Chapter 4
Biologically Active Components of the Western Ghats Medicinal Fern *Diplazium esculentum*

Ammatanda A. Greeshma, Kandikere R. Sridhar, and Mundamoole Pavithra

Abstract The riparian fern *Diplazium esculentum* is nutritionally and medicinally valuable in the ethnic population of the Western Ghats of India. Fiddle heads of this fern are nutraceutically versatile and consumed similar to other leafy vegetables. The present study has addressed biologically active compounds (total phenolics, tannins, flavonoids, vitamin C, phytic acid, L-DOPA, trypsin inhibition and haemagglutination) and antioxidant potential (total antioxidant activity, ferrous ion-chelating capacity, reducing power, DPPH and ABTS radical-scavenging activities) in uncooked and cooked fiddle heads. Fiddle heads were devoid of L-DOPA as well as haemagglutinin activity. Total phenolics and flavonoids contents were not influenced by cooking, while tannins, vitamin C, phytic acid and trypsin inhibition activity were higher in uncooked than cooked fiddle heads. Among the antioxidant properties, total antioxidant activity and ferrous ion-chelating capacity were not influenced by cooking, whereas reducing power, DPPH and ABTS radical-scavenging activities were higher in uncooked than cooked fiddle heads. The principal component analysis was performed to ascertain the link between bioactive components and antioxidant potential of uncooked and cooked fiddle heads. Vitamin C and trypsin inhibition activity of uncooked fiddle heads influenced the ABTS radical-scavenging activity, while total phenolics, flavonoids and tannins of cooked samples influenced the total antioxidant activity, ferrous ion-chelating capacity and reducing power. Cooking has differentially influenced the bioactive components as well as antioxidant potential of fiddle heads. There also seems to be geographical difference in quantity of bioactive components (phenolics, flavonoids and vitamin C) as well as antioxidant potential (reducing power). Further insights are warranted to utilize different parts of the ethnically valued fern *D. esculentum* for nutritional and therapeutic advantages.

Keywords Antioxidant potential · Bioactive compounds · Ethnic value · Leafy vegetable · Non-conventional food · Nutraceutical potential · Riparian fern

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4.1 Introduction

Consumption of nutrient-rich vegetables is one of the alternatives to overcome the malnutrition as well as nutrition-dependent human ailments (Ruma 2016; Sridhar and Karun 2017). Various ethnic groups are formulating and utilizing plant-based nutrients as well as medicines for several generations worldwide. In the recent past, ethnobotanical studies are advancing rapidly towards evaluation of traditional knowledge of wild plant sources as food and medicine (Jain 1987a, b, 1991; Sridhar et al. 2016; Sridhar and Karun 2017). Up to 80% of world's foods are derived from plants belonging to 17 families, and the most important families include Brassicaceae, Fabaceae and Poaceae (Fici 2016). However, pteridophytes (ferns) are largely ignored and untapped natural resource for food as well as medicine (Singh and Singh 2012). Ferns are well known for food value (nutritional), medicine (homeopathic, ayurvedic and unani), insecticide (anti-herbivory) and antibiotic properties (Benniamin 2011).

Pteridophytes and their allies of the Western Ghats include up to 256 illustrated forms (Dudani et al. 2012). One of the nonconventional edible and medicinal ferns of the Western Ghats is *Diplazium esculentum*, which is riparian and commonly occurs on the banks of streams and rivers. It is an important delicacy of multi-ethnic population of the Western Ghats (Archana et al. 2013; Greeshma and Sridhar 2016; Greeshma et al. 2018). Rinsed fiddle heads of *D. esculentum* are pan-frayed by seasoning with edible oil, spices and grated coconut to serve as a starter dish. Tender leaves of *D. esculentum* are consumed with hot sauce in Uttarkhand, India (Alderwerelt 1989). It also serves as an ingredient in culinary dishes in the Philippines (Tongco et al. 2014).

Apart from nutritional qualities, *D. esculentum* is also known for biologically active constituents (Kaushik et al. 2012; Archana et al. 2013; Tongco et al. 2014; Greeshma et al. 2018). Its foliage is traditionally used to cure headache, pain, fever, wounds, dysentery, glandular swelling, diarrhoea and skin infections (Akter et al. 2014). Roy et al. (2013) demonstrated through in vitro assays that the extract of *D. esculentum* possesses significant quantity of natural antioxidants, which prevents progression of various oxidative stress-associated diseases. The tribal communities and ethnic groups of the Western Ghats are utilizing different parts of this fern (e.g. rhizome, stem, fronds, pinna and spores) in treatment of many human ailments (Akter et al. 2014). Dried rhizomes of *D. esculentum* also serve as insecticides, and its decoction is useful in curing haemoptysis as well as cough (Anderson et al. 2003). In Albino mice model, aqueous extract of fresh leaves of *D. esculentum* at low doses (100 mg/kg) served as significant CNS stimulant against standard caffeine (Kaushik et al. 2012). Fiddle heads of *D. esculentum* being known for nutritional and medicinal values, the present study envisaged to emphasize some of the biologically active components, antioxidant potential and their interrelationships.

