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Evaluation of the Anti-oxidative, Erythrocyte Membrane Stabilizing Effect and Nutritional Status of *Neolamarckia cadamba* Fruit

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Abstract *Neolamarckia cadamba*, (Roxb.) is commonly grown in different regions of Bangladesh, India also in some other parts of the world. The present study was carried out to evaluate the anti-oxidative and erythrocyte membrane stabilizing effect of fruit extract along with the nutritional values of ripen fresh *N. cadamba* fruit. The antioxidant potential of the extract was assessed by its 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging activity, anti-hemolysis and anti-lipid peroxide assay. Membrane stabilizing effect of *N. cadamba* extract was assessed by using hypotonic solution- and heat-induced hemolysis of erythrocytes. Nutritional values such as total protein, lipid, carbohydrate, crude fiber, etc., along with minerals (Na, K, Mg and Fe) were analyzed by different established chemical methods. The water and ethanolic extracts of ripen fruit showed antioxidative and membrane stabilizing activity, and the extracts were found rich in polyphenols, flavonoids, β-carotene, lycopene and Vitamin-C. The fruits were also found rich in minerals. Thus, *N. cadamba* fruit could be used as a good source of food nutrients. Due to its antioxidative and membrane stabilizing property, this fruit could have a promising role to avert hemolysis-related anemia, as seen inevitable during famine-related malnutrition.

Keywords: *Neolamarckia cadamba, antioxidants, membrane stabilization, nutritional value, minerals*

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1. Introduction

The world's population would be ~9 billion at the 2050s of this century. Because of deforestation, the obtainability of the natural foods are dropping day by day. Food scientists are thus continuously searching for new edible foods from insects to plants for the wellbeing of the human population. Ripen *N. cadamba* (locally known as Kadam fruit) is such a fruit to which a minimum attention has yet not been paid especially from the nutritional point of view, although it might be a promising source of nutrition and bio-medicines. *Neolamarckia cadamba* (Roxb.) is native to Bangladesh including South and Southeast Asian countries [1,2]. The people of north Bangladesh eat Kadam fruit for years, but this is not commonly accepted as fruit in the most part of the country. Foods used by humans obviously symbolizes their social status and also establishes the relationship between people and their environment. This northern part of Bangladesh was once a drought- and famine-stricken region. Humans need to obtain nutrients from foods in order to survive. Therefore the habits of consuming Kadam fruit might have been influenced by economic, culture, and other factors. Also the nutritional status of this fruit is not well known. This fruit has been reported to be rich in phenolic compounds [1], and thus possibly possess antioxidant activities.

Oxidative stress is one of the most important contributing factor to the pathogenesis of inflammatory diseases, ischemic heart diseases, hypertensive disease, hypercholesterolemia, strokes, hepatic diseases, degenerative diseases of neurons, and tobacco smoking related diseases [3], and in the impairments of erythrocyte membrane functions, including hemolysis [4]. An extra oxidative force can lead to the oxidation of lipids and proteins, which is connected to the changes in their structures and functions. Antioxidant compounds are thus medicinally important to reduce oxidative stress and maintain good

health of the individuals. Plants are rich sources of naturally occurring antioxidant ingredients like polyphenols, terpenoids and pro-vitamins, which received much attention as alternative therapeutic agents to fight against oxidative stress induced diseases [5,6]. A stabilized membrane is obligatory to prevent oxidative damage and related inflammatory actions triggered by free radicals. Erythrocyte membrane stability test is a widespread study, which highlights the effect of synthetic and herbal anti-inflammatory mediators on erythrocyte membrane that is exposed to hypotonic solution and heat [7]. To treat the consequences of oxidation and inflammation, there are innumerable anti-inflammatory mediators or drugs in the marketplace [8]. As these drugs are legally responsible to intestinal side effects and mucosal erosions, scientists have focused on medicinal plants for finding natural anti-inflammatory drugs with reduced side effects [9] and durable antioxidant potential. Animals are strictly dependent on nutrients, such as proteins, carbohydrates, lipids and minerals for growth and reproduction. Energy compulsory for metabolism is obtained from these nutrients and can be used by the body when needed [10]. So the objective of this research was to investigate nutritional values, anti-oxidative and membrane stabilizing activities of this fruit, and finally was to familiarize this fruit to the third world as well as developing countries as a source of nutrition and medicine.

