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Phenolic content and Antioxidant Activity of some Underutilized Wild Edible Fruits of the Sikkim Himalaya

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Abstract

In the present study, nine potential wild edible fruits viz., *Elaeagnus latifoila, Diploknema butyracea, Eriolobus indica, Spondias axillaris, Machilus edulis, Baccaurea sapida, Ficus hookeri, Elaeocarpus Sikkimensis and Cyphomandra betacea* were investigated for antioxidant activity (AOA), total phenolic contents (TPC) and free radical scavenging activities (FRSA). Among different fruits analyzed, *Spondias axillaris* extract was found to possess the highest antioxidant activity (73.9%) and total phenolic content (69.4 mg gallic acid equivalent/g extract). *Spondias axillaris* extract also showed best free radical scavenging activity in terms of IC_{50} value (inhibitory concentration); EC_{50} (efficiency concentration; mg/mg DPPH); ARP (anti radical power) and reducing power (ASE/ml). The study thus signifies the potential of *Spondias axillaris* as a source

of antioxidant compounds.

Keywords: Antioxidant, polyphenol, wild fruits, 1,1-diphenyl-2-picryl hydrazyl radical

Introduction

Fruits are important sources of minerals, fiber and vitamins, which provide essential nutrients for human health. Increased consumption of fruit and vegetables significantly reduces the incidence of chronic diseases, such as cancer, cardiovascular diseases and other age-related disorders. Various compounds such as polyphenols, carotenoids (pro-vitamin A), vitamins C and E (tocopherol) present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases[1,2,3]. Polyphenols express many biological activities, such as antifungal, antibacterial, antiviral, anti-inflammatory, anticancerous and antioxidative[4,5,6,7,8], and hence the continued identification of fruits with high polyphenol content is of considerable interest and importance to the scientific community due to the potential health benefits of these compounds[9]. In the Sikkim Himalaya, several varieties of locally available wild fruits are commonly consumed and are considered an integral part of ethno-culture globally. Many of them have been used traditionally as medicines and also made in to sauces, jellies, jams or pickles for human consumption[10,11,12]. The antioxidant properties of cultivated edible fruits and plants are well investigated, however, there is little information available about such properties of underutilized wild edible fruits.

The aim of the present investigation was to evaluate the antioxidant potential of nine commonly used wild edible fruits species growing in the Sikkim Himalayan region of India viz., *Elaeagnus latifoila, Diploknema butyracea, Eriolobus indica, Spondias axillaris, Machilus edulis, Baccaurea sapida, Ficus hookeri, Elaeocarpus Sikkimensis* and *Cyphomandra betacea* by three different antioxidant test systems; β-carotene/linoleic acid, reducing power and DPPH, and to determine their total phenolic content (TPC).

Materials and Methods

The selected nine fruits *viz. Elaeagnus latifoila, Diploknema butyracea, Eriolobus indica,*

Spondias axillaris, Machilus edulis, Baccaurea sapida, Ficus hookeri, Elaeocarpus Sikkimensis and *Cyphomandra betacea* were collected from different parts of the Sikkim Himalaya from November 2006 to June 2007 (**Figure 1**).

Description of the selected wild edible fruits

Detailed study of the nine selected wild edible plants of the region performed in terms of their general characteristics, locational details, plant parts used and other relevant information are given in **Table I**.

1. ***Elaegnus latifolia*** **L. (syn. *Elaeagnus conferta* Roxo**., EIaeagnaceae; local name: muslendi 'bastard oleaster') is a dense thorny shrub or small bushy tree found in the lower temperate zone. The fruit is long elliptical about 4 cm long, reddish with light freckles**,** and sour in taste. Fruits are commonly available in the markets during February-March.

2. ***Diploknema butyracea*** **Roxb (syn. *Bassia butymcea* Roxb.*, Aesandra butyracea*** (Roxb.) Baehni, Sapotaceae; local name: chiuree 'Indian butter tree') is a robust tree up to 15 m high, growing in the temperate zone between 600-1,500 m. The fruits are scented and pulp is juicy and sweet. The seeds are also a source of low-grade oil and mayonnaise.

3. ***Eriolobus indica*** **Schn. (Rosaceae**; local name: mehal 'Indian crabapple') is a straggly tree up to 12 m high, growing between 1,200-1,800 m. Fruits are available during November –December, which are roundish, about 1 inch in diameter, light greenish, spotted. These are sour in taste and eaten fresh, made into pickles or turned into a concentrate known as the melko chook. The chook acts as food condiment and also doubles up as a cure for diarrhea.

