International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 6; November 2017; Page No. 01-09

Nutraceutical potential of two edible wild fruits, *Bischofia javanica* Blume and *Ficus cunia* Buch.-Ham. ex Roxb. from Sikkim Himalaya

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Abstract
Background and Objective: The present study encompassing nutraceutical, nutritional and antioxidant properties of two wild edible fruits *Bischofia javanica* and *Ficus cunia from Sikkim Himalaya* dwells on the study of local plants from Sikkim Himalaya that is traditionally used.
Materials and Methods: Proximate parameters of the selected fruits were analysed by standard methods. The content of phenolic components, ascorbic acid, tannins and antioxidant parameters were analysed. In addition, some mineral constituents were also estimated.
Results: The presented studies established *Bischofia javanica* as an excellent protein supplement while the other fruit, *Ficus cunia* as a good supplement for vitamin-C. Furthermore, the *Ficus cunia* fruit due to its higher content of phenolics, flavonoids and flavanols is an excellent source of antioxidant which has been proven by several anti-oxidant assays.
Conclusion: The antioxidant activity shown by both the fruits is due to the significant presence of ascorbic acid, tannins and phenolic components like flavonoids, flavonols and total phenols etc. These fruits may be included in the local diet together so that one will address the malnutrition due to protein deficiency while other will take care of vitamin and antioxidant deficiency of the modern diet.

Keywords: antioxidant activity, phytochemical analysis, nutraceuticals, proximate composition, *bischofia javanica, ficus cunia*

1. Introduction
Wild fruits are used for human consumption because of their assumed health benefits for which they could be categorized as medicinal foods and nutraceuticals. Knowledge of such foods is there in the cultural tradition of many ethnic communities and as such considered as traditional knowledge [1]. Low fruit and vegetable consumption is regarded as one of the main risk factor for mortality in the world [2]. However, wild edible fruits and vegetables are known to be excellent source of nutrients such as minerals, vitamins, carbohydrates etc. And they may contribute an important part of diet providing health and nutrition while also serving as an appetizer [3]. In view of this, wild foods could become useful vehicles for improved nutrition and increased food supply [4].
Studies have shown that there is a connection between the intake of fruits and vegetables and a reduced rate of heart disease, mortality, common cancers and other degenerative diseases as well as aging, and this is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds [5].
Bischofia javanica Blume (BJ) belongs to family Euphorbiaceae, locally known as Bishop wood (Eng), Kainjal (Nep) and Sumong-kung (Lep) is an evergreen tree, upto 30m tall with palmately 3-foliate somewhat fleshy leaves. Flowers tiny, green, without petals and fruits globose or subglobose, berrylike, fleshy, upto 10 mm in diameter, brown or blue-black in color. *Ficus cunia Buch.*-Ham. exRoxb. (FC) of family Moraceae locally known as Khasray-Khaniu (Nep) and Tungshee-kung (Lep) is a tree which grows upto 10 m in height. The Bark is thick, reddish brown and contains milky juice. Fruits are borne on special shoots arising near the base of the trunk. Fruits about 20 mm in diameter and brownish-red in color. Both these plants grow in the Sub-Himalayan tract of Sikkim Himalaya at an altitude between of 300-1400 m amsl and in both the cases the ripening of fruits takes place during April-June. In traditional medicine, the leaves and buds of BJ are used in tonsillitis and throat pain whereas infusion of ground bark is used for abortion [6] and the decoction of tree bark is used for curing diarrhoea and dysentery [7]. The major phyto-constituents isolated from *Bischofia javanica* are tannin, β amyrins, betulinic acid, friedelan-3α-ol, epifriedelinol, friedelin, luteolin, quercetin, beta-sitosterol, stigmosterol and ursolic acid [8].
Similarly, in the the local traditional medicine across the Himalaya, FC is used in various ways. The latex of the plant is used to cure boils [9], it is also drunk to cure fever [10] Raw fruits are eaten in diarrhea and the decoction of the bark is taken against dysentery and liver complaints [11]. The dried leaves of FC have shown the presence of condensed tannins (+)-catechins, flavonoids quercetin, quercitrin [12], terpenes and shikimic compounds [13].
Wild edible plants are used as supplements to the cultivated crops and as famine foods during the lean season in this Himalayan region. Although these fruits are known to be edible, the nutritional information for the fruits is not available. Poor knowledge of the nutrient composition is one of the reasons for low fruit and vegetable consumption in

developing countries [14]. Therefore, the documented information on the wild edible fruits may serve as baseline data for introduction of new food items for improved nutrition and health.

The objective of the present work is to assess and compare different phytochemicals present in two edible wild fruits, BJ and FC growing in same altitude and fruiting at the same season which are commonly eaten by the local community. The phytochemical analysis, antioxidant activity, reducing power and total phenolic contents of both the fruits were evaluated. The study proved that these wild edible plants may have the potential to be valuable food sources and could easily be part of a strategy for food and nutritional security in this part of the world.

2. Materials and methods

Fresh fruits of *Bischofia javanica* Blume and *Ficus cunia* Ham. exRox. were collected from the forest of Central Pandam in Sikkim (ca 1000m amsl.). The samples were collected within 9.00 AM in ice bag and immediately brought to the laboratory for analysis.

2.1 Proximate analysis

The fruits samples of *Bischofia javanica* (BJ) and *Ficus cunia* (FC) were washed thoroughly, sliced and oven dried at 70°C until they are completely dry. The dried sample were ground into a fine powder using a mechanical grinder and stored at room temperature in desiccators until further analysis.

To determine moisture content, 25g of fresh fruit sample each was weighed and placed in an electric oven for 12-18 hours at 105°C in triplicate. The percentage loss in weight was expressed as percentage moisture contents. To determine ash content 2g of dried powder of fruits in triplicate were placed in a muffle furnace at 550 ± 5°C for 8 hours until ash was obtained. The ash content was then calculated by measuring the weight loss percentage [15].

