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ORIGINAL ARTICLE

Antioxidant activity of raw, cooked and *Rhizopus oligosporus* fermented beans of *Canavalia* of coastal sand dunes of Southwest India

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Abstract The raw and processed (cooked and cooked + solid-state fermented with *Rhizopus oligosporus*) split beans of two landraces of coastal sand dune wild legumes (*Canavalia cathartica* and *Canavalia maritima*) of the southwest coast of India were examined for bioactive compounds (total phenolics, tannins and vitamin C) and antioxidant potential (total antioxidant activity, ferrous-ion chelating capacity, DPPH free radical-scavenging activity and reducing activity). One-way ANOVA revealed significant elevation of bioactive compounds as well as antioxidant activities in fermented beans compared to raw and cooked beans in both legumes ($p<0.001$). The EC_{50} values in fermented beans of both legumes were significantly lowest compared to raw and cooked beans ($p<0.001$). In principal component analysis, total phenolics along with antioxidant activities (total antioxidant, ferrous-ion chelating and free radical-scavenging activities) of fermented beans of *C. cathartica*, while total antioxidant and free radical-scavenging activities of fermented beans of *C. maritima* were clustered. The present study demonstrated that split beans of coastal sand dune *Canavalia* fermented by *R. oligosporus* endowed with high bioactive principles as well as antioxidant potential and thus serve as future nutraceutical source.

Keywords Antioxidant activity · Bioactive compounds · *Canavalia* · Fermentation · *Rhizopus oligosporus* · Seeds

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Introduction

Animal and plant cells are often exposed to several challenges those are responsible for oxidative stress leading to production of oxygen free radicals and reactive oxygen species responsible for oxidative damage. Such damages are prevented by natural antioxidants, which are effective against chronic diseases like atherosclerosis, neurodegenerative disorders and cancer (Sulaiman et al. 2011). Antioxidants are known to protect the cells by reducing the formation of free radicals, scavenging the free radicals, converting existing free radicals into less harmful molecules and interrupting the radical chained reactions (Du et al. 2009). The antioxidant activity of various fruits, vegetables and legumes are due to the combined effect of various bioactive compounds (e.g. polyphenols, vitamin C, vitamin E and carotenoids) (Blomhoff et al. 2006). Seeds of legumes possess economically viable nutritional constituents such as proteins, carbohydrates and fibres (Vietmeyer 1986; Kris-Etherton et al. 2002; Lewis et al. 2005; Das et al. 2012). Several studies suggest that regular dietary intake of food legumes are associated with health-promoting effects due to the presence of various bioactive compounds (e.g. Bhat and Karim 2009; Sridhar and Seena 2006; Vadivel and Biesalski 2011). Besides major nutrients, wild legumes serve as source of nutraceuticals by reducing the risks of diabetes, cardiovascular diseases and cancer (Kris-Etherton et al. 2002; Vadivel and Biesalski 2010).

Indigenous landraces of wild legumes belonging to the genus *Canavalia* (*C. cathartica* and *C. maritima*) in coastal regions of the Southwest India exhibit fast growth, high seed yield, tolerance to salinity and resistance to diseases (Seena and Sridhar 2006). Several studies on the seeds of *Canavalia* have demonstrated their nutritional adequacy especially in proteins, amino acids, fatty acids and fibre