4. ***Spondias axillaris*** **Roxb. (syn. *S. acuminata* (L.f.) Kurz.*, Choerospondias axillaris*** (Roxb.) Burtt. & Hill, Anacardiaceae; local name: lapsi 'hog-plum') is a robust tree attaining up to 35 m height and growing between 300-1,500 m elevations. It commonly grows in lower hill forests. The longish ovoid fruit are available during October- December. The fruit is mostly pickled for future use and also eaten fresh.

5. ***Machilus edulis*** **King (syn. *Persea fructifera* Kost**., Lauraceae; local name: pumsi) is an evergreen large tree with dense crown reaching height up to 24 m or more. It grows between 1,500-1,800 m elevations. It is considered to be a local variety of avocado

(*Persea americana*). Fruits are commonly found in the markets during December-February. Fruit is globose, 2.5-4.5 cm diameter, greenish and the outer fleshy pulp that comes out attached with the skin is scooped out and eaten.

6. ***Baccaurea sapida*** **Muell. (syn. *B. ramiflora* Lour.,** Euphorbiaceae; local name: kusum 'sapida') is an evergreen tree reaching 10 m height or more and found at the deeper valleys closer to the streams and Terai. The yellowish fruits are sweet and about 1 inch in diameter

Figure-1. Some important wild edibles used in the study are; 1. Muslendi (*Elaeagnus latifoila*), 2. Chiuree *(Diploknema butyracea*) Lam), 3. Mehel *(Eriolobus indica*.) Decne), 4. Lupsi (*Spondias axillaris*.), 5. Pumsi (*Machilus edulis*), 6. Kusum (*Baccaurea sapida*), 7. Nebara (*Ficus hookeri*), 8. Bhadrase (*Elaeocarpus Sikkimensis),* and 9. Rukh tamatar (*Cyphomandra betacea).*

and are available from June-July.

7. ***Ficus hookeri*** **Nebara N (Moraceae)** is an evergreen tree reaching 12 m height. It grows mainly in subtropical to upper temperate region of the Sikkim Himalaya. Its fruits are available during June-September.

8. ***Elaeocarpus sikkimensis*** **Roxb (syn.** ***Elaeagnus conferta*** **Roxo.,** Elaeagnaceae; local name: muslendi 'bastard oleaster') is a large evergreen tree up to 30 m height of the upper temperate zone and grows at lower elevations in the Sikkim Himalaya. Its fruit is oblong 4 cm long, with tips tapering towards both ends, greenish in colour and available in the market in March-May. The seeds within the hard shell are also eaten.

9. ***Cyphomandra betacea*** **Rukh tamatar N (Solanaceae)** is a small deciduous tree (3m high). This plant is found in subtropical regions of Sikkim, Nepal and Darjeeling hills. Fruits are commonly found in the markets during June-December and mostly pickled for future use.

Fruit extraction and antioxidant assays

Fruits collected from different natural habitats of the Sikkim Himalaya were chopped, dried, powdered (40-mesh) and stored in polythene bags at 4° C. The powdered material (100 mg) was extracted with 50% MeOH : H_2O (1:1, 2 X 10 ml), overnight at room temperature. The total phenolic contents (TPC) in different extracts were estimated by the method of Ragazzi and Veronese[13] and expressed as gallic acid equivalent (GAE) mg/g

on dry weight basis. The antioxidant activity (AOA) of plant fruit extracts was assayed by auto-oxidation of β-carotene and linoleic acid[14] and expressed as per cent inhibition relative to control.

Free radical scavenging activity (FRSA) of the extracts (1.0 mg/ml methanol) was assayed by using 1, 1-diphenyl-2-picryl- hydrazil (DPPH) radical (6×10^{-5} M in MeOH) according to Yen and Duh[15]. The inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti radical power (ARP) were estimated as described by Kroyer[16]. Reducing power of fruit extracts (1.0 mg/ml in MeOH) was determined[17] by ferric reducing - antioxidant power assay and by using quercetin as standard. Reducing power was expressed as ascorbic acid equivalent (1mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power.

Statistical analysis

All experiments were performed in triplicate. The statistical analysis was done by analysis of variance (ANOVA) and the data were recorded as means ± standard deviations.

