.5	8.05±2.5	4.61±1.2	5.13±1.2	6.47±1.9	1.8
Mn	7.9±1.5	7.9±1.7	9.0±1.6	8.25±1.2	7.07±1.5	5.32±2.1	0.6
Ca	152.5±2.5	205.5±2.5	202.8±2.4	191.16±2.8	215.96±7.5	118.81±1.7	7.6
Mg	71.8±1.7	61.7±1.3	69.9±4.5	90.55±0.5	63.69±1.7	94.97±4.5	4.4
Fe	5.77±0.4	4.6±0.1	8.4±0.8	12.59±0.4	9.16±0.3	21.59±1.4	1.4
Zn	5.74±0.2	5.9±0.7	4.6±0.1	4.97±0.5	3.71±0.1	3.82±0.3	0.5
Cu	0.43±0.1	0.57±0.02	0.30±0.05	0.48±0.03	0.47±0.04	0.41±0.01	0.1

The results of *in vitro* study for antioxidant activity of Indian spinach types are presented in Figure 2. Significant (p<0.05) difference observed between DPPH and ABTS activities. Leaf fraction had significantly (p<0.05) higher DPPH and ABTS activities than stem extract. Both methods showed higher activities for ascorbic acid (88.1%; 78.4%) and rutin (78.2%; 70.5%). Among the extracts, it was highest for leaf extract of CARI Poi Selection (DPPH, 83.5%; ABTS, 75.0%) while red type had lowest activities (60.5% and 58.9%, respectively). It might be due to high content of carotenoids, tannins and phenolics in CARI Poi Selection. Ascorbic acid and chlorophyll also support quenching of free radicals. Low antioxidant activity of stem fraction of three types could be attributed to poor phytochemical profile.

The RP-HPLC chromatogram for carotenoid analysis of leaf extract of CARI Poi Selection generated more peaks (16) than 'CIARI Shan' (10) and 'Intermediate type' (7) (Figure 3a). The identified carotenoids were lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and remaining unidentified peaks. The results support high content of total carotenoids in CARI Poi Selection besides confirming earlier reports of Raju *et al*. (2007) [20] on *B. alba* and Lakshminarayana *et al*. (2005) [15] in *B. rubra* along with new information on 'Intermediate type'. In phenolics analysis, the study observed 10 peaks for red types, seven for 'Intermediate type' and five for CARI Poi Selection (Figure 3b). The identified compounds were caffeic acid, chlorogenic acid, ellagic acid and naringin in CARI Poi Selection and

'Intermediate type' while red type had sinapic acid, rutin, and four unidentified peaks. The RP-HPLC analysis of leaf part for anthocyanin (Figure 3c) identified only petunidin in CARI Poi Selection while 10 peaks in each of 'Intermediate type' and 'CIARI Shan' representing cyanidin, petunidin, pelargonidin, peonidin, malvidin and two unidentified peaks.

