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Biochemical and Molecular Analysis of Wild Endemic Fruits of the Manipur Region of India

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Manipur state of India is endowed with enormous genetic diversity in local and indigenous fruits, but nutritional attributes of some have not been scientifically investigated earlier. Nineteen endemic, endogenous fruits from 11 families were selected for biochemical and molecular analyses. Ascorbic acid, antioxidant activity, and correlation analysis revealed high and varying quantities. Phyllanthus emblica *had the highest vitamin C (375.68 mg/100 g) and antioxidant activity (IC_{50} 181.21 μg/ml). High-quality genomic DNA isolation was standardized, showing excellent spectra, efficient restriction digestion, and PCR amplification. Here, nutritional screening and molecular analysis of the endemic fruits and their potential nutritive value to hinder malnutrition is reported.*

KEYWORDS vitamin C, antioxidant, DNA isolation, Averrhoa carambola, Calamus latifolius, Citrus medica, Citrus species phouheiri, Docynia indica, Elaeagnus pyriformis, Elaeocarpus floribundus, Hodgsonia macrocarpa, Phyllanthus emblica, Prunus armeniaca, Prunus persica, Prunus triflora, Punica granatum, Pyrus communis, Spondias pinnata, Zizyphus mauritiana

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INTRODUCTION

Malnutrition is one of the most devastating problems worldwide and is inextricably linked with poverty. Eliminating hunger and malnutrition is one of the most fundamental challenges facing humanity (Lomborg, 2004). It is the condition that results from an unbalanced diet in which certain nutrients are lacking, in excess (too high an intake), or in the wrong proportion (Arthur et al., 2003). Fighting malnutrition, mostly through fortifying foods with micronutrients (vitamins and minerals), improves lives at a lower cost and in a shorter time than other forms of aid, according to the World Bank. The Copenhagen Consensus, which looks at a variety of development proposals, ranked micronutrient supplements as number one (Kristof, 2009). This can be achieved effortlessly from various fruits that are widely distributed, but oblivious in certain regions.

Fruits are rich in a variety of antioxidant compounds, such as ascorbic acid, tocopherol, glutathione, and carotenoids, all of which contribute to protection against oxidative damage by reactive oxygen species (ROS) (Bloknina et al., 2003). About 5% or more of the inhaled oxygen (O_2) is converted to ROS by univalent reduction of O_2. Antioxidants act by scavenging ROS, inhibits their formation by blocking activation of phagocytes, binding transition metal ions, and preventing formation of OH and or decomposition of lipid hydroperoxides by repairing damage (Niwa et al., 2001).

Ascorbic acid (vitamin C) is the principal vitamin supplied by fruits in the diet. About 90% of a person's dietary vitamin C requirement is normally obtained from fruits and vegetables. An adult human being on average requires about 50 mg of vitamin C per day (Salunkhe et al., 1991). It is needed during various growth stages of life and being a strong reducing agent, it helps to tie up free radicals and thus protect the body from their deleterious effects (Sumati et al., 2003).

In the recent past, rapid progress in plant molecular biology and biotechnology has opened up interesting and challenging possibilities in characterizing and evaluating diversity and conservation of plant genetic resources. Procuring a good quality, intact, high molecular weight genomic DNA is a primary requisite for all kinds of DNA-based molecular biology applications, such as PCR, RAPD, RFLP, Southern blotting, library construction, DNA barcoding, etc., in plants (Karthikeyan et al., 2010; Ibrahim, 2011).

Northeast India is endowed with enormous genetic diversity in a number of crops like citrus, banana, mango, rice, maize, and a wide range of fruit species. The Manipur region of northeastern India covers an area of 22,327sq. km and is the natural home of many citrus species (Sheo Govind and Ghosh, 1997). The state lies between the geographical position of longitude 98° 03′ E and 94° 78′ E and latitude 23° 83′ N and 25° 68′ N having a border of about 854 km of which 352 km are in the international border with upper

Myanmar in the east and Chin hills in the southeast, which offers enormous trade potential with other Asian countries (Ramanathan et al., 2007). The state is a part of the Indo-Myanmar hotspot, which is one of the areas of the world rich in biodiversity (Myers et al., 2000). The agro-climatic conditions of the region are different from the rest of the country having an average rainfall of 1507 mm, much higher than the Indian average. It belongs to the catchment areas of two major river systems, namely, the Brahmaputra-Barak and the Irrawaddy-Chindwin systems.