The crude protein contents of the fruits were estimated by using auto Micro Kjeldhal apparatus (Kjeldac-Foss A 8200). For this 1g of dried fruit powder was mixed with 3 grams of digestion mixture (96.5g Na_2SO_4 + 2.5g $CuSO_4$ +1g Se) to which 30 ml of conc. H_2SO_4 was added and digested in Kjeldahl digestion at 400ºC for 80 minutes until the mixture was clear. The digest was filtered into a 100 ml volumetric flask and the solution was diluted up to the mark with distilled water. Ammonia in the digest was steam-distilled from 10 ml of the digest to which 20 ml of 45% NaOH solution had been added. The ammonia liberated was collected in 50 ml of 20% boric acid solution. Ammonia was estimated by titrating with standard 0.01 M HCl solution using a mixed indicator (0.01 g of methyl red and 0.03 g of bromocresol green in 100 ml of alcohol). Crude protein was estimated by multiplying the value obtained for percentage nitrogen content by a factor of 6.25 as per AOAC [16].

Crude fibre contents of the two wild edible fruits were estimated by acid-base digestion [17] with 1.25% H_2SO_4 and 1.25% NaOH solutions. Approximately 2g (W_1) dried powder sample was put into a 600 ml beaker and 200 ml of boiling 1.25% H_2SO was added. The contents were boiled for 3 min, cooled, filtered and the residue was washed three times with 50 ml aliquots of boiling water. The washed residue was further digested by boiling in 200 ml of 1.25% NaOH for 30 min. The residue from the digest was washed three times with 50 ml aliquots of boiling water and finally with 25 ml ethanol. The washed residue was dried in an oven at 130°Cto constant weight and cooled. The residue was scraped into a crucible, ashed at 550°C for 2 hours, cooled in desiccators and reweighed. Crude fibre content (%CFb) was expressed as percentage loss in weight on ignition.

$$\%CFb = (W_2-W_3) / W_1 \times 100$$

Where, *W*1 = Weight of sample
*W*2 = Weight of dried residue
*W*3 = Weight of ash

The crude fat contents was determined from two grams of dried fruit powder which was placed in a thimble of a Soxhlet apparatus (Borosil) above a pre-weighed receiving flask containing petroleum ether (b.p. 40-60ºC). The flask was heated for eight hours, the thimble was removed and the solvent distilled off. The flask containing the crude lipid was heated in the oven at 100ºC for 30 minutes to evaporate the solvent, cooled and reweighed. The difference in weight was expressed as percentage crude lipid content.

The carbohydrate content was obtained by indirect method difference method [18] by subtracting the values obtained for moisture, crude protein, crude fats, crude fibre and ash from 100 as per the following formula:

Carbohydrates =
100 – (%Moisture + %CP + %CF + %CFb + %Ash)

where,
CP = crude protein,
CF = crude fat
Cfb= crude fibre.

Energy value in kilocalorie per gram (kcal/g) was estimated by multiplying the content of crude proteins, crude fats and carbohydrates by the recommended factors of 4, 9 and 4, respectively and then taking the sum of the values. The value was then converted to kilojoules by multiplying by 4.2 [19].

$$\text{Energy value (kcal/g)} = (CP \times 4) + (CF \times 9) + (Carb. \times 4)$$

2.2 Phytochemical analysis

Crude alkaloid was determined as per Harborne [20]. Briefly, 2.5 g of the sample was added with 100 ml of 10% acetic acid in ethanol and covered and allowed to stand for 4 h. This was filtered and the filtrate was concentrated on a water bath to one-quarter of its volume. Concentrated ammonium hydroxide was added dropwise to the extract until complete precipitation. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

To extract and estimate crude saponin, ten grams of dried fruit powder was defatted with petroleum ether for 1h at 40 °C in a water bath and then extracted with methanol for 1 h with mild heating. It was then filtered and centrifuged at 5000g for 10 min. In order to get crude saponin extract, the methanolic

extract was dissolved in mixture of methanol and acetone (1:5 v/v) to precipitate the saponins. It was filtered and dried. Crude saponin was collected and weighed and the crude saponin content is expressed in mg/g of dried fruit powder [21].
Tannin was extracted from 5g sample which was mixed with 50 ml distilled water and shaken for 1 hr. After that, 5 ml of filtrate was pipetted out and mixed with 3 ml of 0.1 M $FeC1_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. It was estimated by measuring the absorbance at 605 nm within 10 min using tannic acid (10-100µg) as standard [22].
From the fruit samples which were dried at 70 °C for 48h in an electric oven (Rivotek) and ground into fine powder a two step extraction process was adopted for the hydrophilic and hydrophobic contributions in this study [23]. First the sample were extracted with water (hydrophilic part) and then with acetone (hydrophobic part). 10.0g of dried powdered sample was mixed with 100ml of distilled water and this homogenate was centrifuged at 6000 rpm in Hermle Labortechnik Z 32HK centrifuge machine for 15 minutes and supernatant was collected in a clean test tube. This extraction procedure was repeated 3 times and supernatants were pooled in a flask (WE). The solid residue was further extracted thrice in acetone (1:10 v/v) and supernatants were also pooled (AE).
The total phenol contents was determined from both water and acetone extracts following the method of Lin *et al.* [24]. Aliquots of 1.0 ml of water and acetone extracts (10 fold dilute each) were mixed with 5 ml of 10 fold diluted Folin-Ciocalteau reagent and 4ml of 7.5% Na_2CO_3. The mixture was incubated at room temperature 90 minutes and the absorbance was measured at 760 nm in UV-vis spectrophotometer. The phenol content was determined using Gallic acid as standard for the calibration curve. Results were expressed as mg GAE (Gallic acid equivalent/100g FW) and the calculations were done by using the following formula:

$$TPC = C \times V/m$$

Where,
TPC= total phenol content
C= concentration of Gallic acid (mg/ml)
V= volume of plant extract (ml) and
m= weight of pure plant extract (g)