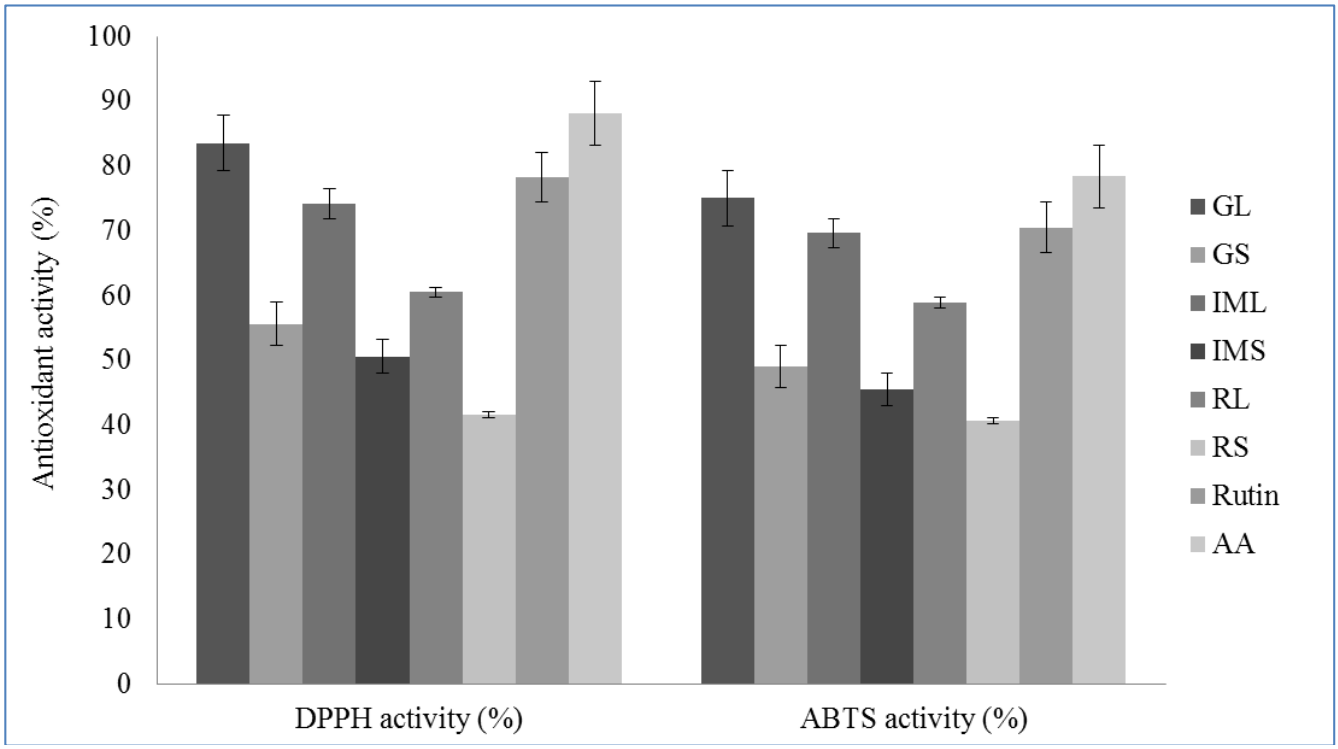
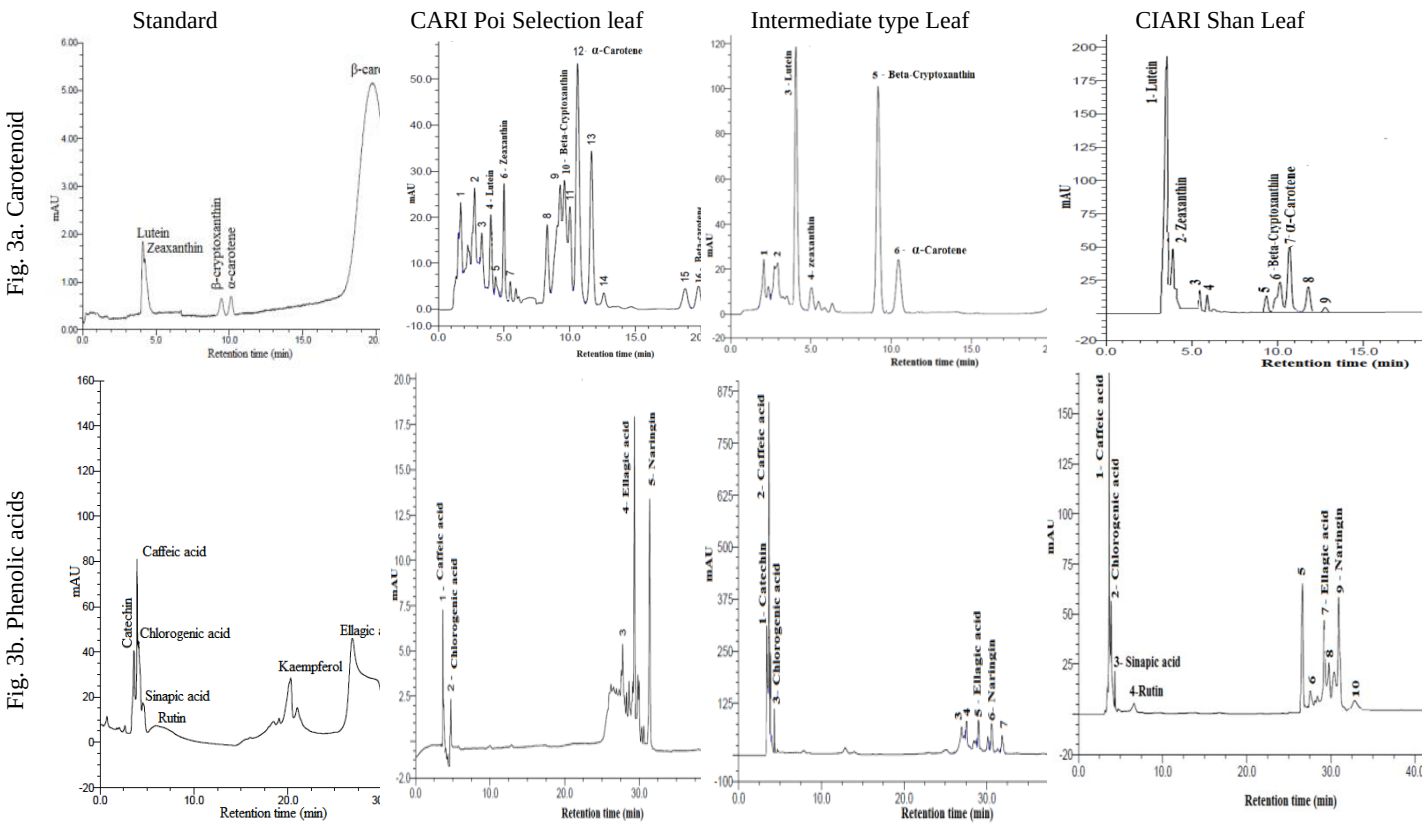