Unfortunately, due to deforestation and urbanization, the local wild fruits of Manipur are in danger of extinction and will be out of the scenario unless proper identification and exploration for conservation is initiated. Although a few reports have documented the antioxidant activities from some wild fruits of Manipur (Haripyaree et al., 2010; Sushma and Shantibala, 2010), currently no study has been reported at the molecular level for these wild nonconventional fruits. Keeping mind the above significant values of vitamin C and antioxidant properties in human health, the present work was focused on estimating some nutritive values of nineteen wild fruits from different families found in different regions of the state. And as a prelude to the molecular studies, herein, we describe standardization of DNA isolation protocols for the wild fruits for downstream molecular studies.

MATERIALS AND METHODS

Plant Materials

Nineteen test samples of wild fruits were harvested from various locations in Manipur for biochemical analysis. For biochemical study the fruits were washed and shredded into small pieces and dried at 55 to 60°C in a hot air oven. Samples for ascorbic acid determination were harvested on the day of the analysis. For nucleic acid isolation fresh young leaves were used.

Chemicals

Chemicals for molecular biology work were obtained from Sigma (St. Louis, MO, USA) and Bio Basic (Canada), restriction enzymes, Taq DNA Polymerases, DNA Ladders were obtained from New England Bio Lab (Ipswich, MA, USA), RNaseA from Invitrogen (Carlsbad, CA, USA).

Isolation of DNA and PCR Analysis

DNA was isolated from fresh leaf samples using CTAB (cetyl trimethyl ammonium bromide) extraction method (Murray and Thompson, 1980). In brief,

100 mg of leaf tissue was ground in liquid nitrogen. Freshly prepared extraction buffer containing β-mercaptoethanol was added. The suspension was incubated at 60°C for 30 min with intermittent mixing. Then an equal volume of chloroform:isoamylalcohol (24:1) was added and centrifuged at 12000 rpm for 5 min. DNA from an aqueous layer was precipitated by adding 0.6 volume of isopropanol. The mixture was centrifuged at 13000 rpm for 10 min to collect the DNA pellet. The pellet was washed with 70% ethanol and centrifuged at 13000 rpm for 10 min. After drying the pellet at 37°C, it was dissolved in Tris-Cl-EDTA (pH 8.0) buffer and checked in a 0.8% agrose gel.

PCR amplification of DNA with housekeeping Actin gene primers from rice was carried out in a final 50 ul volume of reaction mixture. A reaction contained 50 ng DNA, 1 u of Taq DNA polymerase, 10 mM dNTP mix, 1X Taq DNA polymerase buffer, and 10 pmol each forward 5′ AGCGAGTCTTCATAGGGCGATTGT 3′ and reverse 5′ TAGCTCTGGGTTCGAGTGGCATTT 3′ primer. The reaction conditions were 95°C, 3 min; 35 cycles at 94°C, 50 sec; 68°C, 50 sec; 72°C, 2 min; and a final extension at 72°C for 10 min. PCR product was subjected to 1.2% agarose gel electrophoresis in 0.5 XTBE buffer, stained with ethidium bromide and photographed in a gel documentation system (Alpha imager).

Ascorbic Acid Estimation

Ascorbic acid was determined titrimetrically by the modified Tillmann's method (Pauel and Pearson, 1967) using 2, 6-dichlorophenol, indophenol reagent. In all cases, samples for analysis were prepared in 0.4% oxalic acid solution.

Antioxidant Activity Determination

The antioxidant activity of the wild fruit was examined using the chemical assays of DPPH (Krings and Berger, 2001), using ascorbic acid as the standard. The reaction mixture consisted of 0.004% of 0.1 mM DPPH methanol with 50–250 μg/ml of the fruits extract or 0.01 mM of vitamin C. After 30 min incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm. Percentage inhibition was determined by comparison with a methanol-treated control group. The percentage of DPPH decoloration was calculated as follows:

$$\%\text{DPPH decoloration} = \left(1\text{-O.D. sample/O.D. control}\right) \times 100.$$

The degree of decoloration indicates the free radical scavenging efficiency of the fruits and the IC_{50} value shows the potential of antioxidant

activity, which was co-related by plotting a graph of concentration sample versus the % of DPPH inhibition. Pearson coefficient correlation was use for correlationship analysis between vitamin C and antioxidant activity.

