Proximate and mineral composition of Kadamba (*Neolamarckia cadamba*) fruit and its use in the development of nutraceutical enriched beverage

Arti Pandey^{1,3} · Attar Singh Chauhan¹ · Devendra Jaganath Haware² · Pradeep Singh Negi^{1,3}

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Abstract *Neolamarckia cadamba*, commonly known as Kadamba, is one of the economically important trees, which is being exploited for paper, pulp and wood industries, however uses of its fruits are not reported. In the present investigation the *N. cadamba* fruits were analysed for their proximate and mineral content at different stages of maturity, and the ripe fruit was used for nectar preparation. Proximate analysis of the ripe edible fruit showed that it is rich in fat (2.4%) and proteins (2.1%), and has calorific value of 103.7 kcal/100 g. The nectar prepared from the ripe fruits showed mean overall acceptability score of more than 6 indicating its suitability for nectar preparation. The shelf life of nectar was found to be 150 days at ambient conditions (28 ± 3 °C). The nectar was rich in minerals and antioxidants, and can be recommended for consumption by various age groups. However, studies are required to ascertain its physiological effect on consumers.

Keywords Antioxidant · Kadamba · Minerals · Nectar · *Neolamarckia cadamba* · Storage stability

Introduction

Underutilized plants are the richest bio-resources for folk and traditional medicines; and are also used as food supplements, nutraceuticals and pharmaceuticals (Padam et al. 2014). Because of the accelerated local, national and international interest in recent years, the demand for medicinal and aromatic plants has increased manifolds and pharmaceutical industry is gearing up to utilize the vast potential of these resources. One such underexplored plant is an evergreen tropical tree *Neolamarckia cadamba* (Roxb.) Bosser Syn. *Anthocephalus cadamba* (Roxb.) Miq. (Family: Rubiaceae), which grows in India, Bangladesh, Nepal, Myanmar, Sri Lanka, Philippines, Indonesia, and Papua New Guinea. It was revered as holy tree by the Kadamba Dynasty (345–525 AD), and is commonly known as Kadamba in India. The tree bears globular, sweetly fragrant flowers. The fruit occurs in small, fleshy capsules packed closely together to form a fleshy yellow-orange ball like structure, which contains numerous seed coats. Flowering and fruiting periods of *N. cadamba* varies according to climatic conditions, and the fruits are available throughout the year in one or the other part of the world. The traditional healers worldwide have used different parts of *N. cadamba* in treatment of various diseases such as hoarseness of throat, eye inflammation, stomatitis, fever, urinary infections and skin infections (Kirtikar and Basu 1933; Pandey and Negi 2016).

Neolamarckia cadamba is one of the economically important trees, which is being exploited for paper, pulp and wood industry, however its food uses are not popular. The fruit has been traditionally used as an expectorant and blood purifier (Khare 2011) and have anthelmintic potential (Islam et al. 2015). Many reports on other pharmacological and medicinal potential of *N. cadamba* fruit exist in

✉ Pradeep Singh Negi
psnegi@cftri.res.in

¹ Fruit and Vegetable Technology Department, CSIR-Central Food Technological Research Institute, Mysore 570 020, India

² Food Safety and Analytical Quality Control Laboratory, CSIR-Central Food Technological Research Institute, Mysore 570020, India

³ Academy of Scientific and Innovative Research (AcSIR), New Delhi 110 001, India

literature (Pandey and Negi 2016), and various compounds in fruit extracts of *N. cadamba* have been identified as terpenoids, tannins, phenolics and alkaloids (Alam et al. 2008; Acharyya et al. 2013; Pandey and Negi 2018). It has been reported that the fruits of *N. cadamba* are edible, but to the best of our knowledge no published reports on nutritive value and product development from the fruits are available. Therefore, the present study was aimed to investigate nutritive value of *N. cadamba* fruits and develop edible product in order to explore possibilities of its utilization as a food source. The nutritional profiling of the fresh fruits of *N. cadamba* was also done at various maturity stages. Further, process conditions were standardized for nectar preparation and its quality was evaluated during long term storage (150 days) at ambient conditions (28 ± 3 °C).

Materials and methods

Reagents

All organic solvents used (AR grade) were purchased from Qualigens fine chemical, Mumbai, India. The 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) and 2, 2'-azino-bis (3-ethylbenzothiazoline- 6-sulphonic acid) (ABTS), the standards used in antioxidant assays were from Sigma–Aldrich (Bengaluru, India).