Fig. 4.1 Side view of grown-up *Diplazium esculentum* (**a**), close-up view of fiddle heads with tender pinna (**b**) and different swirling patterns of fiddle heads (**c**–**f**)

4.2 The Fern

The fiddle heads of fern *Diplazium esculentum* (Retz.) Sw. (family, Athyriaceae) were sampled from five different locations of the Western Ghats of Karnataka (Bethri, Kiggal, Mekeri, Murnad and Nelji) during southwest monsoon season (June–August, 2014) and brought to the laboratory in cold packs. The identity of the fern was confirmed by taxonomic descriptions by (Beddome 1865; Manickam and Irudayaraj 1992) (Fig. 4.1). Five fiddle head samples were independently processed within 6–8 h of sampling by rinsing in distilled water to remove the debris followed by pressing with paper towel to remove surface water. Each sample was divided into two groups, the first group was dried in an oven (50–55 °C), while the second group was pressure cooked without addition of more water followed by oven drying. The dried samples were milled (Wiley Mill, mesh # 30), and powder samples were preserved in refrigerator for further analysis.

4.3 Assessment

4.3.1 Bioactive Components

The fern samples were assessed for eight bioactive components like total phenolics, tannins, flavonoids, vitamin C, phytic acid, L-DOPA, trypsin inhibition and haemagglutination.

Total Phenolics Total phenolics content of fern flour was assessed by the method outlined by Rosset et al. (1982). To fern flour (100 mg) methanol (50%, 10 ml) was added, mixed, incubated in water bath (95 °C, 10 min), cooled and centrifuged (2000 rpm, 20 min) and the supernatant recovered. The process was repeated, and the pooled final volume of supernatant was made to 20 ml. Flour extract (0.5 ml) was mixed with equal volume of distilled water, incubated (10 min, room temperature) on adding sodium carbonate (prepared in 0.1 N NaOH, 5 ml). On addition of Folin-Ciocalteu reagent (dilution 1:2, 0.5 ml), the absorbance was read (725 nm; UV-VIS Spectrophotometer-118, Systronics, Ahmedabad, Gujarat, India). The content of total phenolic was expressed as standard mg tannic acid equivalents/gram fern power (mg TAEs/g).

Tannins Tannin content of fern flour was evaluated based on the procedure by Burns (1971). To fern powder (1 g) methanol (50 ml) was mixed to extract tannins and shaken on a rotary shaker (28 °C, 24 h) followed by centrifugation (1500 rpm) to sample the supernatant. To the supernatant (1 ml) vanillin hydrochloride was added (5 ml: 4% in methanol +8% concentrated HCl in methanol; 1:1) followed by incubation (20 min, room temperature), and the absorbance was measured at 500 nm. The catechin dissolved in methanol served as standard, and tannin content was expressed in mg catechin equivalents (mg CEs/g).

Flavonoids Total flavonoids content in fern flour was detected by the method outlined by Chang et al. (2002). Fern flour (1 mg) was extracted with methanol (1.5 ml), aliquots of extract (0.5 ml each) were mixed with aluminium chloride (10%, 0.1 ml) + potassium acetate (1M, 0.1 ml), and the final volume was made to 3 ml in distilled water followed by incubation (30 min, room temperature). The standard used was quercetin dihydrate, and absorbance was measured (415 nm) and expressed the flavonoids in mg equivalents/gram fern powder (mg QEs/g).

Vitamin C Vitamin C content of fern powder was evaluated based on the procedure by Roe (1954). Powder flour (1 g) was extracted using trichloroacetic acid (TCA, 5%, 10 ml), and aliquots of extract (0.2 ml) were made up to 1 ml using TCA (5%) and mixed followed by addition of chromogen (1 ml) (dinitrophenyl hydrazine thiourea copper sulphate solution: 5 parts of 5% thiourea +5 parts of 0.6% copper sulphate + 90 parts of 2% 2,4-dinitrophenylhydrazine in H_2SO_4). The mixture was incubated (boiling water bath, 10 min), cooled, and on addition of H_2SO_4 (65%, 4 ml) further incubated (room temperature, 10 min), and absorbance was measured (540 nm). The standard used was ascorbic acid for quantification of vitamin C and represented in mg ascorbic acid equivalents/gram fern powder (mg AAEs/g).

Phytic Acid Phytic acid in fern powder was determined based on the method by Deshpande et al. (1982) and Sathe et al. (1983). Fern powder (2 g) was extracted with sodium sulphate (10 ml, 10% in 1.2% HCl) and stirred (room temperature,

2 h). On centrifugation (3000 rpm, 10 min), the supernatant was made up to 10 ml in sodium sulphate. The extract (5 ml) was blended with ferric chloride (2 g in 16.3 ml of 12N HCl, diluted to 1 L) and vortexed followed by centrifugation (3000 rpm, 10 min). Filtered the supernatant (Whatman # 1), and the filtrate was made to 10 ml using distilled water. Free soluble phosphorous was determined by vandomolybdophosphoric acid method by potassium dihydrogen phosphate as reference to express phytic acid in percentage.

$$\text{Total phosphorus}\left(\text{g}/100\,\text{g}\right)=\frac{M\times V\times F}{10{,}000\times W\times V}\times\Delta A_{\text{p}} \tag{4.1}$$

[M, average of phosphorus standard (μg/ΔA_{p}); V, original sample in ml; F, dilution factor; ΔA_{p}, absorbance; W, weight of sample (g); V, volume of sample (ml)]

$$\text{Phytic acid}\left(\%\right)=\frac{\text{Phosphorus}\left(\text{g}/100\,\text{g}\right)}{0.282} \tag{4.2}$$

(0.282, factor used to convert phosphorus into phytic acid as it contains 28.2% of phosphorus).