RESULTS AND DISCUSSION

The 19 wild fruits (Fig. 1) were identified at Botanical Survey of India (BSI), Eastern Regional Central, Woodlands, Laitumkhrah, Shillong, Meghalaya, India. The taxonomic name and species with local variation referred to here as local varieties are shown in Table 1. Most of the wild fruits showed good content of vitamin C and antioxidant activity. A strong correlation was also obtained between the vitamin C and antioxidant activity and good quality genomic DNA was isolated.

FIGURE 1 Photo plates of all the wild fruit samples selected for the present study. The numbers of the fruits are in accordance with Table 1.

TABLE 1 List of the Wild Fruits Collected and Identified from Various Regions of Manipur, India

Sl. No.	Botanical name	Family	Vernacular name	Local/Manipuri name
1.	*Averrhoa carambola* Linn.	Oxalidaceae	Star fruit	Heinajom
2.	*Calamus latifolius* Roxb.	Arecaceae	Cane	Heiri
3.	*Citrus medica* Linn.	Rutaceae	Citron	Heijang
4.	*Citrus species* phouheiri	Rutaceae	—	Phouheiri
5.	*Docynia indica* Wall. Decne.	Rosaceae	Indian crab apple	Heitup
6.	*Elaeagnus pyriformis* Hk. f.	Elaegnaceae	Silverberry	Heiyai
7.	*Elaeocarpus floribundus* Blume.	Elaeocarpaceae	Indian olive	Chorphon
8.	*Hodgsonia macrocarpa* Blume. Cogn.	Cucurbitaceae	Chinese lard fruit	Kathai
9.	*Phyllanthus emblica* L.	Phyllanthaceae	Indian gooseberry	Heikru
10.	*Prunus armeniaca* L.	Rosaceae	Apricot	Malhei
11.	*Prunus persica* L. Batsch.	Rosaceae	Peach	Choombheri
12.	*Prunus triflora* Roxb. var. kalenheikha.	Rosaceae	Plum	Kalenheikha
13.	*Prunus triflora* Roxb. var. mangomix.	Rosaceae	Plum	Mangomix
14.	*Prunus triflora* Roxb. var. applemix.	Rosaceae	Plum	Applemix
15.	*Prunus triflora* Roxb. var. maoheikha.	Rosaceae	Plum	Maoheikha
16.	*Punica granatum* Linn.	Puniaceae	Pomegranate	Kaphoi
17.	*Pyrus communis* Linn.	Rosaceae	Pear	Naspati
18.	*Spondias pinnata* L. f. Kurtz.	Anacardiaceae	Hog plum	Heining
19.	*Zizyphus mauritiana* Lamk.	Rhamnaceae	Indian zuzube	Boroi

Ascorbic Acid Content (AAC) of Manipur Fruits

Ascorbic acid is a water-soluble vitamin, which is found in many biological systems and fresh vegetables and fruits. From the analysis, vitamin C was found to be highest in *Phyllanthus emblica* having 375.68 mg/100 g of FW (fresh weight) and lowest in *Prunus armeniaca* containing 6.91 mg/100 g (Table 2). Three types of amlaki fruit used as a main ingredient in the preparation of famous ayurvedic tonic '*chavyanpras*' showed variable content of ascorbic acid like big amalaki 245 mg/100 g, medium amalaki 275 mg/100 g, and small amalaki 350 mg/100 g (Vaishali et al., 2003). Mobasseri (2004) and Garg et al. (2008) have reported that amla (*Emblica officinalis*) juice has 20 times more vitamin C than orange juice. It is a nutritionally and medicinally important fruit, which can correlate with our result of *Phyllanthus emblica* that are grown wild in Manipur have a high content of vitamin C 375.68 mg/100 g. The reason for this could be that ascorbic acid content (AAC) increases as the fruit ripens (Lim et al., 2006). The increase in AAC as the fruit mature is due to the breakdown of starch to glucose, which is used in the bio-synthesis of ascorbic acid. At the same time, *Spondias pinnata* had

TABLE 2 Vitamin C Antioxidant Profile and Correlation Analysis of Wild Fruits of Manipur, India