Raw material

Ripe, mature and immature fruits of *N. cadamba* were collected from areas in and around Mysore city, Karnataka, India; and identified by Dr. K. A. Sharvani, Assistant Professor, Department of Botany, Yuvaraja's College, University of Mysore. The specimen has been deposited in the Herbarium Centre, Department of Botany, Yuvaraja's College, University of Mysore, Mysore (Accession No. 0263). The fruits were divided into two lots, one lot of fresh fruits were washed and pulped using fruit mill and pulper (B. San and Berry, New Delhi, India) and stored at -20 °C until further use. The pulp was used for the analysis of physicochemical parameters, proximate composition, and nectar preparation. The other lot of fruits was washed, oven dried at 60 °C after loading on the perforated stainless steel trays (2.5 kg per tray) in the cabinet hot air drier till constant weight (approx. 8 h), ground to coarse powder using hammer mill (sieve size 0.32 inches), and the powder was utilized for mineral analysis.

Physicochemical parameters and mineral content of fruits and nectar

The physicochemical parameters determined were pH using calibrated pH meter (Cyberscan, Eutech Instrument, Singapore), total soluble solids by calibrated digital refractometer (ATAGO RX-5000, Japan) and titratable acidity by titration using 0.02 N sodium hydroxide solution and phenolphthelin indicator (Ranganna 1997). The ascorbic acid content of fruit pulp was determined by 2,6-dichlorophenol indophenol titration method, and β -carotene content was analysed by spectroscopic method as described by Rodrigued-Amaya (2001). The proximate composition including percentages of moisture, total ash, crude protein, crude fat and carbohydrate by difference was determined. Moisture content was determined as per standard procedure using hot air oven drying till a constant weight was obtained. The protein content was determined as per standard micro kjeldahl method. The amount of protein present was then calculated from the nitrogen concentration of the fruit of *N. cadamba* using factor of 6.25. Fat content was determined by using soxhlet extractor (Ranganna 1997). Ash content was determined after ashing the powder in muffle furnace by incinerating at 450 °C until light grey ash was obtained. This ash was used to prepare ash solution for estimation of minerals. The mineral analysis was done using Atomic Absorption Spectroscopy method for zinc, iron, copper, calcium, magnesium, sodium, potassium and manganese as per standard procedures (AOAC 2003). The calorific value of *N. cadamba* fruits and nectar was calculated by summing up the product of multiplication of percent crude protein, crude fat and carbohydrate present in the sample by 4, 9, and 4, respectively (AOAC 1990).

Development of beverage (fruit nectar)

Different formulations of fruit nectar were developed using varying concentration of fruit pulp (10–20% w/v) at all three stages of maturity with the addition of required quantities of pectin, citric acid, sugar and water; and the nectar with 10% of ripe fruit pulp was found to be the most acceptable in initial sensory trials. Therefore, for storage study, various formulations with 10% of ripe fruit pulp varying in natural flavours, such as black pepper, cumin, cinnamon and cardamom were prepared, and sodium benzoate (120 ppm) was added as preservative along with carmosine (50 ppm) as colouring agent. The nectar was pasteurized for 1 min at 90 °C, hot filled (70 °C) in pet bottles and stored at ambient temperature (28 ± 3 °C).

Quality evaluation of fruit nectar during storage

The changes in the physicochemical and antioxidant characteristics of nectar with and without various flavours were analysed at the interval of 30 days during storage at ambient temperature (28 ± 3 °C). Physicochemical attributes such as total soluble solids, pH, acidity, ascorbic acid content, β -carotene content, and mineral content were evaluated as described above. The colour of nectar was measured in terms of L, a, b colour values (Hunter Lab colour measuring system, Lab scan XE, Virginia, USA), and 'DE' value was used to analyse changes in colour during storage. The consistency of the nectar was determined in terms of Bostwick value obtained using Bostwick consistometer.

