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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME WILD EDIBLE FRUITS OF MEGHALAYA STATE IN INDIA

TAPAN SEAL

Botanical Survey of India, Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, Howrah, India. Email : kaktapan65@yahoo.co.in

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ABSTRACT

The present communication deals with the *in vitro* antioxidant studies of the acetone and aqueous methanol extracts of four wild edible fruits e.g. *Morus indica*, *Parkia roxburghii*, *Prunus nepalensis* and *Terminalia bellerica*, collected from Meghalaya state in India. The total phenol varied from 10.49±0.14 to 95.40±0.74 mg/g in the aqueous methanol extract and 9.21±0.23 to 130.48±0.97 mg/g in the acetone extract of the fruits. Flavonoids content were between 2.14±0.02 and 7.07±0.01 mg/g in aqueous methanol extract and varied from 2.21±0.14 to 10.37±0.10 mg/g in the acetone extract. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging was observed in the acetone extract of *M. esculenta* with IC_{50} = 0.058±9.72E-05 mg dry material. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by the acetone extract of *T. bellerica* . Flavonol content was observed highest in the acetone extract of *P. nepalensis* (14.12 ± 0.05 mg/g) and least in the aqueous methanol extract of *P. roxburghii* (2.83±0.11 mg/g). The reducing power of the extracts of the plants were also evaluated as mg AAE (ascorbic acid equivalent)/g dry material. The results indicate that these wild edible fruits can be utilized as natural antioxidant.

Keywords : Wild edible fruits, Meghalaya, Phenolic, Flavonoids, Flavonol, Reducing power, DPPH

INTRODUCTION

Antioxidants refers to compounds that can delay or inhibit the oxidation of lipids or other molecules. Oxidation reactions can produce free radicals and these radicals are responsible to many disorders and diseases in humans such as infections, diabetes, arthritis, ischemia and reperfusion injury of many tissues, gastritis, cancer, coronary heart diseases and AIDS [1]. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers [2]. It is established that consumption of antioxidant substances has been linked to the reduction in the incidence of oxidative-stress related diseases [3]. The use of currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) has been limited due to their toxicity and side effects. They are suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Hence strong restrictions have been placed on their application and therefore research for the determination of the natural oxidants source is important [4].

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude palnt drugs. Flavonoids, flavonols and other phenolic compounds are especially common in leaves, fruits, stem and barks. They are important in the plant for normal growth development and defense against infection and injury. Flavonoids an other phenolic compounds have been suggested to play a preventive role in the development of cancer and heart diseases. With the development in techniques and recent researches, it has been proved that certain non-nutritive chemicals in plants such as terpenoids, flavonoids and phenolic compounds which were earlier thought to be of no importance to human diet, possess antioxidant properties [5]. The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agent, hydrogen donators and singlet oxygen quenchers [6].

It is widely accepted that fruits and vegetables have many healthful properties and consumption in sufficient amount has been associated with protection against various forms of cancer, a number of chronic diseases, such as neoplasm, cardiovascular diseases, inflammation, neurodegenerative pathologies, cataracts, diabetes as well as the ageing process. It has also been established that fruits are the major sources of dietary antioxidant vitamins such as vitamin A, B, C and E and phenolic compounds which are also act as antioxidant [3].

Natural antioxidants especially phenolics, flavonoids and flavonols from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements [1]. Though many other fruits and vegetables have been investigated in the search for novel antioxidants in the past few years but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, the main objective of this study was to determine the antioxidant activity of different wild edible fruits grown in the Meghalaya state in India.

The present communication deals with the evaluation of antioxidant activity of four wild edible fruits like *Morus indica* Linn. (Moraceae), *Parkia roxburghii* G. Don (Mimosaceae), *Prunus nepalensis* Ser (Steud) (Rosaceae) and *Terminalia bellerica* Roxb (Combretaceae),collected from different places of Meghalaya state, India. The main target of our research was to examine the total phenolic content, flavonoid content, flavonol content and radical scavenging capacity related to antioxidant potential of these four wild edible plants. The antioxidant activity of these plant extracts has been determined as the free radical scavenging ability using stable 2, 2-diphenyl- 1-picrylhdydrazyl (DPPH) and ascertained by measuring reducing power. The traditional use and ethnobotanical importance of these plant has also been mentioned.