The flavonoid contents in water and acetone extracts were measured as per the method of Lin *et al.* [24]. A volume of 5 ml of water or acetone extract was transferred to the test tube, mixed with the 0.3 ml of 5% sodium nitrite for 5 minutes. Then 0.3 ml of 10% aluminium chloride was added. After 6 min, reaction was stopped by addition of 2 ml sodium hydroxide. The mixture was further diluted with distilled water up to 10 ml. The absorbance of the mixture was immediately measured at 510 nm. Rutin was used as standard and the flavonoid contents were calculated and expressed as rutin equivalents (RtE).
To measure the total flavonol contents in the water and acetone extracts 2.0 ml of each sample was taken and 2.0 ml of 2% $AlCl_3$ and 3 ml sodium acetate (50 g/L) solutions were added. The mixture was incubated for 2.5 h at 20°C and the absorption was measured at 440 nm. Total flavonols content was calculated and expressed as as RtE (mg/g) as per the method reported by Kumaran and Karunakaran [25].
Ascorbic acid was determined according to the method of Klein and Perry [26] with slight modifications. Water and acetone extracts were re-extracted with meta-phosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through What man No 4 filter paper. The filtrate (1.0 ml) was mixed with 9 ml of 2, 6-dichloroindophenol (0.8 g/1000 ml) and the absorbance was measured at 515 nm within 30 minutes. Ascorbic acid content was calculated on the basis of calibration curve of L-ascorbic acid and result was expressed as ascorbic acid equivalents (AAE).

2.3 Antioxidant Analysis
DPPH scavenging activity of the wild edible fruits was determined according to Yu *et al.*, [27] with slight modification**.** Briefly 2.0 ml of the sample extract or standards was added to the 5 ml of 0.5 mM DPPH solution (20mg of DPPH powder dissolved in 100ml of methyl alcohol and vortexed. The mixture was then incubated in dark for 30 minutes at room temperature and the decolourization of DPPH was measured against blank at 517 nm. Results expressed as % inhibition was calculated by following formula:

$$\% \text{ of inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

Where,
$A_{C(0)}$ = Absorbance of control at the time of start of reaction
$A_{A(t)}$ = Absorbance of sample extract after 30 minutes

The hydroxyl radical scavenging activity of water and acetone extracts were calculated as described by Yu *et al*., [27] with slight modification. This assay is based on Fenton reaction. Briefly 2.0 ml of 0.2 M phosphate buffer (pH 7.2), 0.04 ml ferrous sulphate (0.02 M), 2 ml of extract and 1 ml of 1, 10-phenanthroline (0.04 M) were taken in a test tube. The Fenton reaction was initiated by addition of 0.1 ml of 7 mM H_2O_2. Absorbance was measured at 560 nm after 5 minutes incubation at room temperature. The relative hydroxyl radical scavenging activity (%) was calculated as:

$$\text{Scavenging Activity } (\%) = (A_{blank} - A_{sample}) / (A_{blank}) \times 100$$

Hydrogen peroxide scavenging activity of the water and acetone extracts was determined by [28] method with slight modification. The extract (4 ml) was mixed with 2.4ml of 4mM H_2O_2 solution prepared in 0.1M phosphate buffer (pH 7.4) and incubated for 10 minutes at room temperature. The absorbance was measured at 230 nm against blank, containing the extract without H_2O_2. Scavenging activity (%) was calculated by:

$$\text{Scavenging Activity } (\%) = (A_{blank} - A_{sample}) / (A_{blank}) \times 100$$

2.4 Quantification of Selected Metals
For each fruit three sample powder (1.0 g each) was weighed and digested with nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$) (20:4:2) solution for 40 minutes on hot plate in fume hood until the reddish brown fumes disappeared[29] and a clear solution was obtained. The samples were left to cool and contents were filtered. A blank digest

was also carried out in the same way. Each sample solution was made up to a final volume of 50 ml with distilled water and stored in a refrigerator before the analysis. An atomic absorption spectrophotometer (Perkin Elmer) was used for the quantification of iron, copper and calcium concentrations [15] while the phosphorous content of measured with a spectrophotometer using K_2HPO_4 as standard [30].

3. Results and discussion

3.1 Proximate compositions

The proximate analysis showed the moisture content of the fruits to be 69.16 and 80.52 for BJ and FC respectively (Table-1). The high level of moisture content in these fruits indicates the low shelf life of the fresh fruit. The level of crude fiber in food can be an indicator of the level of non-digestible carbohydrate and lignin. The crude fibre obtained were from 1.05 and 1.53% of FW for BJ and FC respectively (Table 1) which is quite appreciable and comparable to that of some berries having a value between 0.85 to 1.48 % [31]. High crude fiber content is responsible for the prevention of diseases like cancer, coronary heart diseases, obesity etc. [32, 33]. Dietary fibre also contributes to beneficial effects related to indigestibility and constipation. Both the fruits has very low amount of fat, 0.35% in FC and 1.15% in BJ respectively. This type of food composition of high fibre and low fat makes an ideal diet for overweight people. In addition, an appreciable amount of crude alkaloids and crude saponins present in both the fruits may be responsible for its medicinal properties. Alkaloids are good spasmolytic and anesthetic agents while saponins helps in boosting the immune system, lowering the cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Moderate quantity of alkaloids and saponins was found in both the fruits (Table-1). Ash content indicates the content of the total mineral in food. The ash content of BJ and FC was found to be 0.57 and 0.20 % respectively which is comparable to those of *S. theezans* fruit which showed a value of 0.48 ± 0.03% [34] for the same. A relatively appreciable level of proteins was found in both the species of fruits. Protein synthesis is necessary for the general development of the plants. In the present case, BJ showed twice the amount of protein (2.18%) as compared to the FC (0.89%). However, alkaloid content was found more in FC (0.22mg/g) as compared to that in BJ (0.16mg/g) [Table 1]. Could the proteins have developed for the above reasons or the value expressed also takes into account the nitrogen from secondary metabolites is an open question.