Fig 2: Antioxidant activity of different parts of Indian spinach genotypes. (GL-CARI Poi Selection leaf, GS- CIARI Poi Selection stem, IML-Intermediate type leaves, IMS-Intermediate type stem, RL- CIARI Shan leaves, RS- CIARI Shan stem), (Reference Rutein and AA- Ascorbic acid)



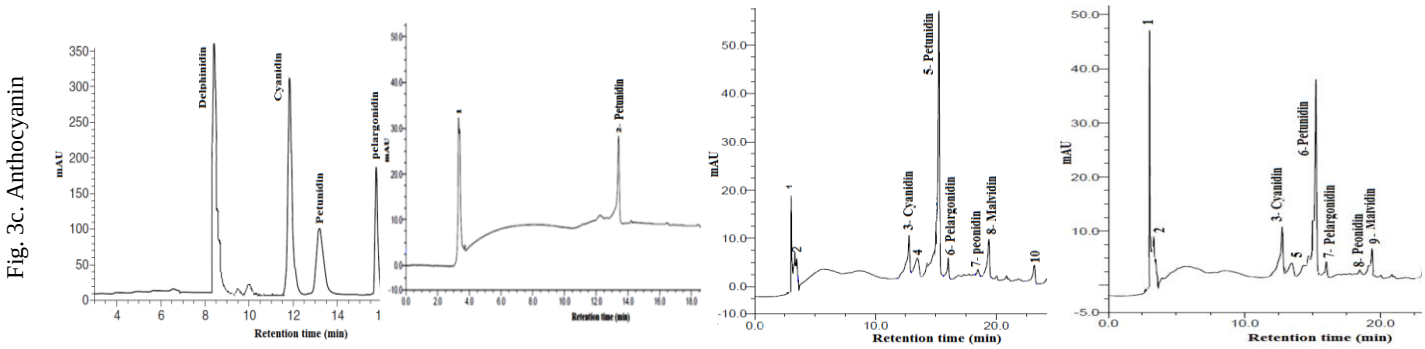


Fig 3 (a-c): HPLC chromatogram of leaf samples of three types of Indian spinach types