The antioxidant activity of different nectars prepared from *N. cadamba* fruit was evaluated using the DPPH free radical scavenging assay (Blois 1958). Different quantity of juice (5–80 μ L in final volume of 1.0 mL made up with distilled water) were added to 2.0 mL of 0.1 mM DPPH solution in methanol. After vortexing, the mixture was incubated for 20 min at room temperature in the dark and the absorbance was measured at 517 nm using methanol as blank in an UV–visible spectrophotometer (Shimadzu UV-1600, Kyoto, Japan). Butylated hydroxyl anisole (BHA, 0.5–2.0 μ g) was used as standard. Control was prepared without adding standard or test compound. The IC₅₀ value (the concentration required to scavenge 50% DPPH free radicals) was calculated from the graph of concentration verses % inhibition, and it was determined in triplicate.

The ABTS⁺ radicle scavenging activity was estimated according to the protocol given by Re et al. (1999). ABTS radical cation was produced by reaction of ABTS stock solution (7 mM) and potassium persulfate (2.45 mM) in the dark at room temperature for 12 to 16 h. The ABTS radical cation solution was diluted with distilled water to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. Fruit nectar (5–100 μ L) or Trolox (1.5–35 nM) in 300 μ L quantity was added to 2.7 mL of diluted ABTS radical cation solution, vortexed and absorbance was taken at 734 nm after 6 min using UV–visible spectrophotometer (Shimadzu UV-1600, Kyoto, Japan). ABTS radical scavenging activity was determined in triplicate, and expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

Microbiological quality

The fresh as well as stored samples of nectar were analysed for their microbial load using standard methods as described by Binduheva and Negi (2014). Nectar (1 ml) was inoculated onto Plate Count Agar and Potato Dextrose Agar for analysing its total bacterial counts, and yeast & mold counts, respectively; and the presence/absence of

Escherichia coli was checked by plating on HiChrome *E. coli* Agar using pour plate technique. The colonies developed in Plate Count Agar (24 h at 37 °C), and Potato Dextrose Agar plates (25 °C for 2–5 days) were counted and expressed as log CFU/mL. The HiChrome *E. coli* Agar plates were incubated for 48 h at 37 °C and the presence/absence of typical *E. coli* colonies was noted.

Sensory quality

The sensory scores were used as a criterion to select best flavour among the different natural flavours added to *N. cadamba* fruit nectar in freshly prepared samples. The nectars were scored on colour, flavour, texture, sweetness, sourness and overall acceptability attributes by semi-trained panelists (n = 15) using a 9- point hedonic scale.

Statistical analysis

Data were presented as mean value $\pm$ standard deviation. ANOVA was performed and means were compared using Tukeys test at 5% level of significance. *T* test was used to compare the changes in various parameters of nectar during storage.

Results and discussion

Proximate composition and physicochemical attributes of *N. cadamba* fruits

Proximate analysis showed (Table 1) that the ripe edible fruit contains 2.39% fat, 2.11% proteins, and 1.46% total ash, which was found to be higher than mature and immature fruits. The moisture and carbohydrate content of *N. cadamba* at different maturity stages differed significantly ($P < 0.05$). Mature fruits have highest moisture content (80.60%) among all the stages. The calorific value of ripe fruit was highest (103.70 kcal/100 g fruit) among the three maturity stages of fruits. The fresh pulp of ripe *N. cadamba* fruit had 10.69% total soluble solids which was significantly ($P < 0.05$) higher than mature (7.38%) and immature (6.90%) fruits. The ripe fruits had 3% titratable acidity and pH of 3.31, indicating acidic nature of *N. cadamba* fruits. There is no report on physicochemical properties of *N. cadamba* fruit, however, values observed here were similar to those reported for other acidic fruits (Mishra et al. 2012).

Mineral content of *N. cadamba* fruits

The data on mineral content of fruits showed that 100 g of the edible portion of the ripe fruit contains Zn (11.05 mg),

Table 1 Proximate composition, physicochemical attributes and mineral content of *N. cadamba* fruits at different maturity stages