L-DOPA The L-DOPA (L-3,4-dihydroxyphenylalaninne) of fern powder was determined by method proposed by Fujii et al. (1991). Fern samples were mixed with distilled water (1 ml), incubated (room temperature) for 2 h and centrifuged (1500 rpm, 10 min), and the supernatant is allowed to concentrate to dryness using a rota evaporator. To eliminate high molecular weight compounds, the extract was dissolved in distilled water followed by filtering through ultrafilter overnight. The fraction was purified using ODS extraction mini column (C18 Sep-Pak Cartridge, Waters) with water followed by evaporation to dryness. The L-DOPA was determined in HPLC (Tosoh system DP-8020; UV-8020, 280 nm; Column, Aqua 180 Mightsil; Kanto chemical Co. Inc., Japan) as well as LC-ESI/MS (Positive mode; Waters 181 Associates Inc., Milford, MA).

Trypsin Inhibition Trypsin activity was evaluated according to the method by Kakade et al. (1974). Fern powder (1 g) was stirred constantly with NaOH (0.01N, 50 ml) up to 10 min. The extract (1 ml) was diluted with distilled water (1:1), followed by addition of enzyme standard (2 ml) (2 mg trypsin/100 ml 0.001 M NaOH) and incubated in water bath (37 °C, 10 min), and 5 ml BAPNA (40 mg N_{α}-Benzoyl-L-arginine 4-nitroanilide hydrochloride dissolved in dimethyl sulphoxide and made to 100 ml with Tris-buffer at 37 °C) was added and incubated at room temperature (10 min). Acetic acid (30%; 1 ml) was added to stop the reaction followed by measurement of absorbance (410 nm). Control was prepared as per protocol without addition of the fern extract. Trypsin inhibition (TIu)/mg of fern flour was calculated.

$$\text{TIu / mg} = \frac{\left[\left(A_{c410} - A_{s410}\right) \times 100\right] \text{per ml extract}}{\text{Mg sample per ml of extract}} \tag{4.3}$$

(A_c, absorbance of control; A_s, absorbance of sample)

Haemagglutination The haemagglutinin activity in fern powder was determined by the method outlined by Occeña et al. (2007). The fern extract was prepared by mixing defatted powder (1 g) in NaCl (0.9%; 10 ml) and incubated at room temperature (1 h). Centrifuged (2000 rpm, 10 min), supernatant was collected and filtered to use as crude agglutinin. Heparinized human blood samples were centrifuged (2000 rpm, 10 min) to separate erythrocytes. Erythrocytes (A^+, B^+, AB^+, O^+) were washed repeatedly until the clear supernatant was obtained (1:4; chilled saline, 0.9%). Processed erythrocytes (4 ml) were transferred into phosphate buffer (100 ml; 0.0006 M, pH 7.4) and incubated (37 °C, 1 h) by addition of trypsin (2%, 1 ml) on mixing. On incubation, trypsinized solution was repeatedly washed using saline (0.9%) to remove trypsin content. The erythrocytes were suspended in saline (0.9%) and made to 100 ml. Round bottomed 96-well microtitre plate was used for assay. Phosphate buffer (50 μl) was added in the well # 1–11, followed by the addition of crude agglutinin extract (50 μl) to the well # 1, and mixed, and twofold serial dilution was made up to well # 11. Erythrocytes suspension (50 μl) was added to well # 1–11. The well # 12 served as control for sample. Contents in the wells were gently mixed followed by incubation at room temperature (4 h) to observe haemagglutination in each well. Haemagglutination unit/gram (Hu/g) was calculated.

$$\text{Hu / g} = \frac{D_a \times D_b \times S}{V} \tag{4.4}$$

(D_a, dilution factor of extract in well #1; D_b, dilution factor of well containing 1 Hu is the well in which the haemagglutination was observed; S, initial extract/gram fern powder; V, volume of extract in well # 1).

4.3.2 Antioxidant Properties

Evaluation of antioxidant potential of any plant material has no universal method. In almost all methods, a radical has been generated, and the capability of test sample in quenching the radical is assessed (Erel 2004). According to Wong et al. (2006), it is necessary to evaluate at least two methods for a fair assessment of antioxidant potential of a biological material. In our study, five methods of assessment have been followed to get a fair idea of antioxidant potential of uncooked and cooked fiddle heads of *D. esculentum* (total antioxidant activity, ferrous ion-chelating capacity, reducing power, DPPH radical-scavenging activity and ABTS radical-scavenging activity).