Preliminary phytochemical analysis revealed the presence of phenols and also alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids and triterpenoids. These are important secondary metabolites since they play many biological roles [35]. Hence the analyses of these metabolites are valuable due to their medicinal importance. The variation on the secondary metabolite level may be due to variation in the polarity of the solvents as well as the nature of the species used [36]. The difference in energy content of the two varieties was a function of their composition; FC appeared to contain more moisture, protein, lipid and crude fibre while BJ had higher ash value. It may, therefore be concluded that the differences in the proximate values were a result of variation in species.

Table 1: Proximate analysis of two wild edible fruits, *Ficus cunia* Buch.-Ham. exRoxb. and *Bischofia javanica* Blume from Sikkim Himalayas

Type of analysis	Material	
	Ficus cunia	***Bischofia javanica***
Moisture content (%)	80.52 ± 1.79	69.16 ± 3.54
Ash content (%)	0.20 ± 0.035	0.57 ± 0.034
Crude fat (%)	0.35 ± 0.017	1.15 ± 0.023
Crude fibre (%)	1.53 ± 0.135	1.05 ± 0.060
Total protein (%)	0.89 ± 0.030	2.18 ± 0.100
Total carbohydrate (%)	16.51 ± 0.37	25.89 ± 0.84
Crude alkaloids (mg/g)	0.22 ± 0.025	0.16 ± 0.025
Crude saponins (mg/g)	13.02 ± 1.56	11.88 ± 0.67
Total energy value (kcal/g)	72.75 ± 2.33	122.63 ± 6.94

3.2 Phytochemical compositions

3.2.1 Total phenols

High level of total phenol content is the uniqueness of Himalayan plants. During the present study the level of total phenols was found to be quite high in the both the wild fruit analyzed which provides a high nutritional value to both (Table-2). Phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. Phenolics provide plant defense mechanisms to neutralize reactive oxygen species (ROS) in order to prevent molecular damage and damage by pathogenic microorganisms, insects, and herbivores [37]. Phenolic compounds are the most important class of phytochemicals which are effective antioxidants as free radical scavenging, chelating metals ions and oxygen radical absorbance [38]. Of the fruit extracts, the extract with the highest levels of total phenols (567 mg/100g) was found in the acetone extract from BJ. In general, the two fruits analysed showed total phenolic contents between 189 to 342 mg/100g GAE similar to other wlde fruits like *Rubus ulmifolius* (297.39mg/100g), *Crataegus azarolus* (379.16 mg/100g) and *Crataegus monogyna* (216.61 mg/100g) [39]. The antioxidant activity of phenols from these small wild fruits plays an important role in the protection of macromolecules from oxidative damage, thereby preventing many health problems including cancer, diabetes, cardiovascular diseases, and obesity [40].

Table 2: Estimation of total phenols (mg/100g GAE), flavonoids, flavonols (mg/100g RtE) of two wild edible fruits, *Ficus cunia* Ham. exRoxb. and *Bischofia javanica* Blume from Sikkim Himalayas (Values are mean ± SE, n=3)

Material	Type of extract	Type of analysis		
		Total phenols	**Flavonoids**	**Flavonols**
Ficus cunia	WE	342.56 ± 3.69	151.46 ± 2.35	44.60 ± 2.27
	AE	327.74 ± 18.67	246.70 ± 3.54	52.34 ± 5.81
Bischofia Javanica	WE	258.31 ± 8.50	29.23 ± 1.60	25.76 ± 2.25
	AE	189.62 ± 18.76	351.18 ± 20.87	47.75 ± 3.52

3.2.2 Total flavonoid content

Flavonoids are therapeutic, water soluble polyphenloic compounds in fruits and vegetables having potential health benefits as antioxidant, antiproliferative and chemopreventive agents [41]. The important characteristics of flavonoid is that they constitute one of the major groups of phenolic antioxidants that reveal anti-proliferative activities in numerous cancer cell lines and inhibit tumor growth in a few animal models. Kaempferol, quercetin, and kaempferol-3-(60-coumaroyl)glucoside, isolated from strawberry extracts showed anti-proliferative activities in cervical, colon, prostate and oral cavity cancer cell lines [42]. The flavonoid content in the investigation presented was found to be slightly higher as opposed to that of total phenol content which is higher in water extract in both the fruits (Table-2). Under the present studies, the falvonoid content in the acetone extrct of BJ (351.18mg RtE/100g) was found to be the very high and comparable to that found in *Malus sylvestris* [43]. Similarly, the water extract of FC showed the flavonoid content to be 151.46 which is comparable to that of *Berberis lycinum* (141.2 mg RtE/100g). At the same time, the water extract of BJ showed a flavonoid content (29.23 mg RtE/100g) one fifth that of FC but comparable to that of *Vitis jacquemontii* (30.29 mg RtE/100g) [44].

3.2.3 Total flavonols

Both the fruits, FC and BJ showed an appreciable amount of total flavonols with their acetone extracts showing greater flavonol content. The maximum level of flavonol at 52.34 and 47.75 mg RtE/100g in acetone extract of FC and BJ respectively which was comparable to that of *Pistacia integerrima* (69.46 mg Rt/100 g) [45]. In contrast, the pods of *Prosopis cineraria*, a tree growing in tropical climate yields almost three times more flavonols in its acetone extract[45] than that of the fruits under the present study.

3.2.4 Ascorbic acid

Ascorbic acid (Vitamin C) acts as potent antioxidant agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor, by donating electrons, it prevents other compounds from being oxidized [46]. During the present studies the water extract of FC showed an appreciable level of ascorbic acid as compared to the other fruit (Table-3). The ascorbic acid content was higher in case of water extract than the acetone extract. The ascorbic acid content of BJ was comparable to that *of Prunus Espinosa* [39], but the water extract of FC showed three times more ascorbic acid content (Table-3). In may be safely said that the ascorbic acid content in both the fruits are in conformity with that of different other wild fruits [47] and the level was also somewhat similar to that in the roots and leaves of *Hypochaeris radiate* [48]. Ascorbic acid also helps in the absoption of iron and phenols. Associated with this, the higher phenolic content of the fruit makes it a better antioxidant.