2. Materials and Methods

2.1. Collection of *N. cadamba* (kadam) Fruits

Ripen *N. cadamba* fruits (Figure 1) were collected from the Botanical Garden of Jahangirnagar University, Savar, Dhaka 1342, Bangladesh. Then the fruits were authenticated by a Botanist of Botany department of the same University. A voucher specimen has been preserved in the herbarium.

Figure 1. Ripen Kadam fruits.

2.2. Preparation of Water and Ethanolic Extract from Fruits

2.2.1. Water Extract

50 g of finely smashed fresh fruits was suspended and extracted with 5 volumes of distilled water with shaking at 4°C for 1 day. Then the extract was filtered through Whatman No 1 filter paper. The residue was re-extracted two more times under the same condition. Concentrated extract was obtained by removing aqueous. Crude extract was kept at -20°C in sterile universal bottles.

2.2.2. Ethanolic Extract

Finely smashed fresh fruit was mixed with absolute ethanol at a 1:10 ratio (50 g in 500 ml solvent) for 3 days. The next steps involved were essentially the same those described for water extract preparation.

2.3. Antioxidant Analysis of *N. cadamba* Fruit

2.3.1. Estimation of Total Polyphenol and Total Flavonoid Contents

Total polyphenol content of the ripen fruit extract was determined by Folin - Ciocalteu's method using Gallic acid as the standard [11] and total flavonoid content of the extract was measured by the aluminum chloride colorimetric assay using quercetin as the standard [11]. The content of both of the polyphenols and flavonoids are expressed as mg/g of extract.

2.3.2. Estimation of β-carotene and Lycopene Contents

β-carotene content was determined following the method of Nagata and Yamashita [12]. The concentration of β-carotene and Lycopene was expressed as μg/g of fresh fruit.

2.3.3. Estimation of Ascorbic Acid (vitamin C)

Ascorbic acid was estimated according to the method of Omaye et. al. [13]. Finely smashed fresh fruit was used for this experiment. The concentration of vitamin-C was expressed as μg/g of fresh fruit.

2.4. *in vitro* Antioxidative Activity of *N. cadamba* Fruit

2.4.1. DPPH Free Radical Scavenging Assay

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was measured according to Choi et. al. [14]. Free radical scavenging activity was expressed as the concentration of the extract required to decrease the absorbance of DPPH (0.2 mM, as final concentration) by 50% (IC50).

2.4.2. Animals

Five-week-old male Wistar albino rats purchased from icddr, b (Dhaka, Bangladesh), were housed in an air conditioned animal room with a 12:12 h dark:light cycle under controlled temperature (23 ± 2ºC) and humidity (50 ± 10%). Rats were provided with a normal pellet diet with water ad libitum. All animal experiments were performed in accordance with the procedures outlined in the 'Guidelines for Animal Experimentation of Jahangirnagar University' and with ethical approval committee of Jahangirnagar University.

2.4.3. *in vitro* Hemolysis Assay

After using deep anesthesia with pentobarbital, rat blood was collected from the inferior vena cava with a heparinized syringe. Erythrocytes were then isolated from the blood as described by Hashimoto et. al. [15]. The resultant purified erythrocytes were subjected to hemolysis. The extent of erythrocyte hemolysis was determined as described previously Hossain, et. al. [16]. In brief, erythrocyte suspensions at 2% hematocrit were

incubated with freshly prepared Fenton's reagent [H_2O_2 (45mM) + $FeSO_4$ (2mM)] in the absence or presence of 100 μL of N. Kadam extract (5mg/mL) at 37ºC for 1 h. At the end of incubation, erythrocytes were pelleted down by centrifuging the samples at 300×g for10 min. Then the extent of hemolysis was quantified by determining the amounts of released hemoglobin (Hb) in the supernatant at 540 nm against hemoglobin standard.