Sl. No.	Name of the fruits	Vitamin C (mg/100 g) FW[z]	AOA (IC_{50} μg/ml)[z]	r ($p < 0.05$)[z]
1.	*Averrhoa carambola*	16.38 ± 2.01	1179.96 ± 104.9	−0.580
2.	*Calamus latifolius*	17.63 ± 3.21	584.24 ± 79.5	−0.142
3.	*Citrus medica*	11.61 ± 2.50	719.20 ± 134.8	−0.307
4.	*Citrus species* phouheiri	36.33 ± 6.56	1701.81 ± 105.0	−0.915
5.	*Docynia indica*	14.84 ± 2.68	1657.28 ± 867.1	−0.259
6.	*Elaeagnus pyriformis*	20.10 ± 4.76	867.84 ± 175.2	−0.896
7.	*Elaeocarpus floribundus*	16.22 ± 2.94	1393.41 ± 593.1	−0.726
8.	*Hodgsonia macrocarpa*	13.21 ± 1.79	2717.46 ± 363.6	−0.039
9.	*Phyllanthus emblica*	375.68 ± 110.6	181.21 ± 2.0	−0.940
10.	*Prunus armeniaca*	6.91 ± 1.21	755.26 ± 45.6	−0.864
11.	*Prunus persica*	10.76 ± 2.18	910.34 ± 100.2	−0.703
12.	*Prunus triflora* var. applemix	8.02 ± 1.02	1147.24 ± 121.2	−0.695
13.	*Prunus triflora* var. kalenheikha	13.18 ± 1.37	1192.83 ± 449.1	−0.633
14.	*Prunus triflora* var. mangomix	8.60 ± 2.15	1379.73 ± 427.1	−0.510
15.	*Prunus triflora* var. maoheikha	13.03 ± 2.59	666.64 ± 33.7	−0.579
16.	*Punica granatum*	14.41 ± 0.88	398.54 ± 47.6	−0.704
17.	*Pyrus communis*	10.34 ± 2.53	1557.09 ± 227.7	−0.522
18.	*Spondias pinnata*	86.16 ± 11.04	518.77 ± 6.9	−0.924
19.	*Zizyphus mauritiana*	11.91 ± 2.28	1378.33 ± 239.8	−0.647

Ascorbic acid content (AAC) mg/100 g FW (fresh weight), antioxidant activity (AOA) IC_{50} μg/ml, and last column shows Pearson Correlation analysis between the AAC and AOA of all the wild fruits. All values are mean ± SD of triplicate of three biological sample determinations.

much higher levels of AAC (86.16 mg/100 g) as compared to values of grape juice, oranges, and strawberries ranging from 38–59 mg/100 g (Arnao et al., 2001), and lime, papaya, mausambi, lemon sweet, and pineapple ranging from 39–63 mg/100 g (Smith and Somerset, 1993). Owolarafe et al. (2006) reported the vitamin C of Nigerian *Spondias mombin* L. as 38 mg/100 g and was found to be very low compared with the *Spondias* species found in Manipur. Citrus species *phouheiri* of the Rutaceae family also has a reasonable amount of vitamin C (36.33 mg/100 g), which is close to that of *Citrus maxima* (36.62 mg/100 g) of fresh juice (Meena Devi et al., 2010), pumelo (36 mg/100 g) (Tarai et al., 2005). Earlier studies indicated (12.04 mg/100 g) of vitamin C of fruit *Elaeagnus umbellate* (Parmar and Kaushal, 1982) and (13.8 to 16.9 mg/100 g) of vitamin C in different fruits of *Elaeagnus umbellate* (Sabir and Riaz, 2005), which was somewhat lower as compared to our investigation in *Elaeagnus pyriformis* (20.10 mg/100 g) found in Manipur. This variation may be attributable to the differences in genetic traits or perhaps the variation in soil nutrients due to variation in soil pH and nutrient levels (Mengel and Kirkby, 1987). Suksri (1999) stated that a variation in soil pH gave variation in amounts of soil nutrients available to plant roots and the uptake in plants could be related to the available amounts or due to climatic factors. Fruit samples of *Averrhoa carambola* having 16.38 mg/100 g

of vitamin C were found to have similar ascorbic acid values as *Averrhoa carambola* (star fruit) found in Kerala and Tamil Nadu (Meena et al., 2010) and in the West Bengal state of India (Tarai et al., 2005). Among the wild plum varieties, *Prunus triflora* var. 'kalenheikha' having 13.18 mg/100 g was found to have differentially higher vitamin C content than *Prunus triflora* var. 'maoheikha' at 13.03 mg/100 g, *Prunus triflora* var. 'mangomix' at 8.60 mg/100 g, and *Prunus triflora* var. 'applemix' at 8.02 mg/100 g. The increase in ascorbic acid might be the result of perpetual synthesis of vitamin C from its precursor until the development of chocolate tint color on the ground surface of fruit (Singh et al., 1981) or perhaps the result of greater synthesis of glucose (-6-) phosphate, a precursor of L-ascorbic acid (Mapson, 1970). Work in wild plum has been reported with similar findings by Yurdugul and Bozoglu (2009).