Table 3: Ascorbic acid and total tannin content (mg/100g dry wt.) in two wild edible fruits, *Ficus cunia* Ham. ex Roxb. and *Bischofia javanica* Blume from Sikkim Himalayas (Values are mean ± SE, n=3)

Material	Type of Extract	Type of analysis	
		Ascorbic acid	Total Tannin
Ficus cunia	WE	92.14 ± 3.10	16.1 ± 1.67
	AE	26.73 ± 0.64	14.5 ± 1.76
Bischofia javanica	WE	30.86 ± 2.93	22.36 ± 2.60
	AE	19.25 ± 2.28	22.96 ± 2.75

WE = Water extract; AE = Acetone extract

3.2.5 Total tannin content

The tannin content was very high in BJ (Table-3).Tannins are known to produce anthelmintic activities as they bind to free proteins in the gastrointestinal tracts of host animals. The content of tannin in both the FC and BJ in all sxtracts found to be quite low ranging from 15 to 13mg/100g dry wt. In comparison, the minimum tannin content was three times more in case of apple fruit [43]. This may be explained by the fact that perhaps both the solvents used-water and acetone may not be good solvents for tannin extraction from these fruits. This idea is also vindicated by the fact that tannin content in two varieties of monkey kola [49] is comparable to that of the present two fruits under study.

3.2.6 Antioxidant enzymes

The activity of anti-oxidant enzymes may be an indication of general vigour and vitality of the plants. The antioxidant enzymes like catalase, peroxidase and superoxide dismutase are absolutely critical for maintaining optimal cellular and systemic health and well-being. In the present study the fruits of BJ showed a higher activity of antioxidant enzymes particularly superoxide dismutase (Fig. 1). Earlier studies on the leaves of *Phaseolus vulgaris* had shown that enzymes catalase and peroxidase showed an inverse relationship [50]. Quite unexpectedly, same type of relationship was noticed in this case with FC showing relatively more catalase and relatively less peroxidase activity while BJ showing just the opposite. Peroxidases participate in a great number of physiological processes, such as the biosynthesis of lignin and ethylene, defence against pathogens and wounding, auxin metabolism and stress response. Peroxidase constitute the first line of defence against reactive oxygene species (ROS) and changes in its indicates the redox status of plants [51]. SOD is an essential enzyme that detoxifies the highly reactive superoxide radical to create hydrogen peroxide. Left unchecked, the superoxide radical is capable of creating numerous other reactive species including peroxynitrite, lipid peroxyl and alkoxyl radicals which can cause destructive havoc throughout the body. The primary functions of this enzyme are to neutralize the superoxide radical and protect cellular proteins, lipids and DNA from ROS-induced destruction. In the presented study, the SOD activity in FC was not that great, however, FC showed an activity of 2.31 units/min/g FW which is comparable to that of banana (2.84

units / min/g FW) [52].

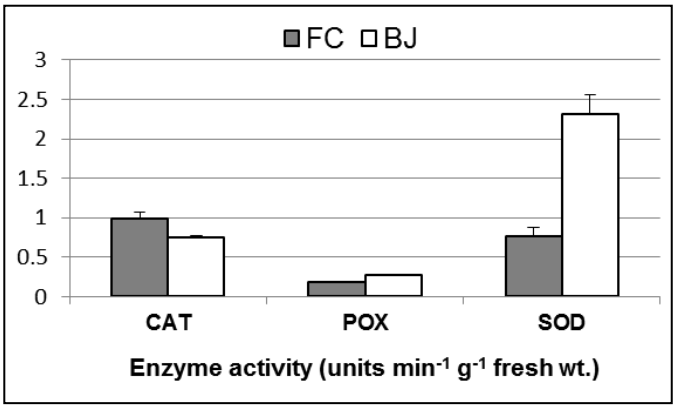

Fig 1: Variations in the activity of different antioxidant enzymes viz., catalase, peroxidase and superoxide dismutase (units/min/gm fresh wt) in two wild edible fruits *Ficus cunia* Ham. ex Rox. and *Bischofia javanica* Blume from Sikkim Himalayas. (CAT=Catalase, POX=Peroxidise, SOD=Superoxide dismutase) (Values are mean± SE, n=3)

3.2.7 DPPH assay

The DPPH method is an easy, rapid, stable and sensitive way to determine the antioxidant activity of a specific compound or plant extracts [53]. In this assay, DPPH free radical accepts hydrogen and gets reduced by an antioxidant. A freshly prepared DPPH• solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant, this colour disappears due to quenching of DPPH• free radicals and converting them into a colourless product 2,2-dipenyl-1-picryl hydrazine [54].

Acetone extract of both the fruits showed higher DPPH scavenging activity with FC showing the highest activity (86.47% inhibition). In contrast, the cold aqueous extract of wild edible mushroom, *Boletus edulis* showed similar activity while the same in alcoholic extract showed a higher activity [55]. Both the water and acetone extract of BJ and FC in this study showed DPPH activity within the range as reported earlier for wild apple in different extracts (8.12 – 78.43 %) by Stojiljkovic *et al*., [43]. High scavenging activity of this extract can be explained on account of its high flavonoid content [48]. Thus, flavonoids contribute to the DPPH scavenging activity of FC fruit extracts.

3.2.8 H_2O_2 scavenging capacity

Endogenous activity of oxidase enzymes or phagocytic activity may generate Hydrogen peroxide in the cells. H_2O_2, may directly or indirectly play a role as a messenger molecule in the synthesis and activation of several inflammatory mediators. When the antioxidant systems scavenge the hydrogen peroxide, the absorption spectrum decreases through the inhibition of peroxidase activity on H_2O_2. The ability of different extracts of the two wild fruits BJ and FC to scavenge H_2O_2 is shown in Table-4. Of the extracts analyzed, the rate of scavenging is moderate in all the cases. However, FC in acetone extract shows and inhibition of about 95%. The H_2O_2 scavenging capacity shown by these two fruits ranging approximately between 65% and 95% is comparable to that of *Crataegus monogyra* (81.04%), *Prunus spinosa* (80.59%) and *Crataegus azarolus* (78.61%) [39].