Results and discussion

Several previous studies have revealed that phenolic contents in plants are associated with antioxidant activities probably due to their redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers[18]. Therefore, the content of total phenolic compounds in the wild edible fruit extracts was determined and the results are presented in **Table II**. The total phenolic content varied from 7.3 to 69.4 mg gallic acid equivalents (GAE)/g extract with lowest being reported for *Ficus hookeri* (7.3 mg/g) and highest for *Spondias axillaries* (69.4 mg/g). The total phenolic content of the selected wild edible fruits are obtained in the order: *Spondias axillaris>Baccaurea sapida>Diplokenema butyracea>Elaeagnus latifoila>Elaeocarpus Sikkimensis>Cyphomandra betacea>Machilus edulis>Eriolobus indica>Ficus hookeri* (**Table II**).

In the present study, three most widely used assays namely β-carotene/linoleic acid test, DPPH free radical scavenging assay and ferric reducing - antioxidant power assay were applied to evaluate the antioxidant capacities of wild edible fruit extracts and the results are given in **Table II** and **Table III**.

In the β-carotene/linoleic acid test, the antioxidant activity of wild edible fruits showed wide variation ranging from 8.6 % (*Eriolobus indica)* to 73.9% (*Spondias axillaries).* Among different fruits analysed, *Spondias axillaris* extract found to possess the highest antioxidant activity (73.9%), and the activity decreased in the order: *Spondias axillaris>Baccaurea sapida> Cyphomandra betacea >Machilus edulis>Elaeagnus latifoila > Elaeocarpus Sikkimensis >Ficus hookeri >Diplokenema butyracea > Eriolobus indica* (**Table II**).

Free radical scavenging activity (FRSA) of fruit extracts was investigated using DPPH free radical assay (**Table III**) in terms of inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti radical power (ARP). The fruits of *Baccaurea sapida, Eleocarpus*

Table I- Details of nine selected wild edible fruit species, their habit, habitat, distribution and plant parts used

Sl. No.	Botanical names	Family	Altitude	Plant habit (height)	Distribution	Availability	Plant parts and Methods of use
1.	*Elaeagnus latifolia* Linn. syn. N-Muslerh**i**	Eleagnaceae	>800m	Evergreen liana (25-35 m)	Subtropical-Temperate Himalaya,, Sikkim, Darjeeling, Khasi hills	Feb-March	Raw, fruits, Pickle, Chutney, Jelly
2.	*Diploknema butyracea* (Roxb.) N-Chiuree	Sapotaceae	600 - 1500 m	Tree (10-15 m)	Throughout Himalaya: Garhwal, Kumaon, Nepal, Sikkim, Darjeeling, and Bhutan	May-June	Raw, Jam
3.	*Eriolobus indica* Schn. N-Mehel	Rosaceae	1200 - 1800 m	Deciduous tree (8-12 m)	Lower temperate forests, community forests	Nov-Dec	Raw fruits, Pickle
4.	*Spondias axillaris* Roxb. syn. *S.* N-Lupsi	Anacardiaceae	300 - 1500 m	Deciduous tree (24-35 m)	Subtropical to temperate forests	Oct-Dec	Raw, Pickle
5.	*Machilus edulis* King. syn. N-Pumsi	Lauraceae	1,500-1,800 m	Evergreen tree (15-24 m)	Nepal to Sikkim, Bhutan, and North Eastern region	Dec-Feb	Raw
6.	*Baccaurea sapida* (Roxb.) N-Kusum	Euphorbiaceae	200-500 m	Subtropical Forest (10 m)	Sub-Himalayan tracts: Nepal to Sikkim, Darjeeling hills, and North east India, Bhutan Burma and Andamans	June-July	Raw, Squash
7.	*Ficus hookeri* Sweet N-Nebara	Moraceae	500-1750 m	Evergreen Tree (12 m)	Subtropical to upper temperate	June-Sep	Raw
8.	*Elaeocarpus Sikkimensis* Mast. N-Bhadrase	Elaeocarpaceae	1830-2450 m	Evergreen Tree (30 m)	Subtropical to upper temperate	March-May	Raw
9.	*Cyphomandra betacea* Sendt. N-Rukh tamatar	Solanaceae	900-1700 m	Semi-deciduous, Small tree (3 m)	Subtropical, Nepal to Sikkim and Darjeeling hills	June-Dec	Pickle

[N – Vernacular names in Nepali]

Table II- Antioxidant activity and total phenolic contents (mg/g plant material expressed as gallic acid equivalent) of fruits and their underutilized parts (Dry weight basis) (after Prakash et al[19]). Values are presented as mean ± SE (n=3).

