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Nutritional composition of the branched murex *Chicoreus ramosus* (Linnaeus, 1758) (Family: Muricidae)

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ABSTRACT

Chicoreus ramosus (Linnaeus, 1758), the branched murex, a species of marine gastropod mollusc collected off the Gulf of Mannar on the south-eastern coast of India was studied for the nutritional composition. The edible portion of *C. ramosus* demonstrated protein content with balanced ratio of essential to non-essential amino acids (~0.94). The C_{20}-C_{22} *n*-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, were predominant in the edible part (15.8 and 17.2% total fatty acids, respectively). Considerably lesser cholesterol content (28.7 mg 100 g^{-1}), greater hypocholesterolemic/hypercholesterolemic index (6.3) and lesser atherogenic (0.2), thrombogenicity (0.1) indices showed its importance as a cardioprotective and antithrombogenic diet. The presence of antioxidative microelement selenium (30.44 µg 100 g^{-1}) along with ascorbic acid and tocopherol (45.5 and 55.8 µg 100 g^{-1}, respectively) demonstrated the value of this foodstuff to impart antioxidative defense in the metabolic system. A lesser sodium/potassium (Na/K) proportion (0.64) in *C. ramosus* could be coupled with a diminished threat of developing hypertension and cardiovascular disease. The aggregate amount of calcium and phosphorus (136.1 mg 100 g^{-1}) showed the beneficial effect of this species in facilitating the recruitment of osteoblasts and bone mineralisation process. The previously undescribed report with regard to nutritional composition of *C. ramosus* appropriately demonstrated this low-value gastropod species as a valuable depot of essential nutritional elements and as a health food for human consumption.

Keywords: *Chicoreus ramosus*, C_{20}-C_{22} *n*-3 fatty acids, Hypocholesterolemic/hypercholesterolemic ratio, Marine gastropod mollusc, Thrombogenicity index

Introduction

New nutritional resources from the oceans are being explored and exploited to meet the nutritional challenge of the increasing world populace. Gastropods have assumed commercial importance as a nutritional resource by virtue of their high quality meat and vast demand in the regional and international markets, particularly in the Mediterranean and Asian countries (Mason *et al.,* 2014). In India, gastropods which were considered as bycatch or was discarded earlier, now forms exclusive gastropod fishery. Muricid gastropods (Family:Muricidae) are gaining therapeutic acceptance as there is much potential to derive high value compounds with pharmacological significance (Naegel and Cooksey, 2002). *Chicoreus ramosus* (Linnaeus, 1758) commonly known as ramose murex or branched murex, is a species of marine gastropod mollusc in the family Muricidae, which forms a major fishery along the coast of Gulf of Mannar and Palk Bay. However, a detailed nutritional profiling of this muricid gastropod species is still nonexistent. Although a preliminary attempt has been done (Ramesh and Ayyakkannu, 1992), the study was limited to the proximate composition analysis without detailing the quality of the lipid, protein and mineral content in the candidate species. The present work is thus a pioneering study on *C. ramosus* encompassing various nutritional parameters and health indices to examine the nutritional attributes of the edible parts of the species.

Materials and methods

Samples and study area

Samples of *C. ramosus* (8 kg) comprising of mature animals were collected from the fishing harbours of Thoothukudi situated along the Gulf of Mannar region which is located amidst India and Sri Lanka (8° 48' 0 N; 78° 09' 0 E) (Fig. 1). The shells were appropriately broken to remove soft body tissues (edible parts) and washed with tap water to remove debris. The tissues were macerated and kept at -80^{O}C for further analysis.