3.2.9 Hydroxyl radical scavenging assay

Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen, and are capable of damaging almost every molecule found in living cells. These radicals have the capacity to join the nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity [56]. Table-4 shows the inhibition of hydroxyl radicals by extracts of wild fruits. The results show that both the fruits had a significantly high antioxidant activity. However, the low activity in acetone extract of BJ may be due to experimental error (Table-4). The range of hydroxyl radical scavenging activity in the two fruits varied between 56.2 to 72.0 % which is somewhat similar to that of the metahnolic extract (60.8%) and ethanolic extract (73.4%) of *Boletus edulis* mushroom [55].

Table 4: Determination of antioxidant potential of two wild edible fruits *Ficus cunia* Ham. exRoxb. and *Bischofia javanica* Blume from Sikkim Himalayas in terms of their DPPH Scavenging Activity, Hydroxyl Radical Scavenging Activity, Hydrogen Peroxide Scavenging Activity, Superoxide anions scavenging activity and Ferric ion Reducing Antioxidant Power (FRAP Assay) (Values are mean ± SE, n=3)

Material	Type of Extract	DPPH Scavenging Activity	Hydroxyl Radical Scavenging Activity	Hydrogen Peroxide Scavenging Activity
Ficus cunia	WE	44.11 ±1.47	59.74 ± 1.61	64.90 ± 2.36
	AE	49.62 ± 3.29	62.18 ± 6.77	94.55 ± 4.08
Bischofia javanica	WE	66.670 ± 5.53	56.2 ± 2.42	74.24 ± 2.17
	AE	86.47 ± 3.14	72 ± 1.73	82.142 ± 4.82

WE = Water extract; AE = Acetone extract

3.3 Minerals

In wild edible plants or any other plants for that matter, mineral elements are an integral part of the architechture of its chemical molecules and structural units. These are responsible for medicinal as well as toxic proprieties of these plants [57]. These trace elements act as enzyme cofactors. The essential enzymes that require the Fe (II) as cofactor in body are pyruvate oxidase, mitochondrial cytochrome, ribonucleotide reductase, tyrosine and proline hydrolase, monoamine oxidase, glucose-6- phosphate.

The presence of vitamin C in FC and BJ will enhance the absorption of iron in humans. Copper is an important enzyme co-factor involved in electron transport chain. The enzymes in the body that require the Cu (II) ion as cofactor are cytochrome-c-oxidase, superoxide dimutase, protein-lysine 6-oxidase [58]. Calcium plays important role in the regulation of membrane permeability for various ions and various metabolic processes. *Endopleura uchi* fruits contained 96mg/100g of

calcium [59] which is slightly higher than that found in FC and BJ. Calcium plays essential role in health of bone and teeth as well as regulating blood pressure.In the present analysis it was found that the contents of iron (4.51mg/g DW) and copper (016 mg/g DW) is higher in FC while that of calcium (71.80mg/g DW) and phosphorous (65.45mg/g DW) is higher in BJ (Figure-2). Interestingly, in both the cases, the calcium: phosphorus ratio was found to be greater than one, indicating that these minerals are present in the correct proportions in both these fruits [49]. Thus, it may be safe to mention that these wild fruits may be included in the diet as a complementary source of these minerals.

4. Conclusion

The world has witnessed growing scientific and commercial interests in wild plants and plant based products, mainly due to their vast economic potential and widespread cultural acceptability, however, less than 5% species have been analyzed as potential medicine, while the rest (95%) of the plants are still there to be analyzed [60]. Food production must be actively combined with evaluation, selection, domestication and greater consumption of under-utilized or wild edible plants that are of local or regional importance to effectively increase nutritional security.

The main antioxidant substances found in fruits and plants include ascorbic acid, carotenoids and phenolic compounds [61]. Besides the phenolic acids and their derivatives, the fruits always contain members from one or more groups of flavonoids, such as glycosylated flavones/flavonols, flavanones, anthocyanins, proanthocyanidins, as the main phenolic components [62].

Considering the activity shown in the different antioxidant assays, we noted a certain association between the ability of fruits to scavenge H_2O_2 and superoxide anions with both the species showing a high H_2O_2 and low superoxide scavenging activity. However the highest scavenging activity was shown by the acetone extract of BJ in case of DPPH scavenging activity (Table). The differences shown by the same fruit in different antioxidant tests may be due to the different reaction media and to the different chemical natures of the radical species generated. The different antioxidant activity of these two fruits may also be due to differential content of antioxidant compounds in fruits, such as phenolic compounds, ascorbic acid and carotenoid etc [39].

In this study certain associations between the phytonutrient concentration and total antioxidant activity were found. In fact, in FC which showed much higher phenolic and and a high concentration of ascorbic acid also showed a much higher capacity for scavenging H_2O_2. The proximate compositions, nutritional and antioxidant activities of these fruits suggested that both BJ and FC contains nutrients, polyphenolic compounds and antioxidant activities that are useful for human health. Both these fruits have potential as a resource for dietary health supplement. Low energy, high moisture, low fat and low protein make the fruit suitable for patients with some physiological conditions.

The present study has positively contributed to some extent towards adding two new fruits from Sikkim Himalaya in food repertoire as a nutritional and Nutraceutical supplement.