2.4.4. Lipid Peroxide (LPO) Assay

After drawing blood, rats were initially perfused with ice-cold saline to remove blood from the brain. Then, the liver was separated from rats, re-perfused with saline and homogenized in phosphate buffer (100mM, pH 7.4) containing 1% phenylmethylsulfonylfluoride (PMSF). The homogenate was centrifuged at 1000×g to remove unbroken tissues and debris and the resultant homogenates were assigned as liver tissue homogenates, which was stored at −20°C until analysis. Oxidative stress was directly induced in the above tissue homogenates by Fenton's reagent, as described previously by Hossain, et. al. [16]. For the determination of antioxidative stress activity, tissue homogenates were divided into three groups (1) tissue homogenate (0.1 ml) alone (control); (2) tissue homogenate plus Fenton's reagent (OS); and (3) tissue homogenate plus Fenton's reagent (OS) plus 100 µL of fruit extract (10mg/mL) (OS + KFE). All samples were incubated at 37ºC for 2h. Then, the level of LPO were determined estimating the thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al. [17]. The TBARS levels were measured in nmoles of malondialdehyde (MDA)/mg of protein.

2.5. H_2O_2-induced Cytotoxicity Test on Brine Shrimp Larvae

Brine shrimp lethality bioassay was performed according to Musini et. al. [18] with slight modification. Naupliis were exposed to H_2O_2 (0µM - 10mM) at 37˚C for 20 min in dark incubators to determine concentration of H_2O_2 for significant cytotoxicity on nauplii. For the determination of protective or toxic activity, naupliis were divided into four groups each with 25 naupliis on four sterile petri dishes, (1) nauplii only with sea water (control); (2) nauplii with 1mM H_2O_2 (Toxic); (3) nauplii with H_2O_2 plus *N. cadamba* extract (500µg/mL) in sea water), (Toxic +Extract), (4) nauplii only with *N. cadamba* extract in sea water (Extract), all of which were incubated at 30°C for 24 hours with constant air and light supply. Naupliis without treatment (H_2O_2 and *N. cadamba* extracts) served as a negative control and naupliis treated with only H_2O_2 (1mM) acted as positive control. Following incubation, alive naupliis were counted manually using a magnifying glass and the mortality was calculated by following equation.

$$Mortality\ \% = \left(Dead\ nauplii \div Total\ nauplii\right) \times 100.$$

2.6. Erythrocyte Membrane Stabilizing Activity of *N. cadamba* Fruit

Erythrocyte membrane stabilizing activity of *N. cadamba* was evaluated by using hypotonic solution and heat prompted hemolysis of erythrocytes by the method developed by Omale and Okafor [19] with slight modification.

2.6.1. Heat-induced Hemolysis

5 ml of the isotonic buffer containing 5mg/ml of ethanolic and water extract of *N. cadamba* were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as a control. 30 µl of erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath. The other pair was maintained at 0-5°C on ice bath. The reaction mixture was centrifuged for 3 min at 1300xg and the absorbance of the supernatant was measured spectrophotometrically at 540 nm (JENWAY 6305, USA). Acetylsalicylic acid (200 µg/ml) was used as a reference standard. Percentage inhibition or acceleration of hemolysis was calculated according to the following equation:

$$\%\ acceleration\ or\ inhibition\ of\ hemolysis = 100 \times \left[1 - \left(OD2 - OD1\right) \div \left(OD3 - OD1\right)\right]$$

Where, OD1= test sample at 5°C, OD2=test sample heated, OD3=control sample heated