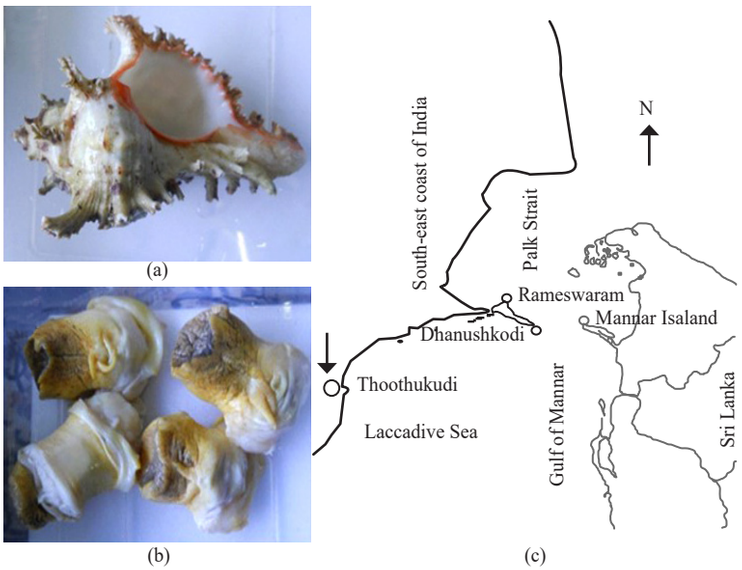

Fig. 1 *Chicoreus ramous* collected from the Gulf of Mannar region. (a) Representative photographs of the morphology of *C. ramous;* (b) Edible portion of *C. ramosus;* (c) Map showing collection site of *C. ramous* in the Gulf of Mannar region in south-east coast of India (8°48'N; 78°09'E)

Analytical methods

Total protein content in the tissues of *C. ramosus* was analysed following previously described method (Lowry *et al.,* 1951). Amino acid content analysis was performed according to Pico-Tag method with suitable modifications (Heinrikson and Meredith, 1984), using reversed-phase binary gradient HPLC (Waters reversed-phase Pico.Tag amino acid analysis system). Total carbohydrate content was determined by dinitrosalicyclic acid (DNS) method as described earlier (Chakraborty and Joseph, 2015). Extraction of lipids was performed as per the Folch extraction method (Folch *et al.,* 1957). Aliquots of the extracted lipids were used to synthesise fatty acid methyl esters (FAME) and evaluated using gas liquid chromatography (GLC) after the method reported by Chakraborty and Joseph (2015). The total cholesterol content in the edible portion of *C. ramosus* was determined colourimetrically as described elsewhere (Wanasundara and Shahidi, 1999) after appropriate modification using o-phthalaldehyde (50 mg 100 ml^{-1} in glacial acetic acid) and the absorbance was measured at a wavelength of 550 nm using UV-visible spectrophotometer (Varian Cary 50). The total cholesterol content was calculated from the standard curve of cholesterol and expressed as mg100 g^{-1} edible portion. The fat soluble vitamins were estimated by the earlier method of Chakraborty and Joseph (2015). Content of vitamin C in the tissues of the gastropod was estimated titrimetrically based on the quantitative discolouration of 2, 6-dichlorophenol indophenol (AOAC, 2005). The mineral content in the edible portion of *C. ramosus* was determined using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES; Thermo Electron IRIS INTREPID II XSP DUO) subsequent to digestion using the di-acid (HNO_3/$HClO_4$) with minor modifications (Chakraborty and Joseph, 2015). The minerals were expressed in mg 100 g^{-1} of edible portion.

Nutritional quality indices

Nutritional quality of the edible portions of *C. ramosus* was assessed using various health indices based on fatty acid and amino acid composition of *C. ramosus*. The health indices such as $\sum n\text{-}6/\sum n\text{-}3$ polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) and total polyunsaturated fatty acids/total saturated fatty acid ($\sum$PUFA/$\sum$SFA) were evaluated to compare with the recommendations of United Kingdom Department of Health (HMSO, 2001). The indices of atherogenicity (AI) and thrombogenicity (TI) were calculated after the methods reported by Ulbricht and Southgate (1991) and Barrento *et al*. (2010). The hypocholesterolemic/hypercholesterolemic (h/H) ratio was determined as suggested by Santos-Silva *et al*. (2002). The amino acid score for the essential amino acids was determined as recommended by Food and Agriculture Organisation of the United Nations (FAO)/World Health Organisation (WHO) (FAO/WHO/UNU, 2007).

Statistical analyses

All analyses were carried out in triplicate. The results were expressed as mean values±standard deviation.

Significance level of the mean of all parameters were set at p=0.05 and analysed by one-way analysis of variance (ANOVA) with Scheffe's post-hoc analysis. All statistical evaluations were done using the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0).