(Seena and Sridhar 2006; Sridhar and Bhagya 2007). However, a few bioactive compounds have been reported from the seeds of coastal *Canavalia* (Seena and Sridhar 2006). Roasted seed powder of *C. maritima* serves as substitute for coffee, while leaves are endowed with L-betonicine and roots are useful in treating ciguatera poisoning (Bourdy et al. 1992; Bhagya and Sridhar 2009). Seeds of *Canavalia* are consumed by the coastal dwellers of Southwest India after processing (e.g. soaking, boiling and removal of coat and testa of ripened beans). Leaves, roots and seeds of coastal *Canavalia* are traditionally used in curing skin diseases as well as skin burns (Chock 1968; Bhagya and Sridhar 2009). Although some studies have been performed on antioxidant activities of other *Canavalia* spp. (*C. ensiformis* and *C. gladiata*) (e.g. Doss et al. 2010; Sowndhararajan et al. 2011; Vadivel et al. 2011, 2012), so far no studies are available on the antioxidant potential of coastal *Canavalia* landraces (*Canavalia cathartica* and *C. maritima*). *Rhizopus oligosporus* is commonly employed to produce fermented oriental foods as it is Generally Regarded As Safe (GRAS). Besides enhancement of nutritional quality, solid-state fermented foods showed enhanced antioxidant properties (Sheih et al. 2000; Starzyńska-Janiszewska et al. 2008; Cai et al. 2012). There is no literature on impact of fungal fermentation on the nutritional and bioactive compounds of *Canavalia* of coastal sand dunes. Therefore, the present study envisaged evaluating bioactive compounds and antioxidant potential of raw, cooked and *R. oligosporus* fermented split beans of *Canavalia* of coastal sand dunes of Southwest India.

Materials and methods

Seed samples and fermentation

The seed samples of *Canavalia cathartica* Thouars and *Canavalia maritima* Thouars were sampled from coastal sand dunes of Someshwara, Southwest India (12°47′ N, 74°52′ E) during summer season (February–March, 2010). Healthy undamaged seeds were selected from dry pods and sun-dried for 2 days. Each seed was cut twice longitudinally using a nut-cracker to have four pieces of split beans and dehusked. Split beans were subjected to two treatments: 1) pressure-cooking, and 2) fermentation of pressure cooked split beans. The split beans (25 g) were transferred to conical flask (250 mL), soaked in distilled water (1:3 w/v) followed by pressure-cooking (6.5 L, Deluxe stainless steel; *TTK* Prestige™, Prestige Ltd., India). The cooked split beans were spread on aluminum foil, dried at 42±2 °C, milled (Wiley Mill, 30 mesh) and stored in air-tight glass containers. Another set of split beans (25 g) was cooked in conical flasks (250 mL capacity), cooled to laboratory temperature, inoculated with two 5 mm plugs of 3-day-old cultures (grown on potato dextrose agar medium) of *Rhizopus microsporus* var. *oligosporus* (MTCC # 556; strain designation # 22959; Institute of Microbial Technology, Chandigarh, India) and allowed for solid-state fermentation up to 1 week at 37 °C. The fermented beans formed tight cake on fermentation and it was spread on aluminum sheets, dried at 42±2 °C, powdered and stored in glass containers.

Assessment of bioactive principles

Total phenolics The total phenolic content was determined according to the method outlined by Rosset et al. (1982). Fifty mg of split bean flours were extracted in 5 mL of 50 % methanol in water bath (95±1 °C) for 10 min, centrifuged (1,500 rpm) and the supernatant was collected. The extraction procedure was repeated for the remaining flour pellet, supernatant were pooled and made up to 10 mL. An aliquot 0.5 mL extract was mixed with 0.5 mL distilled water and treated with 5 mL Na_2CO_3 (in 0.1 N NaOH). After 10 min of incubation, 0.5 mL Folin-Ciocalteu's reagent (diluted, 1:2 v/v) was added and the absorbance was read at 725 nm (UV–VIS Spectrophotometer-118, SYSTRONICS, Ahmedabad, Gujarat, India). Tannic acid was used to prepare standard curve and the results were expressed as mg of tannic acid equivalents (TAEs) per gram of the sample (mg TAEs/g).

Tannins Vanillin-HCl method was employed to determine tannins of split bean flours (Burns 1971). One g of flour was extracted with 50 mL methanol (28 °C, 20–28 h), centrifuged and supernatant was collected. One mL of extract was treated with 5 mL of vanillin hydrochloride reagent mixture (4 % vanillin in methanol and 8 % concentrated HCl in methanol; 1:1). After 20 min, the color developed was read at 500 nm with 50–250 μg catechin (Sigma Aldrich, 98 % HPLC grade, USA) as standard. The tannin content was expressed as catechin equivalents (CEs) in milligram per gram of the sample (mg CEs/g).