Antioxidant Activity (AOA) of Manipur Fruits

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules and recently several studies have indicated that antioxidants prevent the onset of degenerative illness, such as certain cancers, cardiovascular and neurodegenerative diseases, cataracts, oxidative stress dysfunction, and aging (Ritaro et al., 2008). Primary antioxidant properties are generally measured by DPPH assay (expressed as IC_{50}). The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action the higher the antioxidant activity AEAC value (ascorbic acid equivalent to antioxidant capacity), and this is reflected in lower IC_{50} value (Lim et al., 2006). IC_{50} μg/ml values are defined as the concentration of the extracts causing 50% inhibition of absorbance. It is known that fruit ripening continues after harvest and this process leads to significant changes in the contents of the antioxidant (Lim et al., 2006). The same action is found in our sample, *Phyllanthus emblica*, having the lowest IC_{50} value 181 μg/ml (Table 2). From our findings, wild pomegranate fruits also have considerable AOA, having lower IC_{50} at 398.54 μg/ml and *Spondias pinnata* having IC_{50} 518.77 μg/ml AOA compared to other samples. These may be attributed to the fact that in the DPPH assay the antioxidant reacts with the stable free 1, 1-diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1, 1-diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample antioxidant resulting in a decrease in absorbance at 517 nm (Tianpech et al., 2008). Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract (Sujata et al., 2011). The above findings are in close conformity with the works of Bibhabasu et al. (2008) and are somewhat similar with Oki et al. (2006). Fruits like *Calamus latifolius, Prunus triflora* var. 'maoheikha', *Citrus*

medica, Prunus armeniaca, Elaeagnus pyriformis, and *Prunus persica* also have a moderate activity of antioxidants, while the remaining samples show very low antioxidant activity. It may be because it does not have a potent primary antioxidant property. In an independent study of wild fruits of Manipur, IC_{50} 98.87 μg/ml of *Averrhoea crambola*, IC_{50} 102.79 μg/ml of *Spondias pinnata*, IC_{50} 268.98 μg/ml of *Elaeocarpus floribundus* were reported by Haripyaree et al. (2010), which is quite high relative to the determinations in the present investigation. This could be due to a difference in the collection site of the fruits. Further studies using various fruit samples of individual fruit collected from different regions of Manipur will give a clearer picture.

Correlation Analysis between AAC and AOA of the Wild Fruits of Manipur

Ascorbic acid, the most well known antioxidant, is an important molecule in plant tissues to protect plants against oxidative damage resulting from the oxidation of metabolites of photosynthesis and aerobic processes (Smirnoff, 1996). From this analysis, a high content of ascorbic acid was detected in *Phyllanthus emblica*, followed by *Spondias pinnata* and *Citrus* species *phouheiri*, which showed high correlation with their IC_{50} having '*r*' values of −0.940, −0.924, and −0.915, respectively (Table 2). *Elaeagnus pyriformis, Prunus armeniaca, Elaeocarpus floribundus, Prunus persica*, and *Punica granatum* had a favorable amount of ascorbic acid and were found to be correlated with their IC_{50} having $r = -0.896$, $r = -0.864$, $r = -0.726$, $r = -0.726$, $r = -0.703$, and $r = -0.704$, respectively, indicating a correlation between antioxidant activities and ascorbic acid content. Similar findings were observed earlier in guava (*Psidium guajava* Linn.) (Weerasak et al., 2006). Fruit samples of *Zizyphus mauritiana, Averrhoa carambola*, and *Pyrus communis* showed a medium correlation. In spite of having reasonable ascorbic acid contents in some samples, they tend to show antioxidant activities that may be attributed to the presence of some other antioxidant phytochemicals (Brahma et al., 2005). Among the plum varieties, *Prunus triflora* var. 'maoheikha' showed medium correlation. These relationships are comparatively high compared to those observed in cornelian cherry (*Cornus mas* L.) having ($r = 0.322$ and $r = 0.316$) (Rop et al., 2010), but comparable with fruit samples of *Citrus medica* ($r = -0.307$). A very low correlation was observed in *Docynia indica* and *Hodgsonia macrocarpa*, which indicates a big difference in the content of chemical compounds in the fruit (Ros et al., 2004).