Results and discussion

The increasing demand for gastropods in the international market as an important commercial fishery commodity has occurred only during the recent decades as a recognition to their nutritional qualities. Recently there has been a tremendous increase in the market value of the meat of *C. ramosus*, and it has evolved as a potential candidate for sea farming (Nasution and Roberts, 2004). However, structured information concerning the nutritional value of this predominantly available muricid gastropod is unprecedented, except for a short report on its proximate analysis (Ramesh and Ayyakkannu, 1992). Taking into account the promising aspect of utilisation of *C. ramosus* available in the Indian waters, as a plausible health food and the paucity of information in this line, the present study was designed to examine the nutritional composition with respect to vitamins, minerals, amino acids, protein, fatty acid, total carbohydrate and lipids in edible parts of *C. ramosus.*

The lipid content in the edible portion of *C. ramosus* was revealed to be considerably lower (0.39 mg 100 g^{-1} of edible portion, EP w/w) (Table 1) as compared to the lipid contents of other molluscs, such as *Mytilus edulis*, *Perna viridis* and *Perna canaliculus* (Chakraborty *et al.,* 2016) in which the content of total lipid varied from

Table 1. Fatty acid composition, lipid, cholesterol, total carbohydrate and fatty acid-based nutritional indices of the edible part of *C. ramosus* collected from the south-west coast of India

Fatty acids (% total fatty acids)			
Saturated fatty acids		Polyunsaturated fatty acids	
12:0	0.15^{e}±0.01	16:2*n*-4	0.86^{e}±0.03
14:0	0.22^{e}±0.01	16:3*n*-4	0.18^{e}±0.02
15:0	3.11^{c}±0.10	18:2*n*-6	3.56^{c}±0.01
16:0	6.21^{b}±0.50	18:3*n*-6	3.26^{c}±0.01
17:0	0.30^{e}±0.00	18:3*n*-3	0.30^{e}±0.01
18:0	6.88^{b}±0.40	20:2*n*-6	2.52^{c}±0.05
20:0	4.08^{c}±0.20	20:3*n*-6	4.70^{c}±0.01
22:0	7.16^{b}±0.50	20:4*n*-6	0.75^{e}±0.01
24:0	1.04^{d}±0.01	20:5*n*-3	15.83^{a} ±0.03
^{x}∑SFA	29.14	22:5*n*-3	0.63^{e}±0.02
		22:6*n*-3	17.17^{a} ±0.05
		^{z}∑PUFA	49.77
Monounsaturated fatty acids			
14:1*n*-7	0.11^{e}±0.01		
15:1*n*-7	1.49^{d}±0.05	∑*n*-3	33.93
16:1*n*-7	7.01^{b}±0.20	∑*n*-6	14.52
18:1*n*-7	0.91^{e}±0.01	∑*n*-3/∑*n*-6	2.33
18:1*n*-9	2.35^{e}±0.01	Σ C18 fatty acids	7.12
20:1*n*-9	3.06^{e}±0.20	DHA + EPA	33.00
22:1*n*-9	6.00^{b}±0.02	EPA/AA	21.11
24:1*n*-9	0.02^{f}±0.00	DHA/EPA	1.12
^{y}∑MUFA	20.94	∑PUFA/∑SFA	1.67
Fatty acid-based nutritional indices		Other nutritional parameters	
Atherogenicity index	0.20±0.01	Lipid (%)	0.39±0.02
Thrombogenicity index	0.11±0.01	Cholesterol (mg 100 g^{-1} EP)	28.7±1.05
h/H ratio	6.31±0.01	Carbohydrate (mg 100 g^{-1} EP)	5.86±0.50

All samples were analysed in triplicate (n=3) from pooled sub-samples, and expressed as Mean±SD. Means followed by different superscripts within the same column (a-f) indicate significant difference ($p<0.05$). ND: non detectable