The fern powder samples each of 0.5 g were extracted with 30 ml methanol using a rotary shaker (150 rpm, 48 h). After the samples were centifuged, the supernatant

was transferred to a preweighed Petri dish and allowed to evaporate at room temperature. The extract weight was assessed gravimetrically and dissolved in methanol at concentration 1 mg/ml to assess antioxidant potential.

Total Antioxidant Activity Total antioxidant activity (TAA) was determined by the method by Prieto et al. (1999). To methanolic extract of fern (1mg/ml; 0.1 ml) added the reagent mixture (28 mM sodium phosphate +4 mM ammonium molybdate in 0.6 M sulphuric acid) followed by incubation (95 °C, 90 min). The absorbance was measured (695 nm), and the TAA was expressed in µM equivalents of ascorbic acid/gram (µM AAEs/g).

Ferrous Ion-Chelating Capacity Ferrous ion-chelating capacity of the methanolic extract of fern was determined by the protocol by Hsu et al. (2003). On mixing methanol extract (1 ml) with 2 mM ferrous chloride (0.1 ml) + ferrozine (5 mM, 0.2 ml), the volume was made to 5 ml (in methanol) followed by incubation (room temperature) for 10 min, and the absorbance was measured (562 nm). Control was prepared similar to the sample without addition of fern extract to calculate percent ferrous ion-chelating capacity.

$$\text{Ferrous ion-chelating activity}\left(\%\right)=\left(1\ \frac{A_{s562}}{A_{c562}}\right)100 \tag{4.5}$$

(A_s, absorbance of sample; A_c, absorbance of control).

Reducing Power Reducing power of the fern extract was detected following the method by Oyaizu (1986) with a slight modification. Different concentrations of fern extract (0.2–1.0 mg/ml) were prepared in phosphate buffer (0.2 M, pH 6.6), and potassium ferricyanide (1%, 2.5 ml) was added and incubated (50 °C) up to 20 min. The TCA (10%, 2.5 ml) was added to the mixture followed by centrifugation (3000 rpm, 10 min), and supernatant (2.5 ml) was mixed with double-distilled water (2.5 ml) followed by addition of $FeCl_3$ (0.1%, 0.5 ml), and absorbance was measured (700 nm).

DPPH Radical-Scavenging Activity Radical-scavenging activity of the fern extract was determined according to Singh et al. (2002). Different concentrations of fern extract (0.2–1.0 mg/ml) were made up to 1 ml using methanol, and reagent was added (0.001 M DPPH in methanol, 4 ml). On mixing it was incubated in dark room temperature up to 20 min. The reagent devoid of extract served as control, and the absorbance was measured (517 nm).

$$\text{Free radical-scavenging activity}\left(\%\right)=\left(\frac{\left[A_{c517}-A_{s517}\right]}{A_{c517}}\right)\times 100 \tag{4.6}$$

(where A_c, absorbance of control; A_s, absorbance of sample)

ABTS Radical-Scavenging Activity The ABTS [(2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] cationic radical decolourization assay was performed based on the procedure by Adedapo et al. (2008). Stock solution ($ABTS^+$, 7.4 mM, and potassium persulphate, 2.6 mM) and working solution (mixing stock solutions 1:1; allowed to react at room temperature, for 12 h in dark) were prepared. The working solution was diluted with methanol to get suitable absorbance (1 ± 0.01 units at 734 nm). Different concentrations of fern extract (made up to 62 μl using absolute alcohol) were treated with $ABTS^+$ (188 μl, in dark, 30 min) followed by measurement of absorbance (734 nm) to determine percent inhibition.

$$\text{Inhibition percentage}(\%) = \left(\frac{\text{Assay control} - (\text{Test} - \text{Control})}{\text{Assay control}}\right)100 \quad (4.7)$$

(Assay control, ethanol + ABTS reagent; control, sample + ethanol + methanol).

4.3.3 Data Analysis

The distinction between uncooked and cooked fern flours in assays was assessed by t-test (StatSoft Inc. 2008). To establish the relationship between the bioactive components (total phenolics, tannins, flavonoids, vitamin C, phytic acid and trypsin inhibition activity) and antioxidant potential (total antioxidant activity, ferrous ion-chelating capacity, reducing power assay, DPPH radical-scavenging activity and ABTS radical-scavenging activity), the principal component analysis (PCA) was employed (SPSS version 16.0: www.spss.com).

4.4 Observations and Discussion

4.4.1 Bioactive Components

Total Phenolics Total phenolics content of the fiddle heads of *D. esculentum* was not influenced by pressure cooking ($p > 0.05$) (Fig. 4.2a). Turkmen et al. (2005) observed increased content of phenolics in cooked vegetables such as green beans, pepper and broccoli. The phenolic contents of fiddle heads of *D. esculentum* in the present study are higher than the fiddle heads from Assam, while opposite for the young pinna reported from Bangladesh, India (Darjeeling, Maharashtra) and the Philippines (Das et al. 2013; Roy et al. 2013; Akter et al. 2014; Tongco et al. 2014; Saha et al. 2015). Moderately high quantity of total phenolics in the study is comparable with an earlier report by Archana et al. (2013). Phenolic compounds are well known for their importance as antioxidants, antimicrobial agents and insecticidal potential (De Britto et al. 2012).