Nucleic Acid Isolation from Wild Fruits of Manipur

As a first step towards molecular analysis, isolation of good quality DNA and RNA is essential. The fruits studied from the region in this investigation

have not been involved in any kind of molecular investigations. This is the first report of DNA isolation and their quality check. The DNA bands of the 19 wild endemic fruits selected for molecular analysis showed good quality of DNA bands in 0.8% of agrose gel electrophoresis above 10 kbs and quantities ranging from 8.6–13.63 μg/100 mg of tissues (Fig. 2A and B). Upon restriction digestion with different enzymes like EcoR1, Hindi III, and Nco1, 18 wild fruit samples shown good digestibility (Fig. 3). The isolated DNA used for PCR analysis with housekeeping Actin gene primers showed the desired 200 bp amplicon of all the fruits (Fig. 4). This verifies that the isolated DNA of these wild fruits was of excellent quality. For diversity analysis

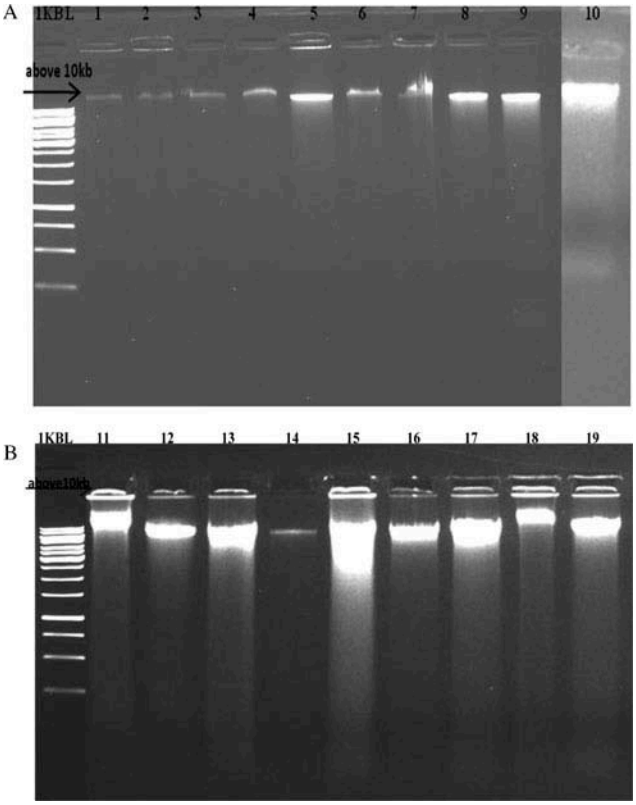

FIGURE 2 (A and B) Electrophoretic analysis of high quality genomic DNA from wild fruits samples. Lanes: 1KBL-1KB. Ladder: 1. *Docynia indica*, 2. *Prunus triflora* var. mangomix, 3. *Prunus triflora* var. applemix, 4. *Prunus armeneica*, 5. *Punica granatum*, 6. *Averrhoa carambola*, 7. *Elaeocarpus floribundus*, 8. *Hodgsonia macrocarpa*, 9. *Prunus triflora* var. kalenheikha, 10. *Citrus species* phouheiri, 11. *Citrus medica*, 12. *Zizyphus mauritiania*, 13. *Phyllanthus emblica*, 14. *Elaeagnus pyriformis*, 15. *Calamus latifolius*, 16. *Pyrus communis*, 17. *Spondias pinnata*, 18. *Prunus persica*, 19. *Prunus triflora* var. maoheikha. The samples were analyzed on 0.8% agrose gel loading 3 μl of isolated DNA at 50 volts for 2–3 h obtaining high molecular weight bands above 10 kb.