Morus indica Linn known as Soh Lyngdkhur (Khasi) in Meghalaya state, belongs to the family Moraceae. Ripe fruits are black in colour and eaten by the local people and used in the treatment of fever. The decoction of the leaves are used as gargle in inflammation of vocal cords [7].

Parkia roxburghii G. Don. belongs to family Mimosaceae, locally known as Zong Tan in Mizoram and Jong Sak in Manipuri . In Manipur it is considered as the most costly vegetable. Both flowers and pods are eaten as vegetable. The Manipuri takes this vegetable as raw in preparation of "Singju", a typical Manipuri salad. Sometime this may be mixed with fish and in preparation of typical delicious curry the "Iromba". Mizos, Garos, Kacharis, Nagas, Mikirs are also consuming the pods as vegetables. In Malaya, both seeds and pods are valued in medicine. Pods pounded in water are also used for washing the head and face. Bark and the leaves are employed in making lotion for skin diseases and ulcer [8].

Prunus nepalensis Ser (Steud) belongs to family Rosaceae locally known as Soh Iong in Khasi hills of Meghalaya. The fruits are edible and also used to make fruit juice called as Um Soh- Iong in khasi. Fruits are astringent, leaf are diuretic and used in dropsy [9].

Terminalia bellerica Roxb. belongs to family Combretaceae locally known as Humra guti in Meghalaya. The seeds are eaten by local people for curing gastric problem and stomach disorders. The fruits of this plant are used in piles, dropsy, leprosy, biliousness, dyspepsia and headache [7]. The fruit possesses antibacterial properties and myocardial depressive activity.

MATERIALS AND METHODS

Plant materials

The four plant materials e.g the fruits of *Morus indica*, *Parkia roxburghii*, *Prunus nepalensis* and *Terminalia bellerica*, were collected from different market of Meghalaya state, India on March 2010 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under registry no BSITS 26, BSITS 29, BSITS 30, BSITS 31, respectively. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

Extraction of plant material (Aqueous methanol and Acetone extract)

One gram of each plant material were extracted with 20 ml each of aqueous methanol (20%, v/v) and acetone at ambient temperature, with agitation for 18 -24 h. The extracts were filtered and diluted to 50 ml and aliquot were analyzed for their total phenolic, flavanoid and flavonol content, reducing power and their free radical scavenging capacity.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ascorbic acid, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., Folin-Ciocalteus's phenol reagent, gallic acid, potassium ferricyanide, Aluminium chloride, $FeCl_3$ and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Estimation of total phenolic content

The amount of total phenolic content of crude extracts was determined according to Folin-Ciocalteu procedure [10]. 20 - 100 µl of the tested samples were introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Hitachi U 2000 Japan). The total phenolic content was expressed as gallic acid equivalents (GAE) in miligram per gram (mg g^{-1}) of extract.

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordonez *et al*. [11]. To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve : $y = 0.0353x + 0.0566$, $R^2 = 0.9985$, where y was the absorbance and x was the quercetin equivalent (mg/g).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran [12] . To 2.0 ml of sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Hitachi U 2000 Japan) was read after 2.5 h at 20°C. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve : $y = 0.0513x + 0.1658$, $R^2 = 0.9995$, where y was the absorbance and x was the quercetin equivalent (mg/g).

Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu 1986 [13]. Extracts (100 µl) of fruit extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram of dry material.

Determination of free radical scavenging activity

The free radical scavenging activity of the plant samples and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [14]. Aliquots (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm (UV-visible spectrophotometer Hitachi U 2000 Japan). The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg ml^{-1}) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

STATISTICAL ANALYSIS

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and IC_{50} value of each plant material was calculated by using Linear Regression analysis.