Table 5: Determination of minerals, iron, calcium, copper and phosphorous (mg/100g dry wt.) in two wild edible fruits, *Ficus cunia* Ham. exRoxb. and *Bischofia javanica* Blume from Sikkim Himalayas(Values are mean ± SE, n=3)

Minerals	Plant species	
	Ficus cunia	***Bischofia javanica***
Iron	4.51 ± 0.13	2.66 ± 0.015
Calcium	40.53 ± 3.22	71.80 ± 2.60
Copper	0.16 ± 0.012	0.10 ± 0.015
Phosphorus	0.35 ± 0.055	65.45 ± 1.88

5. References

1. Mishra TK, Saha A. Variation of antioxidant properties and phytochemical constitutuents of tea cultivated under various agronomic conditions of North Bengal. NBU Journal of Plant Sciences. 2008; 2:58-66.
2. WHO. Global health risk: Mortality and burden of disease attributable to selected major risks. WHO Press, Geneva, 2009.
3. Orech FO, Hansen JA, Friis H. Ethnoecology of traditional leafy vegetables of the Luo people Bondo district of Western Kenya. Int J Food Sci Nutr. 2007; 58(7):522-530.
4. Olorode O. Conservation of plant genetic resources. African Journal of Traditional, Complimentary and Alternative Medicine. 2004; 1:4-14.
5. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables-The millenium's health. Int J Food Sci Tech. 2001; 36: 703-725.
6. Bourdya G, Walterb A. Maternity and medicinal plants in Vanatu-I: The cycle of reproduction. J Ethnopharmacol. 37:179-196.
7. Purkayastha J, Dutta M, Nath SC. Ethnobotanical plants from Dibru-Saikhowa Biosphere Reserve, Assam. Ind J Trad Knowl. 2007; 6(3):477-480.
8. Whistler WA. Polynesian plant introduction, In Cox PA and Banack SA (eds), Islands, plants and Polynesians: An introduction to Polynesian Ethnobotany, Dioscoroides Press, Portland, Oregon, 1991.
9. Karki L. Documentation of indigenous knowledge on the utilization of plant resource by the Chepang community in Dhading district, Nepal. Central Department of Botany, Tribhuvan University, Kathmandu, 2001.
10. Panthi MP, Chaudhary RP. Angiosperm flora of Arghakhanchi district and adjoining areas, West Nepal. J Nat Histy Mus. 2002; 21(1-4):7-32.
11. Khumbongmayum AD, Khan ML, Tripathi RS. Ethno medicinal plants in the sacred groves of Manipur. Ind J Trad Knowl. 2005; 4(1):21-3.
12. Nguyen VT, Tran VS, Nguyen MC, Nguyen BT. Study on the chemical constituent of *Ficus semicordata.* Tap Chi Hoc. 2002; 40:69-71.
13. Knudsen JT, Eriksson R, Gershenzon J. Diversity and distribution of floral scent. The Botanical Review. 2006; 72:1-120.
14. Grivetti LE, Ogle BM. Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutrition Research. 2000; 13:31-46.
15. AOAC. Official methods of analysis, 16th edition, Association of analytical chemists, Arlington, VA, USA, 1995.

16. AOAC. Official methods of analysis, 15th edition, Association of analytical chemists, Gaithersburg, MD, USA, 2000.
17. Aberoumand A. Nutritional evaluation of edible *Portulaca oleracea* as plant food. Food Anal Methods. 2009; 2:204-207.
18. Bamigboye AY, Okafor AC, Adepoju OT. Proximate and mineral composition of whole and dehulled Nigerian sesame seed. Afr J Food Sci Technol. 2010; 1:76-81.
19. Effiong GS, Udo IF. Nutritive values of four indigenous wild fruits in Southern Nigeria. Elect J Env Agric Food Chem. 2010; 9(7):1168-1176.
20. Harborne JB. Phytochemical methods, Chapman and Hall, London, 1973).
21. Yan W, Ontani K, Kasai R, Yamasaki K. Steroidal saponin from fruits of *Trilubus terrestris*, Phytochem. 1996; 42(5): 1417-1422.
22. Van-Burden TP, Robinson WC. Formation of complexes between proteins and tannic acid. J Agric Food Chem. 1981; 1:177-180.
23. Ji L, Wu L, Gao W, Yang, J, Guo C. Antioxidant capacity of different fraction of vegetables and correlation with the contents of ascorbic acid, phenolics and flavonoids. J Food Sci. 2011; 76:1248-1257.
24. Lin L, Cui C, Wen L, Yang B, Luo W, Zhao M. Assessment of in-vitro antioxidant capacity of stem and leaf extracts of *Rabdosia serra* (Maxim) Hara and identification of the major component. Food Chem. 2011; 126: 154-159.
25. Kumaran A, Karunakaran RJ. Antioxidant and free radical screening activity of an aqueous extract of *Coleus aromaticus.* Food Chem. 2006; 97:109-114.
26. Klein BP, Perry AK. Ascorbic acid and vitamin-A activity in selected vegetables from different geographical areas of the United States, J Food Sci. 1982, 941-945.
27. Yu L, Haley S, Perret J, Harris JW, Qian M. Free radical scavenging properties of wheat extracts, J Agric Food Chem. 2002; 50:1619-1624.
28. Aeyigoro AO, Okoh IA. Preliminary Phytochemical screening and in-vitro antioxidant activities of the aqueous extract of *Helchrysum longifolium* DC. *BMC* Compl Alt Med. 2010; 10:1-8.
29. Jan G, Khan MA, Ahmed M, Iqbal Z, Afzal A, Afzal M, Shah GM, Majid A, Fiaz M, Zafar M, Waheed A, Gul F. Nutritional analysis, micronutrients and chlorophyll contents of *Cichorium intybus* L. J Med Plant Res. 2011; 5(12): 2452-2456.
30. Chen PS, Toribara TY, Warner H. Microdetermination of phosphorous. Anal Chem. 1956; 28:1756-1758.
31. Jeong C, Choi S, Heo H. Analysis of nutritional compositions and antioxidative activities of Korean commercial blueberry and raspberry. J KorSoc Food Sci Nutr. 2008; 37:1375-81.
32. Shahar S, Yusoff A, Arshad F. Association between dietary fibre and cancer: a case-control study in Malaysia. Malays J Nutr. 2004; 10:173-182.
33. Edem CA, Miranda ID. Chemical evaluation of proximate composition, ascorbic acid and anti-nutrients content of African star apple (*Chrysophyllum africanum*) fruit. Int J Res Rev Appl Sci. 2011; 9:146-149.
34. Hyun TK, Song SC, Song CK, Kim JS. Nutritional and nutraceutical characteristics of *Sageretia theezans* fruit. J Food Drug Anal. 2015; 23:742-749.
35. Vishnu R, Nisha R, Jamuna S, Paulsamy S. Quantification of total phenolics and flavonoids and evaluation of in vitro antioxidant properties of methanolic leaf extract of *Tarenna asiatica* - an endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. J Res Plant Sci. 2013; 2(2):96-204.
36. Ghasemzadeh A, Jaafar H, Rahmat A. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe.) extracts. J Med Plant Res. 2011; 5(7): 1147-1154.
37. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation toward LDL oxidation. Free Rad Biol Med. 1997; 23:302-313.
38. Halliwel B, Aeschbach R, Loliger J, Aruoma OI. The characterization of antioxidants, Food Chem Toxicol. 2010; 331:601-617.
39. Egea I, Sanchez-Bel P, Romojaro F, Pretel MT. Six edible wild fruits are potential antioxidant additives of nutritional supplements. Plant Foods and Hum Nutr. 2010; 65:121-129.
40. Paredes-Lopez O, Cervantes-Ceja ML, Vigna-Perez M, Herandez-Perez T. Berries: improving human health and healthy aging, and promoting quality life-a review. Plant Foods Hum Nutr. 2010; 65:299-308.
41. Yao LH, Jinag YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, Chen SS. Flavonoids in food and their health benefits. Plant Foods and Hum Nutr. 2004; 59:113-122.
42. Zhang H, Zhang H, Feng Y, Yu LS, Zhang RG. Analysis of volatile components of 'Jinzhen' durian fruit by TCT-GC-MS. Food Science (Shipin Kexue). 2008; 29:517-519.
43. Stojiljkovic D, Arsic I, Tadic V. Extracts of wild apple fruit (*Malus sylvestris* L. Mill. Rosaceae) as a source of antioxidant substances for use in production of nutraceuticals and cosmeceuticals. Ind Crops and Prod. 2016; 80:165-176.
44. Abbasi AM. Ethnobotanical and nutritional investigation of wild edible fruits and vegetables used by the tribal communities of lesser Himalayas-Pakistan. PhD thesis, Quaid-i-Azam University, p-385, Islamabad, 2013.
45. Malik S, Mann S, Gupta D, Gupta RK. Nutraceutical properties of *Prosopis cineraria* (L.) Drule pods-A component of 'panchkuta'. J Pharmacog Phytochem. 2(2): 66-73.
46. Padayatty SJ, Karz A, Wang Y, Eck P, Kwon O, Lee JH, *et al*. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. J American Coll Nutr. 2003; 22: 18-35.
47. Imran M, Khan H, Shah M, Khan R, Khan F. Chemical composition and antioxidant activity of certain *Morus* species. J Zhenjiang Univ-Sciences B. 2010; 11(12):973-980.
48. Senguttavan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. For in vitro antioxidant activities. Asian Pac J Trop Biomed. 2014; 4(1):S359-S367.