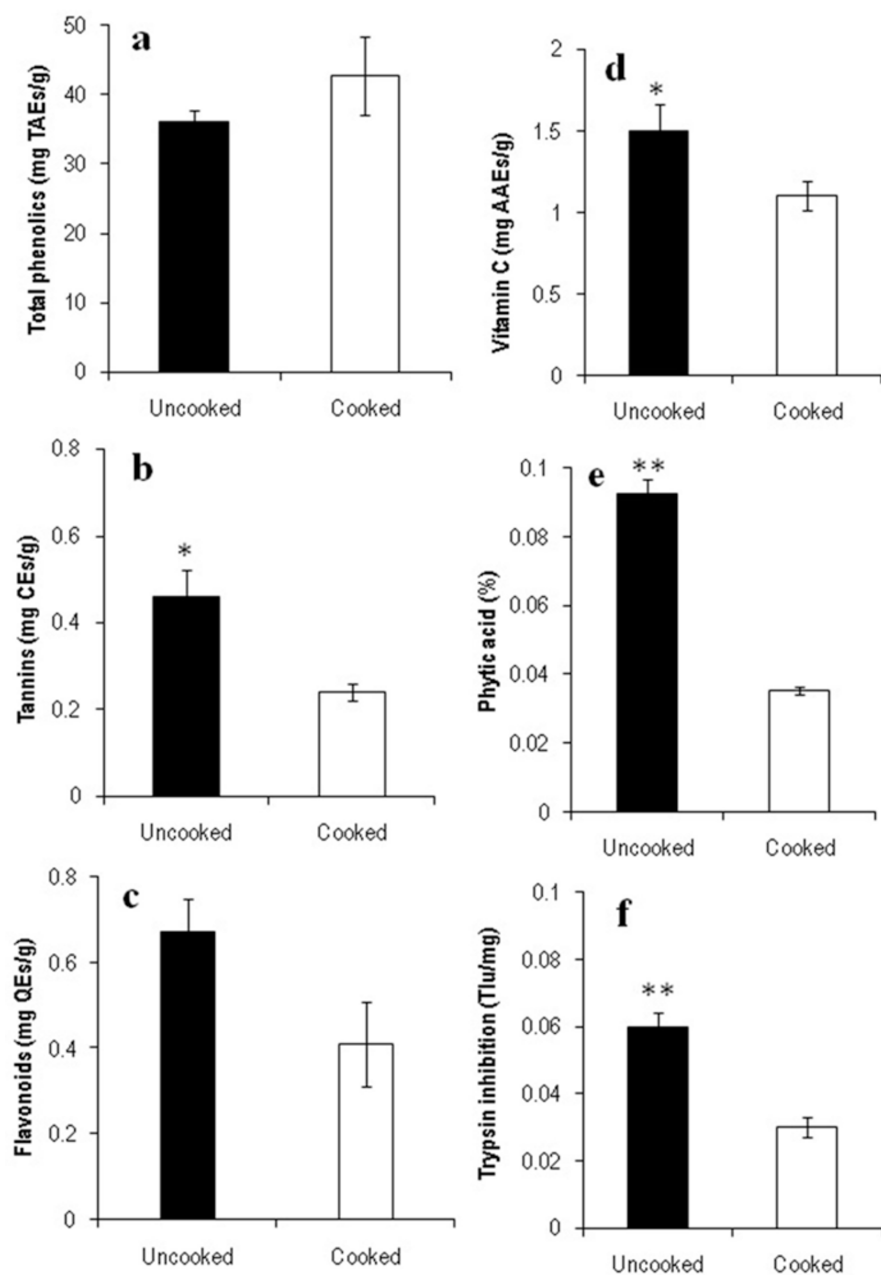

Fig. 4.2 Bioactive principles of fiddle heads of *Diplazium esculentum*: total phenolics (**a**), tannins (**b**), flavonoids (**c**), vitamin C (**d**), phytic acid (**e**) and trypsin inhibition (f) (*t*-test: $*p < 0.05$; $**p < 0.01$)

Tannins Tannins content was significantly higher in uncooked than cooked fiddle heads ($p < 0.05$) (Fig. 4.2b). Its content in uncooked samples in our study is higher than the young pinna from Assam (0.44 vs. 0.1 mg/g) (Saha et al. 2015). Tannins are known for their wide influence on the nutritive values of foodstuffs of humans as well as livestock (Saxena et al. 2013).

Flavonoids Although flavonoids content was higher in uncooked fiddle heads, it was not significantly differed compared to cooked fiddle heads ($p > 0.05$) (Fig. 4.2c). However, Stewart et al. (2000) noted that heat treatment increases the level of free flavonols. Flavonoids content in our study is higher than the fiddle heads sampled from Malaysia, while lower than the young pinna from Bangladesh, India (Darjeeling and Maharashtra) and the Philippines (Miean and Mohamed 2001; Das et al. 2013; Roy et al. 2013; Akter et al. 2014; Tongco et al. 2014). Flavonoids have a wide array of biochemical and pharmacological effects especially anti-oxidation, anti-inflammation, antiplatelet, antithrombotic and anti-allergic effects (Havsteen 1983; Gryglewski et al. 1987; Middleton and Kandaswami 1992; Cook and Samman 1996).