^{x}∑SFA: Total saturated fatty acids

^{y}∑MUFA: Total monounsaturated fatty acids

^{z}∑PUFA: Total polyunsaturated fatty acids

1.06-2.50 mg 100 g^{-1} EP (wet weight). Ramesh and Ayyakkannu (1992) reported the crude lipid content in the edible part of *C. ramosus* as 2 mg 100 g^{-1}. Notably, the previous report recorded crude lipid, which apparently included the non-polar to medium polar compounds, such as terpenoids, steroids, anthocyanidins, other than triglyceride and phosphlipidic components. The lipidic variations could be also due to the period of lipid catabolism that influences the lipid utilisation and storage. This present study recorded the total triglyceride content, which has been considered as nutritionally significant component. The study by Ramesh and Ayyakkannu (1992) did not elucidate the finer components of triglycerides, such as fatty acid composition. However, to establish the high nutritional value of the gastropod's lipid content, we determined the individual fatty acid components of the triglyceride fraction, indicating significantly greater share of PUFAs (48.7% total fatty acids, TFA) among other fatty acid components. Each of the SFAs and MUFAs constituted < 30% of the total fatty acids in the edible part of the species studied (Table 1). The PUFAs in the edible portion of *C. ramosus* were mainly composed of *n*-3 fatty acids (~34% TFA) than *n*-6 fatty acids (~ 15% TFA). The quality of the lipids in the edible part of *C. ramosus* were judged by the fatty acid indices, such as $\sum n$-3/$\sum n$-6 fatty acid ratio that was deduced to be greater than 2, which has been well above the required threshold, and was significantly greater than various marine and freshwater fish species. A greater proportion of $\sum n$-3/$\sum n$-6 fatty acid ratio is proportional to the lipid quality. Diets containing greater *n*-3 PUFA were reported to reduce the plasma concentration of triglycerides and insulin and have been potent inhibitors of inflammatory mediators (Gilroy *et al.,* 2004). The long chain C_{20}-C_{22} fatty acids, such as EPA and DHA contributed major shares (43.65 and 50.60%, respectively) to the *n*-3 PUFAs. The aggregate content of EPA and DHA in the edible portion of *C. ramosus* (~33%) was found to be considerably higher as compared to those found in other species of marine fish and molluscs (Chakraborty *et al.,* 2016). Greater EPA and DHA content in *C. ramosus* are of immense importance due to their contribution in the prevention and therapy of cardiovascular diseases (Dal Bosco *et al.,* 2012). The proportion of these long chain C_{20}-C_{22} *n*-3 fatty acids (DHA and EPA) was also found to be closer to 1, which is significant to meet the requirement of balanced fatty acid nutrition.

The greater content of *n*-3 fatty acids and accordingly greater proportion of *n*-3/*n*-6 fatty acids (~2.3) in *C. ramosus* evidently contributed to the lower values of the AI and TI, which suggested that this foodstuff has been useful in a cardio-protective and anti-thrombogenic diet (Ulbricht and Southgate, 1991). The hypocholesterolemic/hypercholesterolemic (h/H) ratio is a significant index as it considers the consequence of fatty acids on the metabolism of cholesterol and a greater value for this ratio are desirable from a nutritional view point. A greater h/H ratio recorded in *C. ramosus* (greater than 6), appeared to contribute towards its preferred nutritional and health qualities.

The results of the protein content (Table 2) in this gastropod species (12.89 mg g^{-1}) was in conformity with the previous studies on freshwater molluscs *viz*., *Melania tuberculata* (12.36 mg g^{-1}) and *Anisus convexiusculus* (12.93 mg g^{-1}) and greater than that obtained for *Bellamya bengalensis* (8.97 mg g^{-1}) and *Lamellidens marginalis* (6.46 mg g^{-1}) as reported by Baby *et al*. (2010). The biological requirement is for amino acids, though the allowances are expressed as protein. Hence we have focused our study on the quality of protein by evaluating the amino acid indices. The amino acid profile of *C. ramosus* showed that the essential amino acids (EAA) were notably greater in concentrations, when compared to finfishes and other molluscs (Kim and Lall, 2000; Chakraborty and Joseph, 2015), which implied that the proteins in *C. ramosus* had a greater biological value. The EAA dominated the protein content in *C. ramosus* (mean of 1.86 g100 g^{-1}). TEAA in the samples was found to be close to 50%, well above the adequate limits of 11% for ideal protein food for adults, 26% for children and 39% for infants (FAO/WHO/UNU, 1985; FAO/WHO, 1990). The TEAA/TAA percentage contents in *C. ramosus* was found to be closely related to that in egg (50%) (FAO/WHO, 1990). The EAA/NEAA ratio, of 0.94 recorded in the edible part of *C. ramosus*, indicated that *C. ramosus* could provide high quality proteins or well balanced protein deposition. One of the predominant EAAs in *C. ramosus* was found to be lysine (0.26 g100 g^{-1}) and therefore, could serve as an effective alternative to the plant proteins, which are deficient in lysine content. The leucine/isoleucine ratio (>1) of the edible part of *C. ramosus* was found to be close to the optimal ratio laid down by FAO/WHO (1990). Lysine content in the edible portion of *C. ramosus* was comparable with that in reference egg protein (0.63 mg 100 g^{-1}). Arginine was found to be present in an appreciable quantity (0.55 g 100 g^{-1}) in the edible part of *C. ramosus* and was found to involve in metabolic processes and important in the treatment of cardiovascular disorders. Notably, lysine is involved in the growth and maintenance of positive nitrogen balance and also used in protein cross-linking, especially collagen. Comparison between the amino acid composition of the present study and the FAO/WHO (1990) reference pattern showed that all the amino acids met the recommended range of amino acid requirements for adults. Since the accepted average daily requirement for