2.6.2. Hypotonic Solution-induced Hemolysis

The hypotonic buffer solution used for this experiment was 50 mM NaCl in phosphate buffer solution (pH 7.4). 30 µl of stock erythrocyte suspension was mixed with 5 ml of the hypotonic solution containing water and ethanolic extract of *N. cadamba* (5mg/ml) while the control sample was mixed with a drug free solution. The mixtures were incubated for 10 min at room temperature, centrifuged for 3 min at 1300xg and the absorbance of the supernatant was measured at 540 nm. Acetylsalicylic acid (200 µg/ml) was used as a reference standard. Percentage inhibition or acceleration of hemolysis was calculated according to the following equation:

$$\%\ acceleration\ or\ inhibition\ of\ hemolysis = 100 \times \left[\left(OD1 - OD2\right) \div OD1\right]$$

Where, OD1= Control sample, OD2=Test sample

2.7. Nutrition Analysis of *N. cadamba* Fruit

2.7.1. Estimation of Total Protein

The finely smashed fresh fruit was soaked with 0.1N NaOH in glass screw capped test tubes for 24 h with brief sonication in an ultrasonic bath sonicator filled with ice water (at maximum output). Then, the test tubes were vortexed and heated at 80°C in the block heater for 2min. After centrifugation at 2000xg, total protein was measured from the supernatant by the method of Lowry et. al. [20] and was calculated as mg of protein per gram of ripen fresh fruit.

2.7.2. Estimation of Total Lipid

Following the extraction method of Folch, et. al [21], total lipid content of ripe fresh *N. cadamba* fruit was estimated gravimetrically. Smashed fresh fruit was used for this assay and the amount of total lipid expressed as mg of lipid per gram of ripen fresh fruit.

2.7.3. Estimation of Total Carbohydrate

Total carbohydrate was estimated following the phenol-sulfuric acid method of Dubois et. al. [22] Values are expressed as mg/g of ripen fresh fruit.

2.7.4. Estimation of Crude Fiber

Total crude fiber was estimated according to the method of AOAC [23]. Finely smashed fresh ripe fruit was used in this experiment and the crude fiber content was expressed as mg/g of fresh fruit.

2.7.5. Estimation of Moisture

Moisture was determined by the method of Cockerell et. al. [24]. 5 gm of smashed *N. cadamba* was weighed in a covered, flat, aluminum dish and dried to constant weight at 100ºC in an oven fitted with controlled ventilation. After complete drying, weight (wt) was measured again after cooling in desiccator and moisture determined as follows:

$$Moisture\ (\%) = \left[\begin{pmatrix} Wt\ of\ fresh\ sample \\ -Wt\ of\ dry\ sample \end{pmatrix} \div Wt\ of\ fresh\ sample\right] x\,100$$

Where, Wt= Weight

2.7.6. Estimation of Minerals

Samples were digested according to the method of Mello et. al. [25]. Digested sample after appropriate dilution was aspirated into the atomic absorption spectrophotometer (AA-7000, Shimadzu Corporation, Kyoto, Japan) coupled with an auto sampler, ASC 7000; burnt into atomic components and read at their respective wavelength with optimized instrumentation. In the present study, sodium (Na), potassium (K), magnesium (Mg) and iron (Fe) were determined. The standard stock solutions of Na, K, Mg and Fe were prepared (1000mg/L), and the amount of minerals in each sample type was calculated from different concentrations of respective standard based on the slope of the standard curve and expressed as μg/mg of fresh fruit.

2.8. Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). The significant difference among different groups was determined by one-way ANOVA followed by Fisher's PLSD test for post-hoc comparison using Graph Pad Prism software version 5.0 (Graph Pad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Contents of Antioxidants in *N. cadamba* Fruit

Antioxidants that can securely interact with free radicals and terminate the chain reactions before vital molecules are compromised, from fruits, vegetables and beverages play an important role in human health, for example, inhibiting cancer and cardiovascular diseases, and lowering the incidences of different diseases [26]. In our study, we have analyzed total polyphenols, flavonoid, β-carotene, lycopene and vitamin C as antioxidants in *N. cadamba* fruit, and the contents are shown in Table 1.