RESULT AND DISCUSSION

Total phenol, flavonoid and flavonol content of the extracts

The screening of the aq methanol and acetone extracts of four wild fruits revealed that there was a wide variation in the amount of total phenolics ranging from 9.21 ±0.23 to 130.48±0.97 mg GAE/g dry material (Table 1). The highest amount of phenolic content was found in the acetone extract of *T. bellerica* (130.48± 0.97 mg GAE/g dry material), while least amount was observed in the acetone extract of *M. indica* (9.21±0.23 GAE). The Aq methanol extract of *T. bellerica* (95.40±0.74 GAE and the acetone extract of *P. nepalensis* (55.51±0..34 GAE) and *P. roxburghii* (79.63±0.43 GAE) was also found to contain a very good amount of phenolic compounds.

The flavonoid contents of the extracts in terms of quercetin equivalent were between 2.14± 0.02 to 10.37 ±0.10 mg/g dry material (Table 2). Highest amount of flavonoid content was observed in the acetone extract of *T. bellerica* (10.37 ±0.10 mg/g). The aq. methanol extracts of *M. indica* (7.04 ±0.06 mg/g) and *T. bellerica* were also found to contain a very good amount of flavonoid.

In case of flavonol, the highest amount was observed in the acetone extract of *P. nepalensis* (14.12±0.05 mg/g) followed by *T. bellerica* (11.06± 0.10 mg/g) (Table 3). Appreciable quantities of flavonol were found in the acetone extract of *M. indica* (5.33± 0.06 mg/g) and *P. roxburghii* (5.00± 0.06 mg/g) (Table 3).

It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties.

Table 1: Total phenolic content in the fruits collected from Meghalaya

Name of the plant	Local name at Meghalaya	Parts used	Total phenolic content (GAE mg g^{-1} of dry material) (Mean±SEM)	
			Aq methanol extract	Acetone extract
Morus indica	Soh Lyngdkhur	Fruits	24.94 ±0.58	9.21± 0.23
Parkia roxburghii	Zong Tan	Fruits	49.39 ±0.25	79.63± 0.43
Prunus nepalensis	Soh Iong	Fruits	10.49 ±0.14	55.51± 0.34
Terminalia bellerica	Humra guti	Fruits	95.40 ±0.74	130.48±0.97

Values are mean ± SEM (n =3)

Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals [15]. Flavonoids and flavonols are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoids and flavonols show antioxidant activity through scavenging or chelating process[1]. In addition to their antioxidant activities, flavonoids inhibit enzymes such as prostaglandin synthase, lipoxygenase and cycloxygenase, closely related to tumorigenesis, and may induce detoxifying enzymes such as glutathione *S*-transferase. Many kinds of flavonoid have been reported in fruits and vegetables and their types and contents vary with cultivar and maturation [16]. The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants. According to our study, the high content of these phenolic compounds in *T.bellerica*, *P. nepalensis*, *P. roxburghii* and *M. indica* can explain their high radical scavenging activity.

Table 2: Total flavonoid content in the fruits collected from Meghalaya

Name of the plant	Local name at Meghalaya	Parts used	Total flavonoid content (mg g^{-1} of dry material) (Mean±SEM)	
			Aq methanol extract	Acetone extract
Morus indica	Soh Lyngdkhur	Fruits	7.04 ±0.06	2.21± 0.14
Parkia roxburghii	Zong Tan	Fruits	4.05 ±0.03	4.35± 0.06
Prunus nepalensis	Soh Iong	Fruits	2.14 ±0.02	3.74± 0.13
Terminalia bellerica	Humra guti	Fruits	7.07 ±0.01	10.37±0.10

Values are mean ± SEM (n =3)

Table 3: Total flavonol content in the fruits collected from Meghalaya

Name of the plant	Local name at Meghalaya	Parts used	Total flavonol content (mg g^{-1} of dry material) Mean±SEM	
			Aq methanol extract	Acetone extract
Morus indica	Soh Lyngdkhur	Fruits	4.16 ±0.04	5.33± 0.06
Parkia roxburghii	Zong Tan	Fruits	2.83 ±0.11	5.00± 0.06
Prunus nepalensis	Soh Iong	Fruits	3.79 ±0.06	14.12± 0.05
Terminalia bellerica	Humra guti	Fruits	3.68 ±0.04	11.06±0.10

Values are mean ± SEM (n =3)

Reducing power assay

The reducing powers of the four wild fruits were evaluated as mg AAE/g dry material as shown in Table 4.