Vitamin C The vitamin C content of split bean flours was estimated according to Roe (1954) with minor modifications. One gram of sample was extracted in 10 mL of 5 % trichloroaceticacid (TCA). An aliquot (0.2 mL) was made up to 1 mL in 5 % TCA followed by addition of 1 mL of 2,4-dinitrophenylhydrazine (DNPH). The reaction mixture was boiled for 10 min, cooled to laboratory temperature, 4 mL of 65 % sulphuric acid was added, incubated up to 30 min at laboratory temperature and the absorbance was measured at 540 nm. Ascorbic acid (Sisco Research Laboratories, Mumbai, India; purity, 99.8 %) was used to prepare standard curve. The vitamin C content was

expressed as ascorbic acid equivalents (AAEs) in milligram per gram of the sample (mg AAEs/g).

Antioxidant assays

Antioxidant properties have been evaluated by four assays in our study: 1) total antioxidant activity (Reduction of Mo (VI) to Mo(V) by antioxidant compounds); 2) Fe^{2+} ion chelating capacity (Detected by Ferrous ion-Ferrozine complex formation); 3) radical-scavenging activity (DPPH radical absorption on exposure to radical scavengers); 4) reducing power (conversion of Fe^{3+}/ferricyanide complex to the ferrous form). Split bean flour extract was prepared at a concentration of 1 mg/mL (W/V) using methanol. A known amount of the extract was used for the antioxidant assays.

Total antioxidant activity To determine the total antioxidant activity (TAA), 0.1 mL of extract was mixed with 1 mL of reagent mixture (sulfuric acid, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4 mM) (Prieto et al. 1999). The samples were incubated at 95 °C for 90 min, cooled to laboratory temperature followed by measurement of absorbance of phosphomolybdenum complex at 695 nm with methanol as blank. The TAA was expressed as μM equivalent of ascorbic acid per gram of the seed powder.

Ferrous-ion chelating capacity The Fe^{2+} chelating capacity was determined according to the method by Hsu et al. (2003). To 1 mL of the extracts, 0.1 mL each of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine were added. The final volume was made up to 5 mL using methanol. The mixture was incubated for 10 min at laboratory temperature followed by determination of absorbance of Fe^{2+}-ferrozine complex at 562 nm. The sample without the extract served as control and ferrous ion chelating capacity was calculated:

$$\text{Ferrous ion chelating capacity}\,(\%) = [1 - (A_{s562} \div A_{c562})] \times 100 \quad (1)$$

(Where, A_c is absorbance of the control and A_s is absorbance of sample)

DPPH free radical-scavenging activity Free radical-scavenging activity of the extracts was measured according to the procedure by Singh et al. (2002). Different concentrations (0.2–1 mL: 200–1,000 μg) of the test samples were made up to 1 mL using methanol. Four mL of 0.01 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was added and allowed to react at room temperature for 20 min. Reagents without addition of sample served as control and absorbance of the reaction mixture was measured at 517 nm to calculate free radical-scavenging activity:

$$\text{Free radical} - \text{scavenging activity}(\%) = [(A_{c517} - A_{s517}) \div (A_{c517})] \times 100 \quad (2)$$

(Where, A_c is absorbance of the control and A_s is absorbance of sample)

The effective concentration (EC_{50}; concentration of sample necessary to scavenge 50 % of the DPPH radicals) (μg extract mL^{-1}) was obtained by plotting per cent radical-scavenging activity against concentration of the extracts.

Reducing activity Reducing activity of the extract was determined employing the method outlined by Oyaizu (1986) with a slight modification. Different concentrations (0.2–1 mL: 200–1,000 μg) of split bean flour extracted in methanol were taken, 0.2 M phosphate buffer (pH6.6, 2.5 mL) was added followed by addition of 2.5 mL 1 % potassium ferricyanide. The contents were mixed and incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10 % TCA was added and centrifuged (3,000 rpm) for 10 min, 2.5 mL of supernatant was mixed with equal volume of distilled water. To the above mixture, 5 mL of 0.1 % ferric chloride was added and the absorbance was measured at 700 nm. Increase in the absorbance of the reaction mixture indicated increased reducing power.