Table 1. The antioxidant contents of *N. cadamba* fruit

Antioxidants	Sample	Amount per g
Polyphenol	Ethanolic extract	29±.819 mg of GAE
	Water extract	15±.55 mg of GAE
Flavonoid	Ethanolic extract	20±.25 mg of CE
	Water extract	13.8±.19 mg of CE
B-Carotene	Fresh fruit	88±.06 μg/g of FF
Lycopene	Fresh fruit	190.8±.28 μg/g of FF
Vitamin C	Fresh fruit	950±1.57 μg/g of FF

GAE= Gallic acid equivalent, CE= Catechin equivalent, FF= Fresh fruit. Each value is presented as mean ± standard error of mean, SEM (n = 3).

3.2. Antioxidative Activity of *N. cadamba* Fruit

3.2.1. DPPH Free Radical Scavenging Activity

DPPH free radical scavenging assay is a standard method for investigating antioxidative activity, as its results are not affected by substrate polarity [27]. We inspected that water and ethanolic extracts of *N. cadamba* fruits have potential DPPH-free radical scavenging ability (Figure 2A). The IC_{50} values for ethanolic and water extract was 312µg /ml and 589 µg/ml, respectively, while the value of control (Gallic acid) was 6.25 µg/ml.

3.2.2. Protective Effects of *N. cadamba* Fruit Extract against Erythrocyte Hemolysis *in vitro*

Mammalian erythrocytes are an exceptional and interesting cellular model for research on oxidative stress, induced either by reactive nitrogen species (RNS) or reactive oxygen species (ROS), as well as for studies of the molecular mechanisms underlying the protective effects of the antioxidants [28]. The erythrocytes intrinsically are more susceptible to peroxidation due to the heavy gathering of polyunsaturated fatty acids and hemoglobin in them. During respiration erythrocytes are endlessly exposed to high pressure of oxygen, which can prompt oxidative damage. Therefore, we investigated whether Kadam fruit extract possesses any protective effects against oxidative stress-induced hemolysis *in vitro*. For this purpose, erythrocytes were incubated with water and ethanolic extract of Kadam fruit and oxidative stress that leads to hemolysis was induced simultaneously by the addition of Fenton's reagent. Results showed that erythrocytes pretreated with the ethanolic and water extract had reduced degree of Fenton's reagent-induced hemolysis (Figure 2B), endorsing that extract effectively protects erythrocytes from oxidative stress-induced hemolysis *in vitro.*

3.2.3. *N. cadamba* Fruit Extracts Reduce Oxidative Stress in Liver Tissues *in vitro*

The antioxidant potential of the extract or fraction is directly related to the scavenging of hydroxyl radical and

consequently the inhibition of lipid peroxidation. Lipid peroxides may be pro-inflammatory and can damage tissue directly [29]. Protection against free radical-induced lipid peroxidation by plant extracts is of great importance for their traditional use against inflammatory disorders [30]. In our study, we induced oxidative stress in liver tissue homogenates *in vitro* by the use of Fenton's reagent and examined whether oxidative stress in these tissues could be inhibited by *N. cadamba* fruit extracts. Lipid peroxide (LPO) levels were measured in these tissue samples as an indicator of oxidative stress in the absence or presence of fruit extract. As expected, Fenton's reagent significantly increased the levels of LPO in the liver tissues (Figure 2C). However, the levels of LPO significantly reduced in the liver tissues when they were pretreated with fruit extract having a better effect by ethanolic extract.

