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Nutraceutical potential of *Ficus roxburghii* an underutilized fruit of Sikkim Himalayas

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Abstract:
Ficus roxburghii, "Elephant ear fig "or wild fig is one of very popular fruits found growing wild in the hills of North Eastern and North Western Himalayan region. The fruit of wild fig has also been used as medicine by the tribal people of Sikkim and other states of India. Keeping this in view, the present study was conducted at Laboratory of Department of Horticulture, Sikkim University, Sikkim to access the different nutraceuticals properties as nutritional constituent like protein, fat, fibre, carbohydrate and energy value, mineral content *viz.*Ca, K, Mg, Na, Zn, Co, Mo, Fe, Mn and phytochemical content such as total phenols, flavonoid, ascorbic acid, anthocyanin and total carotenoids of *F. roxburghii*. The results of present study revealed that fruit of *F. roxburghii* contains significant amount of nutritional, mineral and phytochemical properties *viz.* protein (3.00±0.06%), fat (0.13±0.04%), fibre (3.06±0.02%), carbohydrate (90.81±0.44 %), energy value (376.45±1.44), Ca (23.69 ± 1.7), Mg (73.09 ± 2.1), K (819.64 ± 12.54), Mo (0.58 ± 0.06), Na (6.73 ± 1.2), Zn (0.34 ± 0.10). Fe (26.55 ± 2.8), Cu (4.22 ± 0.20), Mn (7.11 ± 0.11), total phenols (4.13±0.52 mg GAE/ G), total flavonoid (3.10±0.09 mg GAE/ G), ascorbic acid (3.36±0.27 mg GAE/ G), anthocyanin (1.13±0.15 mg GAE/ G) and total carotenoids (0.68±0.10 mg GAE/ G). It may be concluded that the fruit of *F. roxburghii*is rich in nutraceuticals and must be incorporated in our balanced diet.

Keywords: *Ficus roxburghii,* Minerals, Nutritional, Nutraceuticals, Phytochemicals

INTRODUCTION

Ficus roxburghii is a wild fig belonging to Moraceae family. Plant is small, wide-spreading, evergreen tree growing up to 12 meters tall. *Roxburghii* fig is also known as elephant ear fig because the plant has very large, ovate leaves that can be up to 55 x 30 cm. The tree is harvested from the wild for its edible fruit, medicinal uses and its leaves, which are used as plates. The plant is grown in India and from Myanmar to Vietnam and SW China and Brazil for its edible fruits. The fruit is available almost round the year usually eaten raw or cooked. The fruit is depressed-globose to pear-shaped, up to 8cm in diameter. The roasted fruit is used in the treatment of diarrhoea and dysentery. The fruits are claimed to have an anti-diabetic properties but no proper scientific evidence have been found yet. A huge biodiversity of underutilized fruits and vegetables exists in Sikkim Himalaya but their potentials and proper utilization has still not fully exploited. *F. roxburghii* is one of those valuable underutilized fruits growing wild in Sikkim is being claimed to be rich in nutraceuticals properties. Nutraceutical is associated with the combination of nutrient, minerals, phytochemicals as bio active substances which are of known therapeutic values which substantially contribute towards human health as a mode of balance diet and therapeutic drugs. Underutilized species having high nutritional and nutraceuticals values are major source of protective food which occurs naturally and always played vital role in nutritional security for large section of rural tribes. *Ficus* species are rich source of polyphenolic compounds and flavonoids which are responsible for strong antioxidant properties that helps in prevention and treatment of various oxidative stress related diseases (George *et al.,* 2016). Keeping the above points into con-

sideration, the nutraceuticals constituents viz. nutritional constituents (protein, fat, fibre, available carbohydrate and energy value), minerals, total, phenols, flavonoids, ascorbic acid, anthocyanins and total carotenoids of *F. roxburghii* were determined.

MATERIALS AND METHODS

The present investigation on Nutraceuticals potential of *Ficus roxburghii,* an underutilized fruit of Sikkim Himalayas." was carried out at Laboratory of Department of Horticulture, Sikkim University during the year 2014-2017. Experiment was conducted on matured fruits of *F. roxburghii* which were directly collected from the forest area of different region of Sikkim Himalayas. Nutritional component viz. crude protein, crude fat, crude fibre, available carbohydrate, energy value, minerals and phytochemicals *viz.* total phenols, flavonoid, ascorbic acid, anthocyanin and total carotenoids were estimated using standard method of chemical analysis which are mentioned below:

Nutritional analysis

Crude protein: The crude protein was estimated by Lowry's method (Lowry *et al.,* 1951) by using UV/VIS Spectrophotometer, Perkin Elmer, Lambda 35 UV/VIS spectrometer.