Table 2. Protein, amino acid, vitamins and mineral composition (mg 100 g^{-1} edible portion) including essential amino acid score of *C. ramosus*

Essential amino acids		Essential amino acid score	
His	69.30±0.45	His	282.97^{c}
Arg	551.3^{a}±0.50	Thr	425.19^{a}
Thr	186.3^{b}±1.10	Val	250.34^{c}
Val	112.9^{c}±1.04	Met+Cys	384.58^{b}
Met	106.2^{c}±0.51	Ile	360.27^{b}
Ile	130.0^{c}±0.91	Leu	378.09^{b}
Leu	321.6^{b}±1.13	Phe+Tyr	245.66^{c}
Phe	117.3^{c}±1.21	Lys	351.84^{b}
Lys	263.0^{b}±1.45	Macrominerals	
ΣEAA	1857.9±2.10	Ca	38.10^{c}±0.50
Non-essential amino acids		P	98.00^{b}±1.50
Asp	418.8^{a}±1.15	Na	95.00^{b} ±0.60
Glu	701.5^{a}±1.45	Mg	75.79^{b}±0.20
Ser	176.6^{b}±1.60	K	149.60^{a}±0.47
Gly	198.0^{b}±1.20	Microminerals	
Ala	246.4^{b}±2.00	Zn	1.27^{d}±0.02
Pro	137.4^{c}±0.50	Cu	0.16^{c}±0.01
Tyr	82.20^{d}±0.20	Mn	0.11^{c}±0.01
Cys	17.70^{c}±0.20	Fe	1.65^{d}±0.01
ΣNEAA	1978.6±2.40	Se	30.44^{c}±0.05
Amino acid-based nutritional indices		Mo	0.07^{c}±0.05
∑AA	3836.5	Mineral indices	
∑EAA/∑AA	0.48	Na/K	0.64
∑NEAA/∑AA	0.52	Ca+P	136.1
∑EAA/∑NEAA	0.94	Vitamins	
∑ArAA	268.80	Retinol (A)	21.89^{b}±0.01
∑SCAA	123.90	Cholecalciferol (D_3)	1.21^{c}±0.02
Arg/Lys	2.10	α–tocopherol (E)	55.82^{a}±0.90
Leu/Ile	2.47	Phylloquinone (K_1)	ND
Protein (mg g^{-1})	12.89±0.50	Ascorbic acid (C)	45.45^{a}±0.50

Data expressed as Mean ± SD (n = 3). The means followed by the same superscript within the same column are not significantly different ($p<0.05$) and differently shown superscripts in the same row are significantly different ($p<0.05$). ND: non detectable
∑EAA: Total essential amino acids; ∑NEAA: Total non-essential amino acids; ∑AA: Total amino acids; ∑ArAA: Total aromatic amino acids; ∑SAA: Total sulfur containing amino acids. Tryptophan was not determined. Mineral content was expressed as mg 100 g EP^{-1} except Se (µg 100 g^{-1})
Vitamins were expressed in µg 100 g^{-1}

reference proteins was found to be 0.6 g kg^{-1} per day (NRC, 1989), *C. ramosus* could be considered as a complementary protein source. Thus, on the basis of the non-truncated values of the amino acid scores, the proteins in *C. ramosus* could be recognised as rich source of indispensable amino acids which could be used to supplement the inadequate amino acids in the cereals and other food sources.