3.3. Protective Effect of *N. cadamba* Fruit Extracts on H_2O_2-induced Cytotoxicity on Brine Shrimp

Brine shrimp bioassay is a simple cytotoxicity test of bioactive chemicals. It is established on the killing capability of test compound on a simple zoological organism, *brine shrimp* (*Artemia salina*). H_2O_2, a precursor of various ROS was chosen as toxic reagent (oxidant) in this study. Various concentrations of H_2O_2 (0 µM - 10mM) were used to determine the appropriate dose. With the purpose of evaluating the protective or toxic outcome of *N. cadamba* fruit extract, co-treatment experiment was done and the results are presented in (Figure 2D). Pretreatment of 500 μg/ml fruit water extract alone and simultaneously with 1mM H_2O_2, presented low mortality than H_2O_2-alone treated group. This test ensures the protective and non-toxic effect of Kadam fruit extract against H_2O_2 induced toxicity.

3.4. Membrane Stabilizing Effect of *N. cadamba* Fruit Extracts

Biological membranes facilitate separation between the inside and outside of cells of an organism, and control permeability of substances also enables living organisms to generate energy. In addition, they regulate the flow of messages between cells by sending, receiving and processing information in the form of chemical and electrical signals [31]. So, a stabilized membrane is very important for living animals. Here we examined the erythrocytes membrane stabilizing action of *N. cadamba* fruits under hypotonic/heating conditions, and we found that *N. cadamba* inhibits the hemolysis of erythrocytes induced by both hypotonic solution and heat treatment (Table 2). The erythrocytes membrane stabilizing activity of *N. cadamba* fruit extracts might be due to the increase in the surface area or volume ratio of the cells, which could be brought about by an expansion of membrane or shrinkage of the cell. Moreover, it has also been shown that the deformability and cell volume of erythrocytes is closely related to the intracellular content of calcium [32]. Hence, it may be speculated that the cytoprotective effect on erythrocyte membrane might be due to the ability of the extracts to alter the influx of calcium into the erythrocytes. The present analysis suggests that the membrane stabilizing activity of *N. cadamba* fruit extract might play an important role in-anti-inflammatory activity.