Vitamin C Vitamin C content was significantly higher in uncooked than cooked fiddle heads ($p < 0.05$) (Fig. 4.2d), and its quantity is higher than the fiddle heads from Assam, India (Saha et al. 2015). Archana et al. (2013) also reported presence of higher quantity of vitamin C in mature fronds than young petioles of *D. esculentum*. The vitamin C elicits many functions in the humans especially by boosting the overall health status (Walingo 2005). In addition to vitamin C, presence of β-carotene, tocopherol, thiamine, riboflavin and niacin was also reported from young and mature fronds of *D. esculentum* by Archana et al. (2013).

Phytic Acid Phytic acid content is significantly higher in uncooked than cooked fiddle heads ($p < 0.01$) (Fig. 4.2e), while its content is comparable to the fiddle heads sampled from Assam, India (Saha et al. 2015). Low quantity of phytic acid in *D. esculentum* in our study corroborates with an earlier study by Archana et al. (2013). Phytic acid serves as antioxidant, and it also involves in digestion and absorption of minerals in the intestine (Saha et al. 2015).

L-DOPA In uncooked and cooked fiddle heads, L-DOPA content was below detectable levels. Like L-DOPA, a number of nonprotein amino acids are known to be produced by plants, which possess strong allelopathic properties (Soares et al. 2014). The L-DOPA is also known for its application in treating Parkinson's disease (Hornykiewicz 2002).

Trypsin Inhibition Trypsin inhibition of uncooked fiddle heads was significantly higher than cooked fiddle heads ($p < 0.01$), which is nutritionally advantageous ($p < 0.01$) (Fig. 4.2f). Usually, deficiency of sulphur amino acids has been connected to presence of trypsin inhibitors in food stuffs owing to utilization of sulphur amino

acids for synthesis of trypsin and chymotrypsin (Liener and Kakade 1969). Interestingly, in uncooked fiddle heads, the sulphur amino acids methionine and cystine were significantly higher ($p < 0.05$) than cooked fiddle heads (Greeshma et al. 2018).

Haemagglutination Uncooked and cooked fiddle heads were devoid of haemagglutinin activity against human erythrocytes (A^+, B^+, AB^+ and O^+), which signifies that it is safe for human consumption. Lectins are involved in carbohydrate storage, binding symbiotic rhizobia to develop root nodules, carrier for the delivery of chemotherapeutic agents, and also serve as tumour markers (Kumar et al. 2012). According to Hartmann and Meisel (2007), the lectins in food stuffs are known for immunomodulation processes.

4.4.2 Antioxidant Potential

Total Antioxidant Activity Although total antioxidant activity was higher in the uncooked fiddle heads, it was not significantly higher than cooked fiddle heads ($p > 0.05$) (Fig. 4.3a). Therefore, the total antioxidant activity in the fiddle heads was not influenced by cooking method followed. Evaluation of total antioxidant activity will be helpful to determine the additive effect of antioxidant properties of plant food stuffs (Pellegrini et al. 2003).

Ferrous Ion-Chelating Capacity Ferrous ion-chelating capacity also followed a similar pattern as in total antioxidant activity ($p > 0.05$) (Fig. 4.3b) and not influenced by the method of cooking applied. Metal ion-chelating capacity is one of the significant aspects because it reduces the concentration of the transition metal, which catalyses lipid peroxidation (Mohan et al. 2012).

Reducing Power The present study showed higher reducing power in uncooked than cooked fiddle heads ($p < 0.05$) (Fig. 4.3c). The reducing power of fiddle heads in the present study is higher compared to the fiddle heads of *D. esculentum* sampled from Darjeeling, India (Roy et al. 2013).

DPPH and ABTS Radical-Scavenging Activities The DPPH and ABTS assays estimate the capacity of antioxidant present in the fiddle heads to scavenge free radical. As seen in reducing power, DPPH as well as ABTS radical-scavenging activities were higher in uncooked than cooked fiddle heads ($p < 0.05$) (Fig. 4.3d, e). The DPPH radical-scavenging activity of the present study is lower compared to the fiddle heads of *D. esculentum* of Assam, India (Saha et al. 2015). Method of cooking fiddle heads might have affected the radical-scavenging activities and thus warrants to apply alternate methods of cooking (e.g. microwave, extrusion cooking and steaming) maximizing the radical-scavenging potential.