S. No.	Species	Antioxidant activity (%)	Total phenolic contents (mg GAE /g)
1.	*Elaeagnus latifoila* Linn.	32.1+1.7	24.2+1.9
2.	*Diplokenema butyracea (Roxb)* Lam.	19.6+2.1	37.1+4.1
3.	*Eriolobus indica (Wall.)* Decene	8.6+1.4	10.4+1.9
4.	*Spondias axillaris* Roxb.	73.9+4.2	69.4+.3.6
5.	*Machilus edulis* King	38.1+2.8	12.7+2.5
6.	*Baccaurea sapida (Roxb.)* Muell.-Arg.	64.7+2.7	51.4+3.4
7.	*Ficus hookeri*	21.2+2.5	7.3+1.2
8.	*Elaeocarpus Sikkimensis*	31.8+2.2	18.2+1.4
9.	*Cyphomandra betacea*	50.3+3.3	15.4+2.3

Table III- Free radical scavenging activity (FRSA) measured by using 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) in term of IC_{50} = inhibitory concentration (mg/mg of dry extract); EC_{50} = efficiency concentration (mg/mg DPPH); ARP = anti radical power and reducing power (ASE/ml) (after Prakash et al[19]). Values are presented as mean ± SE (n=3).

S. No.	**Species**	**IC_{50}**	EC_{50}	ARP	ASE/ml
1	*Elaeagnus latifoila* Linn.	0.060	2.61	38.44	0.81
2	*Diplokenema butyracea (Roxb)* Lam.	0.055	2.30	41.83	1.92
3	*Eriolobus indica (Wall.)* Decene	0.081	3.52	28.37	2.51
4	*Spondias axillaris* Roxb.	0.032	1.39	71.94	1.17
5	*Machilus edulis* King	0.102	4.34	23.51	4.63
6	*Baccaurea sapida (Roxb.)* Muell.-Arg.	0.027	1.12	85.40	0.47
7	*Ficus hookeri*	0.230	10.0	10.08	3.25
8	*Elaeocarpus Sikkimensis*	0.044	1.91	52.36	1.52
9.	*Cyphomandra betacea*	0.039	1.69	59.28	1.68
	Quercetin	0.021	0.87	115.01	0.51
	CD at P< 0.01	0.06	0.36	0.24	0.29

sikkimensis, Spondias axillaris, Cyphomandra betaceae showed low IC_{50} ranging from 0.027 to 0.044 mg/mg, low EC_{50} from 1.12 to1.91 mg/mg DPPH and reasonably high values (52.36 to 85.40) of ARP.

The reducing powers of fruit extracts are presented in **Table III**. Extracts of *Baccaurea sapida*, *Elaeagnus latifoila, Spondias axillaries, Eleocarpus sikkimensis* and *Cyphomandra betaceae* exhibited remarkable reducing capacity as evident by their low ASE/ml values. The fruits of *Baccaurea sapida* showed highest reducing power that is in close proximity to standard, quercetin. Further, it was noticed that *Elaeagnus latifolia* fruits exhibited low ARP (38.44) but good reducing power (0.81 ASE/ml).

A correlation between higher antioxidant activity and larger amount of total phenolics was found in *Spondias axillaris* and *Baccaurea sapida* extracts. Though other antioxidants were probably present in these plants, total phenolic compounds could make a significant contribution to the antioxidant activity in these plant extracts.

Conclusion

According to the results of this study, it is clearly indicated that the extract of wild edible species has significant antioxidant activity against various antioxidant systems; moreover, wild edible species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. Phenolic compounds seem to be the main components responsible for the antioxidant activity of all the species extracts. From the wild edible plant studied, *Spondias axillaries* and *Baccaurea sapida* may be interesting plants due to their high total phenol concentration and antioxidant activity. Thus, *Spondias axillaries* and *Baccaurea sapida* are recommended for use in foods as natural antioxidants. To understand further the beneficial values of these fruits and their role in the prevention and treatment of chronic diseases, further research is necessary.

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Authors Column

Dr. K. K. Singh, Scientist 'F' and former Scientist Incharge, GBPIHED, Sikkim Unit has been working in the area of plant physiology and biochemistry for the past thirty five years. He supervised Ph.D. work of two students and published one hundred two research articles in highly reputed peer reviewed international and national journals. His major interest is R&D work currently is on rhododendrons, incorporating biotechnological and conventional methods to counter the threats on survival of these plants.

Dr. Singh worked jointly with Professor Martin Gibbs, Brandies University, Waltham, United States and reported for the first time that intact chloroplasts of *C. reinhardtii* and spinach indicate the respiratory electron transport system functioning in the darkened chloroplasts has many properties in common with the electron transport pathway of mitochondria.

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