Crude fat: Crude fat content was determined by Soxhlet principle with slight modification (A.O. A.C, 1990). Fat from the oven dried fruit sample was extracted in essential oil extractor (model no. Socsplus-SCS 06 DLS, PELICAN) using petroleum ether as solvent then ether is evaporated and determined the weight of the fat recovered using following Eq. 1.

Crude fat (%) $=W_2-W_1$/Weight of the sample X 100 …….Eq. 1

Crude fibre: Crude fibre was analyzed using fibre estimation system, model no Fibra plus-FES 04 AS DLS, PELICAN.2 g of moisture and fat free sample were taken in the crucibles then it was loaded in the instrument. 150 ml of 1.25 % of H_2SO_4 was added from the top and boiled at 500^o C for 30 minutes. Once the boiling was completed the reagents was drained out with the help of fibra flow then 150 ml of 1.25 % NaOH was added from the top and heating the sample at 400^o C for 45 minutes which led to digestion of sample. After completion of digestion reagents was drained out and residue was dried in hot air oven at 90 -100^o C and cooled and weighed the dried residue (W_1) then the residue was kept in pre-weighed porcelain crucible and put in the muffle furnace for ashes at 600^{o}C in 3 hours then it was cooled and weighed (W_2). Crude fibre content was expressed as percentage loss in weight on ignition (A.O.A.C, 1990) and calculated using following Eq. 2:

Crude fibre (%)$=W_1-W_2$/Weight of the sample X 100 ……..Eq. 2

Available carbohydrate: The percentage of available carbohydrate was calculated by: 100-(Percentage of ash+ Percentage of fat + Percentage of fibre + Percentage of protein) (A.O.A.C, 1990).

Energy value/nutritive value: The energy value in kilocalorie per gram (Kcal/g) was determined by multiplying the percentage of crude proteins, crude fat and carbohydrate by the recommended factor 4, 9 and 4, respectively and then taken the sum of values. The value was then converted to kilojoules by multiplying with 4.2 (A.O.A.C, 1990).

Energy value (Kcal/g) = (CP x 4) + (CF x 9) + (Carb. x 4)

Ash content: Ash content was determined by following the method of (A.O.A.C, 1990)For thiscrucible were kept in a muffle furnace at 600^{o}C for 1h. Then they were transferred from furnace and cooled to room temperature and weighed (W_1) asquickly as possible to prevent moisture absorption. 2 g dried fruit sample was takenin crucible and placed in a muffle furnace at 600^{o}C for 6h. Then crucible wastransferred to cooled at room temperature and weighed (W_2), Then the percentage of ash was calculated byusing the following Eq. 3:-

Ash (%) $=W_2-W_1$(Wt. of ash) Weight of the sample X 100 …..Eq. 3

Mineral analysis: ICP-MS (Inductively Coupled Plasma Mass Spectrophotometry) Perkin Elmer Nex ION 300X was used for estimation of some mineral elements. Digested samples were analyzed for the ionic constitution using multi elements standards for detecting the elements such as Ca, Fe, Mg, Mn, Mo, Na, Zn. The micro wave digestion system (Anton par microwave 3000) was used for sample digestion as 0.5 gm sample were along 9ml of 69% nitric acid and 2ml HCl were added into the digestion tube and run the instrument for 40 minutes. The digested samples were then transferred into 50ml volumetric flask when the temperature of the sample was reduced and distilled water was added for making the volume of 50 ml. The liquid sample was transferred into narrow mouth bottle until the minerals were determined in ICP-MS. The values of the elements were expressed as µg/L.

Nutraceutical analysis

Extraction of fruit sample: The matured fruits of *F. roxburghii* collected from different places of Sikkim were washed and cleaned thoroughly in running water. Fruits were then chopped into small pieces and dried at 105 o C for 48 hours in hot air oven. Dried sample were then grind into fine powder using Willey mill and 5 gram of sample each sample was extracted using 50 ml solvent (80 % methanol) for 12 hours at 60^o C temperature in Soxhlet apparatus (essential oil extractor: model no. Socsplus-SCS 06 DLS, PELICAN). After completion of boiling, temperature was increased to 150^{o}C for 45min to evaporate the sol-

vent. The extract were concentrated to dryness in rotary evaporator under reduced pressure and weighed. The extracts were then diluted with known volume (mg/ml) of methanol in air tight small container and kept under refrigerator at 4^{o} C until analysis.