Vitamin E along with ascorbic acid contents were significantly greater ($p<0.05$) in the edible part of *C. ramosus* (45.5 and 55.8 µg 100 g^{-1} edible portion, EP) and was found to be more remarkable than that in other shellfish species (Gopalakrishnan and Vijayavel, 2009). The vitamin A and D_3 were present in appreciably greater quantities than those found in the dried tissue sample of the clam *Meretrix casta* (Srilatha *et al.,* 2013). A greater content of vitamin D_3 (1.2 µg 100 g^{-1}) in addition to significant amount of Ca (38 mg 100 g^{-1}) and P (98 mg 100 g^{-1}) in *C. ramosus* (Table 2) established its significance in preventing osteoporosis in adults. Presence of greater quantity of antioxidative microelement selenium (30.44 µg 100 g^{-1}) along with ascorbic acid and tocopherol (45.5 and 55.8 µg 100 g^{-1}) demonstrated this species as a high value food item to impart antioxidative defense in the metabolic system. The content of total calcium and phosphorus (136.1 mg 100 g^{-1}) in the tissues of *C. ramosus* echoed the beneficial effect of this species in facilitating the recruitments of osteoblasts and bone mineralisation process. The Ca/P ratio was recorded as 0.39, which reflect that the edible

part of *C. ramosus* is of good quality (Erkan and Ozden, 2007). The most abundant microelement in the edible portion of *C. ramosus* was found to be Fe (1.65 mg 100 g^{-1} EP) followed by Zn (1.27 mg 100 g^{-1} EP). The level of Zn estimated in this study was within the range of fish and seafood reported by FAO (FAO/INFOODS, 2013) The Se content in *C. ramosus* were recorded as 30.44 µg 100 g^{-1} which was significantly greater as compared to those reported in various marine finfishes (10-20 µg 100 g^{-1}) (Chakraborty and Joseph, 2015). Increased dietary intake of Se has been associated with the augmentation of antioxidant defense which can counter cellular damage from free radicals and reactive oxygen species (Stephen *et al.,* 2010).

In aquatic molluscs, cholesterol is a prominent sterol having an integral role as secondary messengers in cell signaling and as biosynthetic precursors to vitamins and steroidal hormones. The total cholesterol content in *C. ramosus* was recorded as 28.7 mg 100 g^{-1} EP, which showed lower values as compared to various marine finfish and molluscs (Stephen *et al.,* 2010; Chakraborty and Joseph, 2015). Interestingly, the edible portion of *C. ramosus* recorded significantly lower total cholesterol content than those of beef, pork, poultry and processed meat products (Dinh *et al.,* 2011). These findings demonstrated *C. ramosus* as a preferred food from human health perspective.

The carbohydrate content in *C. ramosus* was found to be 5.9 g 100 g^{-1} EP and is higher than that reported in marine neogastropod *C. melo*, which ranged from 2.59 to 5.14% (Palpandi *et al.,* 2010). Further, high carbohydrate content of 16% was recorded in *C. ramosus* by Ramesh and Ayyakkannu (1992). The carbohydrate content in molluscs has been primarily composed of glycogen and the carbohydrate reserves might fluctuate due to accumulation and usage of glycogen at various stages like gametogenesis and spawning (Ansari *et al.,* 1981).

The present study is the first report to demonstrate this low-value species (*C. ramosus*) as an important source of key nutritional elements essential for the human nutrition and metabolic pool. This marine gastropod species was found to be rich store house of protein with a notable content of essential/non-essential amino acid ratio. More prominent levels of $C_{20\text{-}22}$ long chain *n*-3 polyunsaturated fatty acids, for example, eicosapentaenoic acids, docosahexaenoic acid and greater fraction of *n*-3/*n*-6 fatty acids (2.33), demonstrated that it is good source of well-balanced diets. The ideal atherogenicity/thrombogenicity indices (≤1.0), hypocholesterolemic/hypercholesterolemic ratio and fatty acid/amino acid based health markers in the edible portions qualify this species as a potential health food. The species was also ascertained to be valuable source of several micro and macro minerals and vitamins, which are vital for the metabolic functioning of the body. *C. ramosus* exhibited a lesser Na/K ratio with greater contents of Ca, P and antioxidative mineral Se and could be considered as an important functional food supplement. *C. ramosus* could be considered as a new source of important health food and could potentially be used in formulating various functional food ingredients for pharmaceutical industries.

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