Data analysis

The difference in quantity of bioactive compounds (total phenolics, tannins and vitamin C) among raw and processed (cooked and cooked + fermented) split beans was assessed by One-way ANOVA (SigmaPlot 11; Systat Software Inc., USA). Relationship between bioactive compounds (total phenolics, tannins and vitamin C) and antioxidant capacities (total antioxidant activity, Fe^{2+} chelating capacity and DPPH radical-scavenging activity) was tested by One-way ANOVA. Principal component analysis (PCA) was performed for total phenolics of raw and processed (cooked and cooked + fermented) split beans against antioxidant assays (total antioxidant activity, Fe^{2+} chelating capacity and DPPH radical-scavenging activity) (SPSS 16.0: www.spss.com). The PCA score plot was used to determine whether total phenolics of raw, cooked and fermented split beans of *C. cathartica* and *C. maritima* vs. antioxidant activities (total antioxidant, Fe^{2+} chelating and DPPH radical-scavenging activities) could be grouped in to different classes.

Results and discussion

Health-promoting and disease-resistance value of antioxidants of plant origin attracted major attention than the

synthetic antioxidants (e.g. butylated hydroxytoluene and butylated hydroxyanisole) (Annegowda et al. 2011). Phenolic compounds in biological materials serve as primary antioxidants or free radical terminators. Epidemiological studies showed a strong relationship between the consumption of natural product rich in phenols with low incidence of cancer, coronary heart disease and atherosclerosis (Randhir et al. 2004; Alothman et al. 2009). Total phenolics and tannin content of raw beans of both legumes in our study decreased on cooking, while it was significantly elevated in *R. oligosporus* fermented beans ($p<0.001$) (Fig. 1). The reduction in phenolic content of cooked beans has been attributed to the loss due to leaching and formation of complex with proteins during thermal treatment leading to poor extractability. The cooked beans of both legumes showed decreased total antioxidant activity (TAA) ($p<0.001$), while the fermented beans showed elevated TAA ($p<0.001$) (Fig. 2). Ferrous ion chelating capacity as well as DPPH free radical-scavenging activity were also showed elevation in fermented beans than the raw and cooked beans

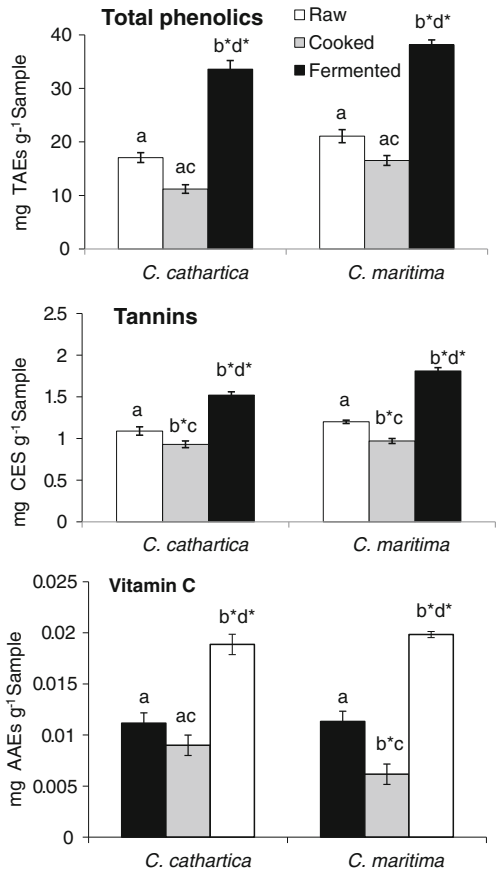

Fig. 1 Total phenolics (TAEs, tannic acid equivalents), tannins (CEs, catechin equivalents) and vitamin C (AAEs, ascorbic acid equivalents) in raw, cooked and fermented beans of *C. cathartica* and *C. maritima* ($n=5$, mean ± SD) (different letters on the bars represent significant difference: *, $p<0.001$, one-way ANOVA)