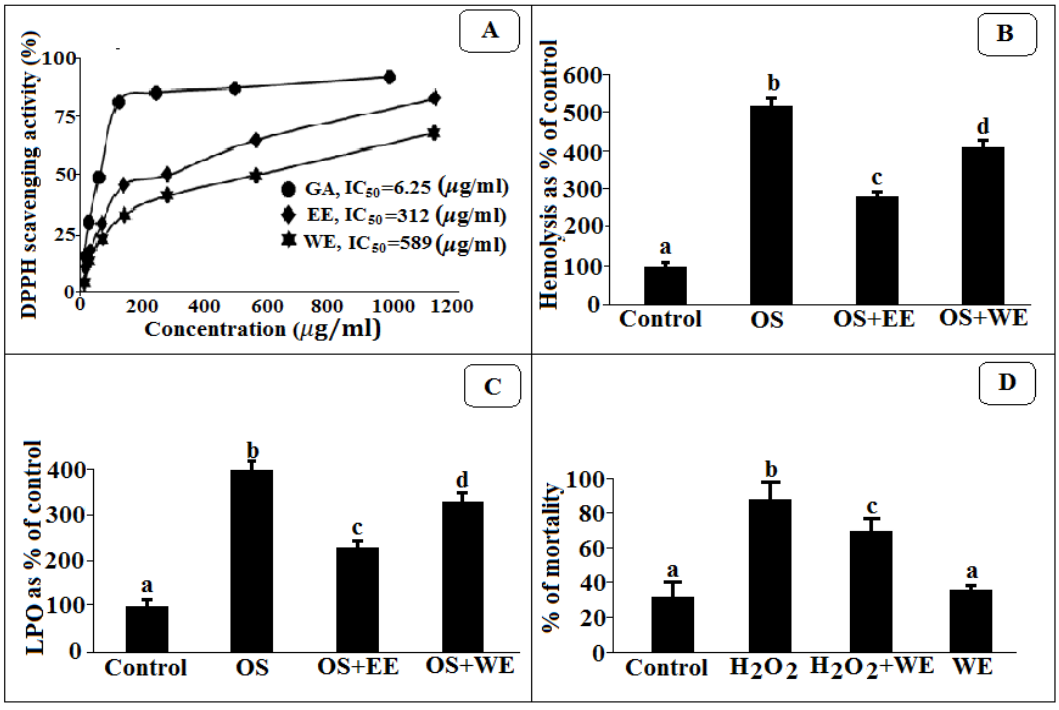

Figure 2. Effects of *N. cadamba* fruit extracts on DPPH radical scavenging activity (A), Fenton's reagent-induced hemolysis of erythrocytes (B), oxidative stress in liver tissues *in vitro* (C) and H_2O_2-induced toxicity in brine shrimp larvae (D). Results are expressed as mean ± SEM, each with duplicate determinations Bars with different letters are significantly different at $P< 0.05$. Data was subjected to one-way ANOVA followed by Fisher's PLSD post hoc test for multiple comparisons. GA=gallic acid, IC_{50}= inhibition concentration 50. LPO= lipid peroxide, OS=oxidative stress, and OS+EE= oxidative stress and ethanolic extract, OS+WE= oxidative stress and water extract, WE= water extract H_2O_2= hydrogen peroxide

Table 2. Effect of *N. cadamba* fruit extract on hypotonic solution and heat induced hemolysis of erythrocyte membrane

Sample/Standard	% Inhibition of hemolysis	
	Hypotonic solution induced	Heat induced
Ethanolic extract	61±0.53	41±.22
Water extract	58±0.28	35±.57
ASA	72.5±0.25	68±.52

ASA= Acetylsalicylic Acid, each value is presented as mean ± SEM (n = 3). Data are found to be significant by testing through one-way ANOVA at 5% level of significance * P<0.05 when compared to the standard.

3.5. Nutritional Value of *N. cadamba* Fruit

The most important nutritional components such as, proteins, lipids, fibers, carbohydrates, moisture, and minerals were found to be in the suitable range in *N. cadamba* fruit (Table 3).

Proteins, lipids and carbohydrates constitute the structural and functional units of cells of all living organisms. Food materials balanced with these nutritional components are good for health, indeed. The crude fiber in fruits and vegetables usually comprises of cellulose, hemicellulose, lignin, pectin, β-glucans and gums [33]. High crude fiber content endorsed the fruits may be a worthy source of phenolic antioxidants. Minerals are indispensable nutrients that are obligatory in small extents to keep us healthy. As our body does not make minerals, to meet our daily requirements, minerals must be taken through foods and drinks. Minerals typically act as cofactors in enzymes and their presence in different body compartments is compulsory for multiple physiological processes (heart rhythm, nerve conduction, muscle contraction, and acid-base balance homeostasis). Additionally, they have structural functions that are principally important for bones and teeth [34,35].

In our study, the results showed that *N. cadamba* fruit is a rich source of major nutrients and some important minerals, and the fruit extracts possess potential antioxidative activity due to the presence of substantial amounts of antioxidants in extracts. Previous studies indicated *N. cadamba* fruit as edible [36,37], very few studies reported on the nutritional values of this fruit, and the information about its medicinal values are rare.

Table 3. The nutritional components and estimated minerals of *Neolamarckia cadamba* fruit

Parameter	Amount per 100 g of fresh fruit
Major nutrients	
Protein	852±0.04 mg
Carbohydrate	26.7±0.18 g
Lipid	3.5±0.2 g
Crude fiber	1.5±0.1 g
Moisture	85±2 (%)
Minerals	
Sodium (Na)	286±.83 mg
Magnesium (Mg)	97±.09mg
Potassium (K)	317±1.2 mg
Iron (Fe)	8.8±.03 mg

Each value is presented as mean ± SEM (n = 3).

4. Conclusion

Although the *N. cadamba* fruit is an edible and an affluent source of nutritional components and antioxidants, it remains neglected, mainly due to the ignorance about its nutritional and medicinal values. To the best of our knowledge, no information exists on the toxicity of this fruit, so, there is a significant scope for its use as food or food supplement and a good source of future medicines, particularly from antioxidant perspective.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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