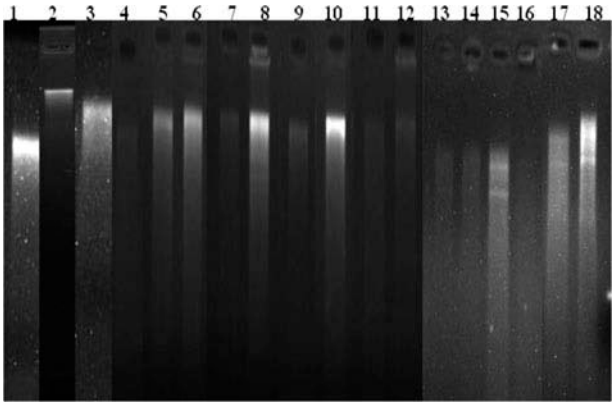

FIGURE 3 Restriction digestion of 18 wild fruits' genomic DNA with various enzymes. Lanes: 1. *Docynia indica*, 2. *Punica granatum*, 3. *Averrhoa carambola*, 4. *Elaeagnus pyriformis*, 5. *Citrus medica*, 6. *Zizyphus mauritiana*, 7. *Elaeocarpus floribundus*, digested with EcoR1, 8. *Hodgsonia macrocarpa*, 9. *Citrus species* phouheiri, 10. *Phyllanthus emblica*, 11. *Pyrus communis*, 12. *Spondias pinnata*, with Hind III, 13. *Prunus triflora* var. mangomix, 14. *Prunus triflora* var. applemix, 15. *Prunus armeniaca*, 16. *Prunus triflora* var. kalenheikha, 17. *Prunus persica*, 18. *Prunus triflora* var. maoheikha with Nco1, electrophoresis on 1.2% agrose gel.

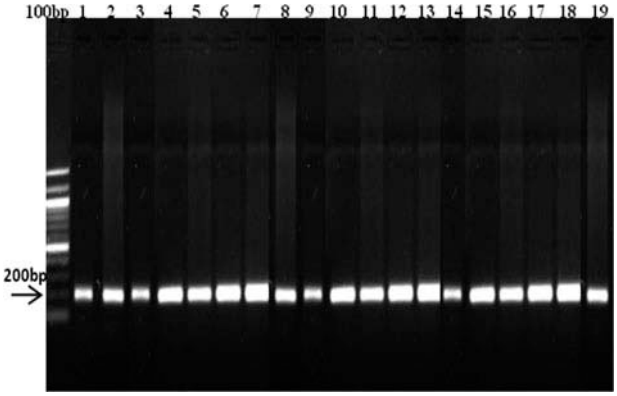

FIGURE 4 PCR analysis with primers for housekeeping gene Actin from rice using 19 wild fruits' genomic DNA; 1.2% agrose gel electrophoresis showed 200 bp amplified fragments. Lanes: 100 bp. DNA ladder: 1. *Docynia indica*, 2. *Punica granatum*, 3. *Averrhoa carambola*, 4. *Elaeagnus pyriformis*, 5. *Citrus medica*, 6. *Zizyphus mauritiana*, 7. *Elaeocarpus floribundus*, 8. *Hodgsonia macrocarpa*, 9. *Citrus species* phouheiri, 10. *Phyllanthus emblica*, 11. *Pyrus communis*, 12. *Spondias pinnata*, 13. *Prunus triflora* var. mangomix, 14. *Prunus triflora* var. applemix, 15. *Prunus armeniaca*, 16. *Prunus triflora* var. kalenheikha, 17. *Prunus persica*, 18. *Prunus triflora* var. maoheikha, 19. *Calamus latifolius*.

and identification of wild unknown fruits using various methods, such as barcoding, high quality DNA is the first input. DNA barcodes are short segments of DNA (<800 bp) that can be easily generated and used to characterize all species on the planet (Savolainen et al., 2005) particularly when

diagnostic morphological features are absent or insufficient (Costion et al., 2011). In fact, the *Citrus* species *phouheiri* and *Prunus* species analyzed here as well as various unexplored fruit species of Manipur could be identified using this method. DNA barcoding, as a molecular tool, has the potential to greatly advance our access to the collective knowledge of the biodiversity. By harnessing advances in molecular genetics, sequencing technology, and bioinformatics, DNA barcoding will allow users to quickly and cheaply recognize known species and retrieve information about them (Schori and Showalter, 2011). It may also speed the discovery of the thousands of species yet to be named.

The results described here will provide a framework for researchers to exploit these wild fruits for various molecular analyses in downstream experiments as well as in nutritional enhancement towards alleviating malnutrition due their desirable phytochemical components.

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