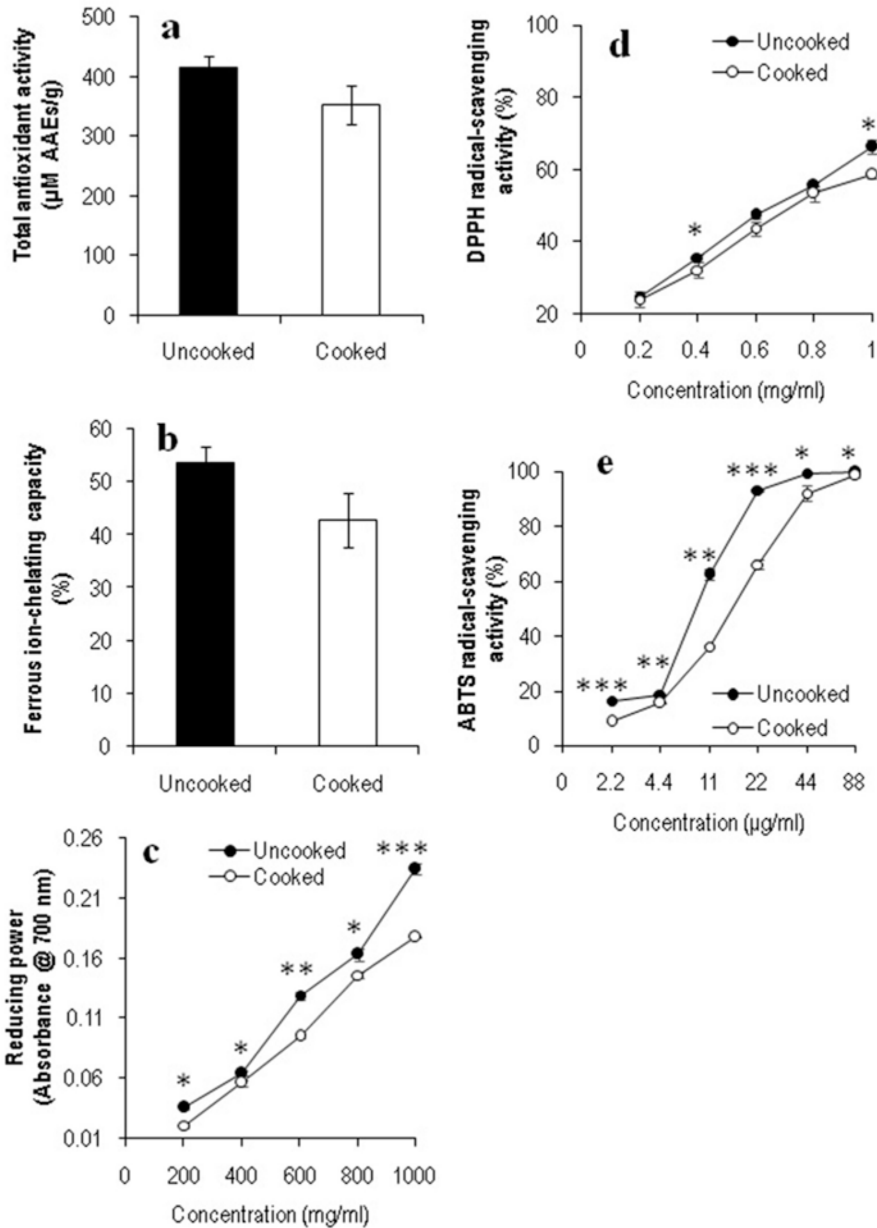

Fig. 4.3 Antioxidant activities of fiddle heads of *Diplazium esculentum*: total antioxidant activity (**a**), ferrous ion-chelating capacity (**b**), reducing power (**c**), DPPH radical-scavenging activity (**d**) and ABTS radical-scavenging activity (**e**) (*t*-test: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$)

4.4.3 Bioactive Components vs. Antioxidant Potential

Principal component analysis (PCA) of bioactive components against antioxidant potential of uncooked fiddle heads of *D. esculentum* resulted in two components with 100% variance. The score plot showed variance 54.5% for component 1 and 45.5% for component 2 (Fig. 4.4a). The bioactive principles vitamin C (VCU) and trypsin inhibition (TiU) were clustered with ABTS radical-scavenging activity (ABTSU) at the right hand corner of the plot. Thus, the quantities vitamin C and trypsin inhibition potential of uncooked fiddle heads have a major role to play in radical-scavenging activity.

The PCA for bioactive principles of cooked fiddle heads against antioxidant capacity yielded two components with 100% variance. The score plot showed variance of 68.1% for component 1 and 31.9% for component 2 (Fig. 4.4b). The bioactive components like total phenolics (TpC), flavonoids (FlC) and tannins (TaC) were grouped with total antioxidant activity (TaaC), ferrous ion-chelating capacity (FiC) and reducing power (RpC) at the right hand corner of the plot. This confirms that although the total phenolics and flavonoids contents were not significantly higher in cooked fiddle heads compared to uncooked fiddle heads, they showed major influence on total antioxidant activity, ferrous ion-chelating capacity and reducing power. Thus, the quantities of total phenolics as well as flavonoids retained in the cooked fiddle heads are mainly responsible for ferrous ion-chelating capacity as well as total antioxidant activity.