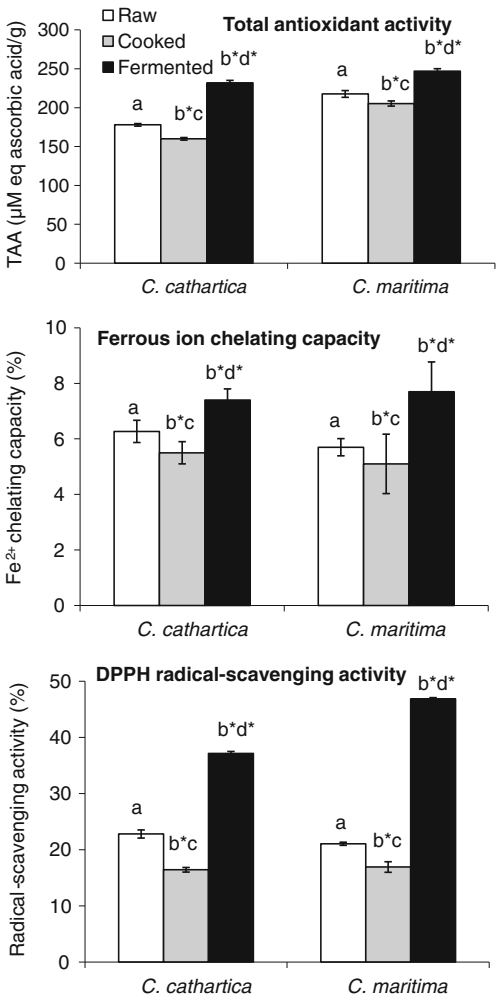

Fig. 2 Total antioxidant activity, Fe^{2+} chelating capacity (200 μg mL^{-1}) and DPPH radical-scavenging activity (200 μgmL^{-1}) of raw, cooked and fermented beans of *C. cathartica* and *C. maritima* ($n=5$, mean ± SD) (different letters on the bars represent significant difference: *, $p<0.001$, one-way ANOVA)

($p<0.001$). The total phenolics of raw beans of *C. cathartica* and *C. maritima* were about 8 to 9–folds higher than the raw beans of other *Canavalia* spp. (*C. ensiformis* and *C. gladiata*) (17.1–21.1 vs. 2.21–2.45 mgg^{-1}), which resulted in relatively high EC_{50} of DPPH free radical-scavenging activities in *C. ensiformis* and *C. gladiata* (18–32.2 vs. 69.8–91.2 μgmL^{-1}) (Doss et al. 2010). Our observations on low yield of phenolics and tannins in cooked beans are in agreement with earlier studies on seeds and leaves of other legumes (Siddhuraju 2006; Xu and Chang 2008; Osman et al. 2010). Similarly, cooking, soaking + cooking and open-pan roasting significantly decreased total free phenolics in *Canavalia* seeds (Seena and Sridhar 2006; Sridhar and Niveditha 2011; Vadivel et al. 2011, 2012). Although cooking sprouted seeds of *C. cathartica* and *C. maritima* decreased the total phenolics (D'Cunha et al. 2009a, b), in *C. ensiformis* and *C. gladiata* sprouting + oil-frying

significantly elevated the extractable free phenolics, antioxidant potential and inhibition of type II diabetes-related enzymes (α-amylase and α-glucosidase) (Vadivel et al. 2011, 2012). On the contrary, extraction of total phenolics and tannins in soaked + autoclaved seeds of *C. ensiformis* were higher than raw seeds (phenolics: 2.2 vs. 1.7 %; tannins: 1.2 vs. 1 %) (Sowndhararajan et al. 2011). Similarly, sprouting wheat, buckwheat, corn and oat seeds for 2 days + autoclaving elevated the total free phenolics by 9 %, 20 %, 27 % and 50 %, respectively (Randhir et al. 2008). This suggests variation of extraction of phenolics and tannins is dependent on the legume species, edaphic factors and methods of extraction. Gazzani et al. (1998) predicted that environmental factors (climatic, growth conditions, ripening stage, temperature and duration of storage) and thermal treatment might influence the antioxidant activity. Although total phenolics and tannins were decreased in cooked beans of both legumes in our study, fermentation of cooked beans with *R. oligosporus* resulted in significant elevation of total phenolics and tannins. A good correlation was seen with the total phenolics, antioxidant and β-glucosidase activities in soybean fermented with *R. oligosporus* (McCue and Shetty 2003). Phenolics are conjugated with sugar moiety through hydroxyl group to form glycosides, which reduces their antioxidant potential due to lack of free hydroxyl groups on the phenolic rings (Robbins 1980). Increased activity of β-glucosidase enzyme during solid state fermentation releases free aglycones (Woodward 1982; Vatten and Shetty 2002), which is clearly evident in our study by highest quantity of extractable phenolics with enhanced antioxidant activity in fermented beans.