Total phenols: The concentrations of total phenol content of methanol extract of fruits were determined in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer) by employing the method (Singleton *et al.,* 1999) with minor modification involving Folin-Ciocalteau Reagentas as oxidizing agent and gallic acid as standard.

Total Flavonoid: The aluminum chloride assay was used for the determination of the total flavonoid content of the fruit extracts according to the method described by (Kumaran and Karunakaran, 2007) with slight modifications in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer).

Ascorbic acid: The ascorbic acid was determined by reduction of 2, 6-dichlorophenol indophenols dye by ascorbic acid as procedure given by(A.O.A.C, 1980) Ten (10) ml of juice was taken and blended with 0.4% HPO_3 and finally volume was made up to 100 ml with 0.4% HPO_3 and then 10 ml aliquot was titrated against standardized dye to obtain a pink colour which persists at least for 15 seconds. Ascorbic acid was expressed regarding mg per 100 gm pulp by using Eq. 4:

Ascorbic acid (mg/100 g pulp) =Dye factor x titre reading x dilution / Weight of sample X 100 ……..Eq. 4

Anthocyanin content: Anthocyanin content was determined by the method described by (Srivastava *et. al.,* 2003) with some modification. Sample was extracted by blending 10 g of finely ground sample with 10 ml of 95 % ethenolic HCL and centrifuged at 10000 rpm for 20 minutes then supernatant was collected and transferred into 100 ml volumetric flask and volume was made up to the mark and solution was stored in the refrigerator at 4^{o} C until analysis. The optical density of the aliquot was determined at 530 nm in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer). The value of total Anthocyanin content was expressed as mg/100 gram. Calculation was done by using Equation No. 5.

Table 1. Nutritional content of *Ficus roxburghii.*

S.N.	Parameters	Values (%)
1.	Crude protein	3.00±0.06
2.	Crude fat	0.13±0.04
3.	Crude fibre	3.06±0.02
4.	Ash	2.99±0.41
5.	Carbohydrate	90.81±0.44
6.	Nutritive value (Kcal/100g)	376.45±1.44

*Each value is an average of 3 determinations.

Total O.D/100g =O.D. x volume made up x100/ weight of sample X100 ……..Eq. 5

Total anthocyanin (mg/100 g) =Total O.D./ 100g/98.2

Total carotenoids: One gram of sample was weighed and grinds it with acetone using acid and alkali washed sand in a pestle and mortar. The extract is decanted into a conical flask. Continue the extraction till the residue was colourless. The acetone extract was transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. About 25 ml of 5% sodium sulphate solution was added. Shaked it and kept for sometimes and yellow colour pigment is transferred into the petroleum ether later. Collected the layer in a volumetric flask and separated acetone layer containing 5 % sodium sulphate. Kept on adding 15 ml petroleum ether to the acetone layer containing Na_2So_4 until thecolour gets transferred into the petroleum ether and measured the colour intensity at 452 nm in a spectrophotometer. And the total carotenoids content was calculated using Equation No. 6:

Total catotenoids (mg/100 g) =3.857xO.D.x Volume made up x 100/weight of the sample X 100 ……..Eq. 6

Statistical analysis: All the experiments were carried out in triplicates and data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Nutritional constituent: Details in respect to nutritional constituents of *Ficus roxburghii* fruits are summarize in Table 1. The present study revealed that the fruit of *F. roxburghii* contains 3.00±0.06 % crude protein which was similar to the finding as revealed by (Bhutia, 2013) while analyzing fruit of *F. roxburghii*. The variable range of protein content from 2.65 % to 5.58 % in different indigenous fruits of Sikkim. As far as crude fat is concerned it was found to be less as it was present in minute quantity i.e. 0.13±0.04 % in fruit pulp (Sundriyal and Sundriyal, 2003). Whereas, Rai *et al.,* (2005) noted 0.9 % crude fat content in *Ficus hookeriana* of Sikkim Himalaya which is similar to our finding. Crude fibre content in present study was observed as 3.06±0.02 %. Fibre is also known to reduce risk of

Table 2. Mineral content of *Ficus roxburghii.*

S. N.	Minerals	Values (µg/L)
1	Calcium	23.69 ± 1.7
2	Magnesium	73.09 ± 2.1
3	Potassium	819.64 ± 12.54
4	Molybdenum	0.58 ± 0.06
5	Sodium	6.73 ± 1.2
6	Zinc	0.34 ± 0.10
7	Iron	26.55 ± 2.8
8	Copper	4.22 ± 0.20
9	Manganese	7.11 ± 0.11

*Each value is an average of 3 determinations.