4.5 Conclusions and Outlook

The present study addressed bioactive components and antioxidant potential of uncooked and cooked fiddle heads of ethnically valued fern *Diplazium esculentum* of the Western Ghats of India. Among the eight bioactive components assessed, the fiddle heads were devoid of L-DOPA and haemagglutinin activity; thus lack of latter activity signifies its nutritional advantage. Cooking has differential impacts on the bioactive components as well as antioxidant potentials of fiddle heads. Total phenolics and flavonoids contents were not influenced by cooking, while tannins, vitamin C, phytic acid and trypsin inhibition was higher in uncooked than cooked fiddle heads. Among the antioxidant properties, total antioxidant activity and ferrous ion-chelating capacity in fiddle heads were not influenced by the cooking, whereas cooking decreased the reducing power, DPPH and ABTS radical-scavenging activities. The principal component analysis indicated that vitamin C and trypsin inhibition activity of uncooked fiddle heads influenced the ABTS radical-scavenging activity, while total phenolics, flavonoids and tannins contents of cooked fiddle heads influenced the total antioxidant activity, ferrous ion-chelating capacity and reducing power. There seems to be geographical difference in phenolics, flavonoids and vitamin C of fiddle heads of the Western Ghats of India against Assam (Northeast

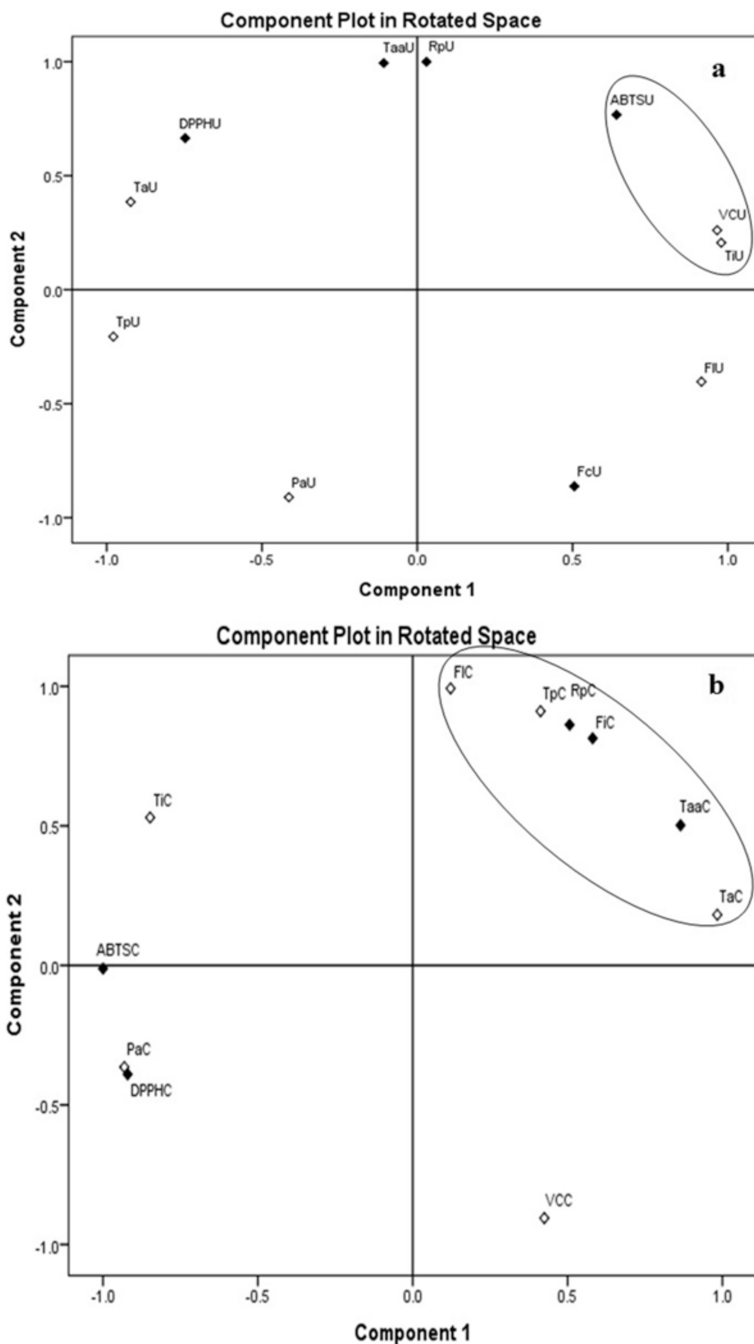

Fig. 4.4 Principal component analysis (PCA) of uncooked (with suffix U) (**a**) and cooked (with suffix C) (**b**) fiddle heads of *Diplazium esculentum*: Bioactive principles (total phenolics, Tp; tannins, Ta; flavonoids, Fl; vitamin C, VC; phytic acid, Pa; trypsin inhibition, Ti) and antioxidant activities (total antioxidant activity, Taa; ferrous ion-chelating capacity, Fc; reducing power, Rp; DPPH radical-scavenging activity, DPPH; ABTS radical-scavenging activity, ABTS)

India) and Malaysia. Similar trend was seen in reducing power of fiddle heads between the Western Ghats and Darjeeling (Northern India). As many vitamins (β-carotene, tocopherol, thiamine, riboflavin and niacin) were assessed qualitatively, future studies are warranted for their quantification. There are ample possibilities to manoeuver the quantity of bioactive components and antioxidant potential of fiddle heads on application of different thermal treatments (microwave, steaming and extrusion cooking) in favour of nutritional and or therapeutic benefits.

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