Vitamin C is known as a potential antioxidant, pro-oxidant and potent radical scavenger simultaneously forms its own ascorbyl radical to promote oxidative reactions (Podmore et al. 1998). Loss of vitamin C in thermally processed samples occurs primarily by chemical degradation involving oxidation of ascorbic acid to dehydroascorbic acid (DHAA) and 2, 3-diketogulonic acid (Gregory 1996). Heat also speeds up the oxidation of ascorbic acid in fruits and vegetables resulting in the loss of vitamin C content. In our study, even though cooking decreased the vitamin C in beans, fermentation by *R. oligosporus* significantly elevated its concentration in both legumes ($p<0.001$) (Fig. 1), which resulted in higher antioxidant potential (Fig. 2).

The DPPH free radical-scavenging assay has been widely employed to evaluate the ability of compounds to serve as radical scavengers or hydrogen donors in plant extracts. The EC_{50} of DPPH free radical-scavenging activity of both *Canavalia* beans was lowest in fermented than raw and cooked beans ($p<0.001$) (Table 1). The radical-scavenging activity was significantly decreased in *Canavalia* beans on cooking in our study corroborates with thermal treatment of *C. ensiformis* and *C. gladiata* seeds (Vadivel et al. 2011, 2012). However, sprouting + oil-frying elevated radical-scavenging activity in *C. ensiformis* and *C. gladiata* seeds (Vadivel et al. 2011, 2012). On the contrary, thermally processed seeds (soaking + autoclaving) of *C. ensiformis* showed elevation of radical-scavenging potential (Sowndhararajan et al. 2011). The *R. oligosporus* fermented beans were more efficient in antioxidant activities (TAA and radical-scavenging activity) compared to raw and cooked beans, which is comparable with earlier study on seeds of two cultivars of *Lathyrus sativus* (Starzyńska-Janiszewska et al. 2008). In our study, the fermented beans showed significant elevation of TAA, Fe^{2+} chelating capacity, DPPH free radical-scavenging activity and reducing power ($p<0.001$) shows the value-addition by the fermentation (Fig. 2). The EC_{50} values were least in fermented beans followed by raw beans and cooked beans (Table 1). The EC_{50} values of raw beans of *C. cathartica* and *C. maritima* are better than *C. ensiformis* and *C. gladiata* (18–32.2 vs. 69.8–91.2 μg mL^{-1}) (Doss et al. 2010). Reducing activity in raw beans of *C. ensiformis* and *C. gladiata* was elevated with increasing concentration, but the absorbance was higher in *C. ensiformis* and *C. gladiata* compared to *C. cathartica* and *C. maritima* (0.31–0.56 vs. 0.08–0.09).