49. Ene-Obong H, Okudu HO, Asumugha UV. Nutrient and Phytochemical composition of two varieties of monkey kola (*Cola parchycarpa* and *Cola lepidota*): An underutilized fruit. Food Chemistry. 2016; 193:154-159.
50. Braber JM. Catalase and peroxidise in primary bean leaves during development and senescence. Z Pflanzenphysiol. 1980; 97:135-144.
51. Schwanz P, Polle A. Differential stress responses of antioxidative systems to drought in pedunculate oak (*Quercus robber*) and maritime pine *(Pinus pinaster)* grown under high CO_2 concentrations. J Exp Bot. 2001; 52 (354): 133-143.
52. Niranjana P, Gopalkrishna KPR, Sudhakar DVR, Madhusudhan B. Effect of precooling and heat treatment on antioxidant enzyme profile of mango and banana. Afr J Food Agric Nutr Dev. 2009; 9(5):1210-1225.
53. Koleva II, Van Beek TA, Linssen JPH. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal. 2002; 13:8.
54. Sumathy R, Sankaranarayanan S, Bama P, Ramachandran J, Vijayalakshmi M, Deecaraman M. Antioxidant and antihemolytic activity of flavonoids extract from fruit peel of *Punica granatum.* Asian J Pharm Clin Res. 2013; 6(2): 211-214.
55. Vamanu E, Nita S. Antioxidant capacity and the correlation with major phenolic compounds: Anthocyanin and tocopherol content in various extracts from the wild edible *Boletus edulis* mushroom. Biomed Re s Int. DOI: 10.1155/2013/313905.
56. Moskovitz J, Yim KA, Choke PB. Free radicals and disease. Arch Biochem Biophys. 2002; 397:354–359.
57. Barnes J. Reports on complimentary health care symposium. Pharm J. 1997; 258:112-119.
58. Manore M, Meyer LN and Thompson J. Mineral and Exercise. In: Sport nutrition for health and performance. Thames and Hudson, New York, 2009.
59. Marx F, Andeade EA, DasGracas B, Zoghbi M, Maia JS. Studies of Amazonian plants, Part-5: Chemical characterisation of Amazonian *Endopleura uchi* fruits. Eur J Food Res Techno, 2002; 214:331-334.
60. Shinwari ZK, Khan J, Naz S, Hussain A. Screening of medicinal plants of Pakistan for their antibacterial activity. Afr J Biotechnol. 2009; 8 (24):7082-7086.
61. Šavikin K, Zdunić G, Janković T, Tasić S, Menković N, Stević T, Đorđević B. Phenolic content and radical scavenging capacity of berries and related jams from certificated area in Serbia. Plant Foods Hum Nutr. 2009; 64:212-217.
62. Peschel W, Bohr C, Plescher A. Variability of total flavonoids in *Crategus*: Factor evaluation for the monitored production of industrial starting material. Fitoterapia. 2008; 79:6-20.