Table 3. Phyto-chemical constituent of *Ficus roxburghii.*

S.N.	Parameters	Composition
1.	Total phenol (mg GAE/g)	4.13±0.52
2.	Total flavonoid (mg QE/ g)	3.10±0.09
3.	Ascorbic acid (mg/ 100g)	3.36±0.27
4.	Anthocyanin (mg/100 g)	1.13±0.15
5.	Total carotenoid (mg/100 g)	0.68±0.10

*Each value is an average of 3 determinations.

some of the world's most prevalent disease like obesity, diabetes, high blood cholesterol, cardiovascular disease, and numerous gastrointestinal disorders (Venn and Mann, 2004);(Tungland and Meyer, 2002). The value of ash content was recorded as 2.99±0.4%. The estimated value of carbohydrate in present study was recorded as 90.81±0.44 %. A relative species *Ficus palmata* fruit was screened and carbohydrate content was recorded as 20.78 % (Saklani *et al.,* 2012) which is lesser than that of current finding. The nutritive value or calorific value of *F. roxburghii* fruit was found to be 376.45±1.44 Kcal/100 g.

Mineral composition: The mineral composition is an important for reliable nutrient informa-tion and its pivotal role in human life provides healthy growth (Anuradha *et al.,* 2013).The data pertaining to mineral composition of *Ficus roxburghii* fruits are presented in Table 2. Out of nine minerals quantified in the fruit of *F. roxburghii* potassium was highest i.e.819.64 ± 12.54µg/L and Zinc (0.34 ± 0.10µg/L) and Molybdenum (0.58 ± 0.06µg/L) were lowest. Good amount of calcium, magnesium and iron was also noted i.e.23.69 ± 1.7 µg/L, 73.09 ± 2.1 and 26.55 ± 2.8, respectively. As human body is not capable to synthesize vitamins, the consumption of diets containing these compounds will be beneficial. Kalita *et al.* (2014) reported that *M. roxburghii* and *S. spirale* were having highest amount of potassium (23.8g % and 23.3g %), followed by *P.pedicellatum*and *G. hirta*. Seal *et at.* (2014) reported highest potassium content (6.14 ± 0.16 to 57.22 ± 0.84 mg/g) among other minerals while analyzing wild fruits.

Phytochemical constituents: Phytochemical constituents such as total phenols, total flavonoid, ascorbic acid, anthocyanin and total carotenoid are presented in table 3. The total phenol content was found to be 4.13±0.52mg GAE/g whereas, the observed value of total flavonoid was 3.10±0.09mg QE/ g. likewise the ascorbic acid, anthocyanin and total carotenoide were found in a range of 3.36±0.27mg/ 100g, 1.13±0.15mg/ 100g and 0.68±0.10mg/100 g, respectively. Prakash *et al.* (2012) found the total phenolic contents (TPC) of 7.3 mg/100g in *Ficus hookeri*, fruits. Aberoumand (2011) evaluated the total phenolic contents in eight plant foods viz. *Alocacia indica Sch, Eulophia Ochreata Lindl, Momordica* dioicia *Roxb., Asparagus officinalis*, *Chlorophytum comosum, Codia myxa, Portulaca oleracia and Solanum indicum* were used as traditional vegetables and fruits. Their phenolic contents ranged from 0.87 to 7.02 mg gallic acid/g. The nutraceuticals value of any plant mainly depends upon the antioxidative properties. Certain compounds like total phenol, total flavonoid, ascorbic acid, anthocyanin, carotenoids etc. can enhance the antioxidant activity.

Nutraceutical content of *F. roxburghii* have found to be rich in major nutrients and phytochemicals and it is also found to be superior as compared to other commercial fruits.

Conclusion

The present investigation suggests that the fruit of *Ficus roxburghii* is a major source of nutritional, mineral, phytochemicals and phenolic compounds. The fruit of *F. roxburghii* found to be rich in nutritive constituents such as protein (3.00±0.06 %), carbohydrate (90.81±0.44 %), crude fibre (3.06±0.02). Mineral like Potassium content (819.64 ± 12.54 µg/L) of the fruit was also observed in good amount and the phyto chemicals constituents like total phenols, flavonoid, Ascorbic acid are also observed in minute quantities. Therefore, the fruits of *F. roxburghii* can be used as a source of nutraceuticals and can be supplemented through a balanced diet which could be much safer and cheaper than commercially available fruits.

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