Study of metal ion chelating capacity is valuable because catalytic activity of metal ions are known to cause lipid peroxidation resulting in the deterioration of food and also cause arthritis and cancer (Gordon 1990; Halliwell et al. 1995). The significant decrease in Fe^{2+} chelating capacity in cooked beans of *C. cathartica* and *C. maritima* corroborates with studies on raw and thermally processed seeds of *C. ensiformis* (Sowndhararajan et al. 2011). Fermentation of black bean (*Glycine max*) using *Rhizopus azygosporus* resulted in the improved metal chelating activity compared to non-fermented beans (Lee et al. 2008). Our observations also showed significant elevation of Fe^{2+} chelating capacity

Table 1 Comparison of EC_{50} (μg mL^{-1}) values for DPPH free radical-scavenging activity of *Canavalia* spp. ($n=5$, mean ± SD)

Standard and sample	Radical-scavenging activity
Ascorbic acid (50 μg mL^{-1})	5.2±0.29
Canavalia cathartica	
Raw	18.0 ± 0.50^{a}
Cooked	$49.2\pm0.29^{b*c}$
Fermented	$16.7\pm0.29^{b*d*}$
Canavalia maritima	
Raw	32.2 ± 2.02^{a}
Cooked	$49.8\pm0.29^{b*c}$
Fermented	$11.8\pm0.29^{b*d*}$

Values in the column with different alphabets represent significant difference: *, $p<0.001$, one-way ANOVA

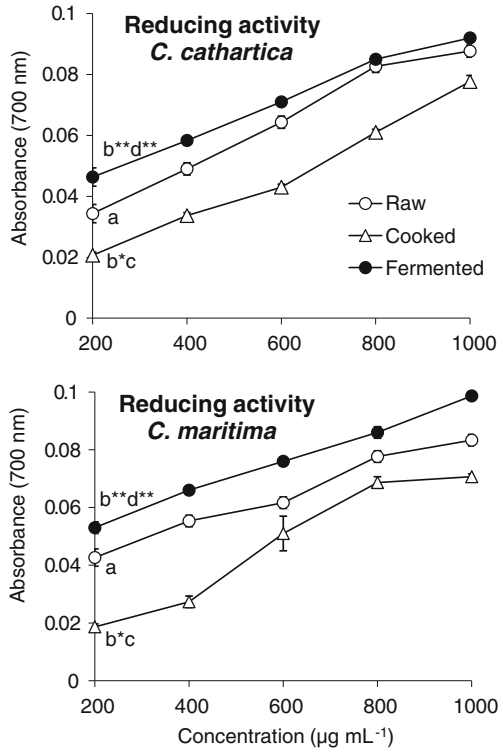

Fig. 3 Reducing power of raw, cooked and fermented beans of *C. cathartica* and *C. maritima* (n=5, mean ± SD) (different letters above 200 µg on each curve represent significant difference: *, p<0.001, one-way ANOVA)

in *R. oligosporus* fermented beans corroborating the studies on black beans by Lee et al. (2008). Gülçin et al. (2004) reported that chelating agents form σ-bonds with metal ions and act as secondary antioxidants by reducing the redox potential of metal ions. Elevation of reducing activity on fermentation might be due to the production of reductants during fermentation (Yang et al. 2000). Such reductants act as intracellular antioxidants and enhance the reducing activity of fermented beans than raw and cooked beans. Various studies reported that fermentation elevates reducing activity in legume seeds and their products (Wang et al. 2004; Lin et al. 2006; Lee et al. 2008). The reducing power of fermented beans of *Canavalia* was higher than raw and cooked beans (p<0.01) (Fig. 3).

The PCA performed on total phenolics vs. antioxidant activities resulted two components (Eigenvalue, < 1), which accounted for 100 % variance. Total phenolics of raw (RPC and RPM), cooked (CPC and CPM) and fermented (FPC and FPM) beans of *C. cathartica* (C) and *C. maritima* (M) with antioxidant activities (total antioxidant activity: RAC, RAM, CAC, CAM, FAC and FAM; ferrous-ion chelating capacity: RFC, RFM, CFC, CFM, FFC and FFM; free radical-scavenging activity: RDC, RDM, CDC, CDM, FDC and FDM) revealed 50.94 % variance for component 1 and 49.06 % variance for component 2 (Fig. 4). The plots in axis 1 and axis 2 showed two groups. In group 1, total phenolics and all the four antioxidant activities tested for fermented *C. cathartica* were clustered indicating the relationship between the bioactive compound and antioxidant activities. Total antioxidant and ion chelating activities of cooked *C. maritima* were clustered together in group 2. The PCA plot showed that the total phenolics and antioxidant activities of raw beans of *C. maritima*, and total phenolics, total antioxidant and ion chelating activities of *C. cathartica* are located to the left in the score plot, which indicates the low antioxidant potential. It is noteworthy that total