The correlation between phytochemicals and antioxidant activity remain good but it depends on nature of sample and on compounds matrix of extract Moure *et al*. (2001) [16]. In present study, significantly positive correlation was observed between DPPH antioxidant activity and phenolics (r = 0.945, p<0.01), flavonoids (r = 0.832, p<0.05) and chlorophyll (r = 0.839, p<0.05) (Table 2). The carotenoids (r = 0.718, p<0.05), tannin (r = 635, p>0.05) and ascorbic acid (r = 0.717, p>0.05) also had positive correlation while anthocyanin (r = -0.630, p>0.05) showed negative correlation with DPPH activity. Similar observations were made for ABTS activity. Strong correlation between ABTS and DPPH methods (r = 0.99, p<0.05) was in the line of reports by Bhoyar *et al*. (2011) in *Capparis spinosa*. Correlation between antioxidant activities and micronutrients in Indian spinach was in conformity with reports of Tarwadi and Agte (2007) [30] on fruits of Indian sub-continent.

Table 2: Correlation matrix of phytochemical contents of *Basella* L. genotypes

Particulars	ABTS activity	DPPH activity	Antho-cyanin	Phenols	Carote-noids	Tannins	Ascorbic acid	Flavo-noid	Chloro-phyll	Co	Mn	Ca	Mg	Fe	Zn
DPPH activity	0.988**	1.000													
Anthocyanin	-0.701NS	-0.630NS	1.000												
Phenols	0.893*	0.945**	-0.378NS	1.000											
Carotenoids	0.718NS	0.630NS	-0.682NS	0.487NS	1.000										
Tannins	0.616NS	0.635NS	-0.054NS	0.673NS	0.173NS	1.000									
Ascorbic acid	0.628NS	0.717NS	-0.207NS	0.867*	0.233NS	0.504NS	1.000								
Flavonoid	0.752NS	0.832*	-0.311NS	0.903*	0.149NS	0.711NS	0.890*	1.000							
Chlorophyll	0.897*	0.839*	-0.617NS	0.720NS	0.904*	0.505NS	0.366NS	0.426NS	1.000						
Co	0.239NS	0.359NS	0.012NS	0.541NS	-0.265NS	0.328NS	0.850*	0.777NS	-0.143NS	1.000					
Mn	0.585NS	0.589NS	-0.703NS	0.513NS	0.314NS	0.318NS	0.646NS	0.659NS	0.300NS	0.624NS	1.000				
Ca	0.156NS	0.144NS	-0.347NS	0.032NS	-0.203NS	0.411NS	0.133NS	0.366NS	-0.115NS	0.372NS	0.682NS	1.000			
Mg	-0.557NS	-0.574NS	0.178NS	-0.559NS	-0.027NS	-0.936**	-0.450NS	0.725NS	-0.345NS	0.413NS	0.495NS	0.693NS	1.000		
Fe	-0.830*	-0.816*	0.633NS	-0.707NS	-0.412NS	-0.779NS	-0.558NS	0.780NS	-0.621NS	0.386NS	0.772NS	0.663NS	0.864*		
Zn	0.529NS	0.492NS	-0.591NS	0.309NS	0.530NS	-0.153NS	-0.064NS	0.077NS	0.581NS	0.359NS	0.041NS	0.362NS	0.205 NS	0.121 NS	
Cu	0.134NS	0.076NS	-0.357NS	-0.176NS	-0.073NS	0.088NS	-0.510NS	0.083NS	0.094NS	0.438NS	0.067NS	0.365NS	0.255 NS	0.263 NS	0.315 NS

The leaves and stem parts of Indian spinach had sufficient dietary microelements like Mg, Ca, Fe, Zn and natural antioxidants like polyphenol, carotenoids, tannin. Though, 'Intermediate type' is less preferred but is found to be a potential source for dietary microelements. Low value of anti-nutrients in red type indicates higher bioavailability of microelements. Strong correlation between phyto-constituents and antioxidant activities in Indian spinach and presence of various compounds are supportive information for its health claims.

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