	Ripe	Mature	Immature
Moisture (%)	75.25 ^a ± 1.10	80.60 ^b ± 0.401	78.46 ^c ± 0.71
Total ash (%)	1.46 ^a ± 0.56	1.31 ^a ± 0.08	1.45 ^a ± 0.11
Crude protein (%)	2.11 ^a ± 0.13	1.74 ^a ± 0.09	1.96 ^a ± 0.23
Fat (%)	2.39 ^a ± 0.68	1.79 ^a ± 0.16	1.82 ^a ± 0.11
Carbohydrate (%)	18.78 ^a ± 0.09	14.56 ^b ± 0.29	16.31 ^c ± 0.68
Caloric value (Kcal/100 g)	103.70 ^a ± 3.90	81.06 ^b ± 2.01	90.15 ^c ± 1.85
pH	3.22 ^a ± 0.17	3.41 ^a ± 0.14	3.15 ^a ± 0.04
TSS (° Brix)	10.60 ^a ± 0.08	7.38 ^b ± 0.10	6.90 ^c ± 0.12
TA (% citric acid on wet weight basis)	3.08 ^a ± 0.07	3.32 ^a ± 0.04	3.33 ^a ± 0.17
Zinc (mg/100 g dry weight)	1.11 ^a ± 0.01	1.04 ^a ± 0.07	1.17 ^a ± 0.08
Iron (mg/100 g dry weight)	2.83 ^a ± 0.30	2.29 ^b ± 0.11	2.73 ^{ab} ± 0.17
Copper (mg/100 g dry weight)	0.42 ^a ± 0.01	0.36 ^b ± 0.02	0.34 ^b ± 0.02
Calcium (mg/100 g dry weight)	123.70 ^a ± 2.26	102.98 ^b ± 3.11	96.84 ^b ± 3.15
Magnesium (mg/100 g dry weight)	7.10 ^a ± 0.52	7.17 ^a ± 0.59	7.14 ^a ± 0.57
Potassium (mg/100 g dry weight)	36.70 ^a ± 1.06	36.96 ^a ± 1.53	37.32 ^a ± 3.46
Sodium (mg/100 g dry weight)	1.07 ^a ± 0.02	5.27 ^b ± 1.07	4.90 ^b ± 0.26
Manganese (mg/100 g dry weight)	1.37 ^a ± 0.46	0.90 ^a ± 0.11	0.80 ^a ± 0.11

Values followed by the same letter in a row are not significantly ($P > 0.05$) different

Fe (28.297 mg), Cu (4.19 mg), Ca (123.7 mg), Mg (71.04 mg), K (36.7 mg), Na (10.7 mg), and Mn (13.7 mg). The ripe fruit was found to have better mineral content than mature and immature fruits. No significant difference was found in Zn, K, Mg and Mn content at different maturity stages of fruit, however, the Fe, Cu, Ca and Na content of ripe fruit were significantly different ($P < 0.05$) from those of mature and immature (Table 1). Almost similar mineral content for *N. cadamba* fruits were reported by Pal et al. (2014), and these values indicate that *N. cadamba* fruits are superior source of the calcium and magnesium as compared to many other popularly consumed fruits.

Physicochemical and sensory characteristics of freshly prepared *N. cadamba* fruit nectars

The physicochemical characteristics of all the nectars i.e. plain and with various flavours were evaluated immediately after preparation. The pH (3.03–3.12), total soluble solids (20.97–21.53°B), and acidity (0.34–0.39% citric acid) were found to be statistically similar in all nectars. Cumin flavoured nectar showed the highest ascorbic acid content (42.06 mg/100 mL) and β -carotene content (1.56 mg/L), however, the consistency of this nectar was the lowest (Bostwick value of 3.93 cm/30 s) as compare to other nectars. Probably, the antioxidant principles present in cumin helped in preventing degradation of ascorbic acid and carotenoids (Brewer 2011). Cumin flavoured nectar also showed the lowest IC₅₀ value (11.88 μ L/mL) for DPPH

(similar to cardamom flavoured), and slightly lower ABTS scavenging activity (TEAC 0.68 mM/mL) value than the cardamom flavoured nectar, probably on account of retention of antioxidant principles after addition of natural flavouring materials. The DPPH radical scavenging activity of wild *Passiflora* infusion reported by Pineli et al. (2014) was lower as compared to the nectar prepared in our study as it showed higher IC₅₀ values than the present study. In another study, the DPPH radical scavenging activity of Japanese plum nectar was reported to be 2595 μ M/L Trolox equivalents, and it is reported that the beverage enriched with lemon and berries had much lower ABTS radical scavenging capacity compared to plain nectar (Beer et al. 2012; Girones-Vilaplana et al. 2014).