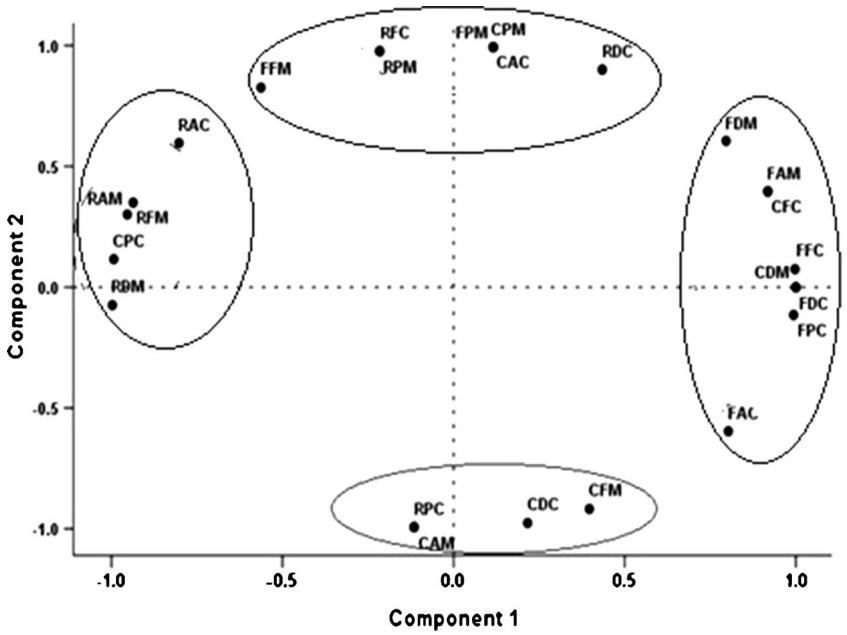

Fig. 4 Score plot of principal component analysis (PC1, 50.94 %; PC2, 49.06 %) for the total phenolics of raw, cooked and fermented beans against total antioxidant activity, Fe^{2+} chelating capacity and DPPH radical-scavenging activity of *Canavalia cathartica* and *C. maritima* [first letter: raw (R), cooked (C) and fermented (F) beans; second letter: total phenolics (P), antioxidant (A), Fe^{2+} chelating (F), DPPH radical-scavenging (D) activities; third letter: *C. cathartica* (C) and *C. maritima* (M)]

phenolics and most of the antioxidant activities of both fermented seeds located on right of the plot indicating their nutraceutical potential.

Besides the bioactive components we tested in the present study, the antioxidant activity of *Canavalia* seeds might have been influenced by other potential compounds like flavonoids, phytates, amino acids, peptides, vitamin E, some fatty acids and minerals (Sridhar and Seena 2006; Vadivel and Biesalski 2010; Sridhar and Niveditha 2011). Further in depth knowledge on bioactive principles and their antioxidant potential is necessary to access benefit from the less known coastal sand dune *Canavalia* spp. distributed widely in pantropical habitats. Further evaluation of antioxidant potential and value addition of coastal sand dune *Canavalia* beans by fermentation with *R. oligosporus* is in progress in our laboratory.

Conclusions

Solid-state fermentation of *Canavalia cathartica* and *C. maritima* with *Rhizopus oligosporus* exhibited significantly highest quantity of bioactive compounds (total phenolics, tannins and vitamin C) and total antioxidant activity (Fe^{2+} chelating capacity, DPPH free radical-scavenging activity, reducing power and the lowest EC_{50} values) suggesting fermentation as an efficient strategy to improve the nutraceutical value. Based on analysis of variance, the antioxidant activity was strongly linked with total phenolics, tannins and vitamin C contents in fermented beans of *Canavalia*. The principal component analysis for total phenolics against antioxidant activities revealed that fermented beans are superior in bioactive compounds as well as antioxidant potential.

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