The calcium, potassium and sodium content (mg/100/mL) of nectar were 4.90, 8.60 and 4.99, respectively, indicating that the nectar was rich source of minerals. Although potassium, magnesium and sodium content differed significantly among various flavor variant of nectar, no significant differences ($P > 0.05$) in calcium content were observed (Table 2). The potassium content of the nectar prepared in the present study was higher than the reported values for mango, peach and ribena fruit juices (Ajai et al. 2014). The calcium content of *N. cadamba* fruit nectar was higher than the mango juice (Mgaya et al. 2014). The *N. cadamba* nectar was also found to contain less sodium than many of fruit juices and roselle-fruit blends, thus it could be a good choice for people with hypertension (Ajai et al. 2014; Mgaya et al. 2014). The overall acceptability values among the treatments showed

Table 2 Physicochemical characteristics of freshly prepared *N. cadamba* fruit nectars

	Plain	Cumin flavoured
pH	3.12 ^a ± 0.04	3.03 ^b ± 0.06
TSS (°Brix)	20.97 ^a ± 0.01	20.96 ^b ± 0.00
Acidity (% citric acid)	0.34 ^a ± 0.01	0.39 ^b ± 0.02
Consistency (cm/30 s)	1.85 ^a ± 0.48	3.93 ^b ± 0.13
Ascorbic acid (mg/100 mL)	35.72 ^a ± 0.84	42.06 ^b ± 1.96
β-carotene (mg/L)	1.43 ^a ± 0.02	1.55 ^b ± 0.01
DPPH scavenging activity (IC ₅₀ in μL/mL)	14.06 ^a ± 0.55	11.88 ^b ± 0.20
ABTS scavenging activity (TEAC in mM/mL)	0.62 ^a ± 0.04	0.68 ^b ± 0.11
Potassium (mg/L)	81.70 ^a ± 3.09	86.00 ^b ± 9.06
Magnesium (mg/L)	14.42 ^a ± 0.85	15.23 ^b ± 0.09
Calcium (mg/L)	54.48 ^a ± 7.76	49.05 ^a ± 10.26
Sodium (mg/L)	25.54 ^a ± 5.32	49.88 ^b ± 0.38
Overall acceptability (n = 15)	6.55 ^a ± 1.26	6.48 ^a ± 0.98

Values followed by the same letter in the columns are not significantly ($P > 0.05$) different

no significant differences ($P > 0.05$), therefore, one representative flavour (cumin) and one nectar without flavour (plain) were studied for their storage stability.

Storage study of fruit nectar

The results of the storage study showed a decline in pH with storage time for both the nectar samples, indicating increase in the acidity of the samples. Similar results were also reported for aloe-vera beverage (Sashikumar 2015), and the pH reduction of carrot juice was attributed to the formation of hydroxyl methyl furfural (HMF) from the reactions involving amino acids and reducing sugars (Wang et al. 2006). However, there was no significant change in colour of the nectars in the present study as observed from delta E values (Table 3). An increasing trend was also observed in total soluble solid content and consistency of the samples. The increase in TSS might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides, and consistency changes may be attributed to the aggregation of various molecules during storage (Sashikumar 2015; Paquet et al. 2014).

The most significant change in quality of nectar during storage was noticed in terms of ascorbic acid content. The ascorbic acid content of the samples declined drastically within one month of storage. Thereafter, there was non-significant change in ascorbic acid content of the nectar. Ascorbic acid is known as most labile vitamin, and therefore the reduction in its levels during storage were expected (Walingo 2005). The β-carotene content of the two nectar samples showed the significant decline with time during storage. Similar results were reported in aloe juice supplemented kinnow nectar (Bhargawa et al. 2014). This decrease may be attributed to the exposure to light and auto

oxidation by reacting with residual oxygen present in the head space of the bottles used for storage (Lin and Chen 2005; Namitha and Negi 2010).

DPPH assay is widely used for the evaluation of antioxidant activity of samples. The working principle of this assay is based on discolouration of DPPH free radical upon reaction with hydrogen donating species *i.e.*, antioxidants present in samples (Krishnaiah et al. 2011). The present study revealed that the addition of natural flavouring had significant effect on the DPPH free radical scavenging activity of the nectar. The initial IC₅₀ value of cumin flavoured nectar (11.88 μL/mL) was found to increase significantly ($P < 0.05$) with increase in storage duration (22.69 μL/mL at the end of storage). Similarly, there was a decline in ABTS value with increase in storage time. Similar changes were also reported during storage of black chokeberry juice concentrate (Walkowiak-Tomczak 2007).

Microbiological analysis

The nectar samples were microbiologically safe for consumption initially as well as during 5 months storage, and visible growth was observed in some of the stored samples afterwards (Data not shown). Presence of *Escherichia coli* was not observed in any of the nectar sample initially as well as during storage. These observations indicated microbial safety of nectar for 150 days at ambient storage. Probably the combination of heat, acid and preservative was able to suppress the microbial growth initially as well as during storage of nectar, and use of various hurdles for preservation of fruit products is widely reported (Moraes et al. 2011).

Table 3 Changes in physicochemical characteristics of *N. cadamba* fruit nectars during storage

	Nectar Variant	Storage days					
		0	30	60	90	120	150
Consistency (cm/30 s)	Plain	1.85 ^a ± 0.48	2.69 ^a ± 0.74	3.13 ^a ± 0.44	2.04 ^a ± 0.32	2.11 ^a ± 0.53	2.57 ^a ± 0.36
	Flavoured	3.93 ^a ± 0.13	3.69 ^{ad} ± 0.23	3.15 ^{bd} ± 0.31	2.84 ^{bc} ± 0.20	2.54 ^c ± 0.14	3.64 ^{ad} ± 0.25
Colour (delta E value)	Plain	96.89 ^a ± 0.59	98.69 ^b ± 0.02	98.68 ^b ± 0.05	98.19 ^b ± 0.09	98.09 ^b ± 0.06	97.38 ^a ± 0.09
	Flavoured	92.65 ^a ± 0.01	92.76 ^b ± 0.00	92.95 ^c ± 0.01	92.56 ^d ± 0.03	92.67 ^a ± 0.02	92.59 ^c ± 0.05
Ascorbic acid (mg/100 mL)	Plain	35.72 ^a ± 0.84	1.87 ^b ± 0.19	1.87 ^b ± 0.09	2.03 ^b ± 0.09	1.54 ^b ± 0.19	1.54 ^b ± 0.19
	Flavoured	42.06 ^a ± 1.96	1.87 ^b ± 0.19	1.60 ^b ± .025	1.56 ^b ± 0.15	1.50 ^b ± 0.17	1.67 ^b ± 0.08
β-carotene (mg/L)	Plain	1.43 ^a ± 0.02	1.06 ^{ac} ± 0.10	1.16 ^{ac} ± 0.09	0.99 ^{bc} ± 0.30	0.82 ^{bc} ± 0.90	0.88 ^{bc} ± 0.13
	Flavoured	1.55 ^a ± 0.02	1.20 ^b ± 0.06	1.23 ^a ± 0.21	1.10 ^b ± 0.12	0.98 ^b ± 0.12	0.90 ^b ± 0.10
DPPH (IC ₅₀ in µL/mL) radical scavenging activity	Plain	14.06 ^a ± 0.55	20.01 ^{bc} ± 0.24	18.17 ^b ± 0.42	21.66 ^c ± 2.10	20.03 ^{bc} ± 0.30	22.41 ^c ± 0.26
	Flavoured	11.88 ^a ± 0.20	12.59 ^a ± 0.52	17.12 ^b ± 3.15	18.03 ^b ± 0.48	19.25 ^{bc} ± 0.28	22.69 ^c ± 0.66
ABTS (TEAC in mM/mL) radical scavenging activity	Plain	0.62 ^a ± 0.04	0.45 ^b ± 0.05	0.43 ^b ± 0.08	0.43 ^b ± 0.03	0.41 ^b ± 0.02	0.33 ^b ± 0.06
	Flavoured	0.68 ^a ± 0.11	0.64 ^{ab} ± 0.02	0.63 ^{ab} ± 0.04	0.49 ^{bc} ± 0.04	0.40 ^c ± 0.05	0.38 ^c ± 0.04

Values followed by the same letter in a row are not significantly ($P > 0.05$) different

Conclusion

It can be concluded that *N. cadamba* fruit is good source of nutrients, as it is rich in protein, fat and minerals as compared to commonly consumed fruits. This study also showed the possibilities of processing of *N. cadamba* fruit into functional food products, e.g. antioxidant and mineral rich nectar, which can be used as an aid in improving the nutritional status of masses. However, the physiological effects of consumption of *N. cadamba* nectar need further investigation.

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Compliance with ethical standards

Conflict of interest All authors declare that they does not have conflict of interest.

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