The reducing abilty of the aq methanol extract of the fruits in descending order was *T.bellerica* > *P. roxburghii* > *M. indica* > *P. nepalensis.* The highest reducing power was exhibited by the acetone extract of *T.bellerica* (76.65 ± 0.09 mg/g AAE) which is also high in phenolic content (130.48± 0.97 mg GAE/g dry material) and acetone extract of *M. indica* showed lowest activity in terms of ascorbic acid equivalent. In this assay, the presence of antioxidants in the extracts reduced Fe^{+3}/ferricyanide complex to the ferrous form. This reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom [3].

Table 4: Reducing power (Ascorbic acid equivalent) of the fruits collected from Meghalaya

Name of the plant	Local name at Meghalaya	Parts used	Ascorbic acid equivalent (AAE) (mg g^{-1} of dry material) (Mean±SEM)	
			Aq methanol extract	Acetone extract
Morus indica	Soh Lyngdkhur	Fruits	13.63±0.04	9.78±0.06
Parkia roxburghii	Zong Tan	Fruits	28.41±0.06	32.25±0.12
Prunus nepalensis	Soh Iong	Fruits	10.19±0.04	37.41±0.24
Terminalia bellerica	Humra guti	Fruits	50.07±0.10	76.65±0.09

Values are mean ± SEM (n =3)

DPPH radical scavenging activity

The evaluation of anti-radical properties of four wild edible fruits was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by the different plant materials was determined (Table 5), a lower value would reflect greater antioxidant activity of the sample.

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [17]. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 2,2- diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased. Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the IC_{50} value will be minimum. In the present study the highest radical scavenging activity was shown

by the acetone extract of *T. bellerica* (IC_{50} = 0.058 ± 9.72E-05 mg dry material), whereas the acetone extract of *M. indica* showed lowest activity (IC_{50} = 0.53 ± 0.009 mg dry material). Strong inhibition was also observed for the acetone extract of *P. roxburghii* (IC_{50} = 0.12 ± 0.0001 mg dry material and aq methanol extract of *T. bellerica* (IC_{50} = 0.20 ± 0.0004 mg dry material). The high radical scavenging property of T. *bellerica* may be due to the hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger. The aq. methanolic and acetone extracts of all of the plants under investigation exhibited different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements. Depending on the values, some plants are more important than some others.

Table 5 : Free radical scavenging ability of the plant samples collected from Meghalaya by the use of a stable DPPH radical (Antioxidant activity expressed as IC_{50})

Name of the plant	Local name at Meghalaya	Parts used	IC_{50} value (mg dry material) (Mean ± SEM)	
			Aq methanol extract	Acetone extract
Morus indica	Soh Lyngdkhur	Fruits	0.21±0.0005	0.53±0.009
Parkia roxburghii	Zong Tan	Fruits	0.23±0.0002	0.12±0.0001
Prunus nepalensis	Soh Iong	Fruits	0.32±0.003	0.27±0.004
Terminalia bellerica	Humra guti	Fruits	0.20±0.0004	0.058±9.72E-05

Values are mean ± SEM (n =3)

CONCLUSION

The result of present study showed that the acetone extract of *T. bellerica* which contain highest amount of phenolic compounds and flavonoids exhibited the greatest reducing power and radical scavenging activity. The acetone extract of *P. nepalensis* contain highest amount of flavonols and flavonols also showed strong radical scavenging activity. The radical scavenging activities of the selected plants extracts are still less affective than the commercial available synthetic like BHT. As the plant extracts are quite safe and the use of synthetic antioxidant has been limited because of their toxicity, therefore, these wild edible fruits could be exploited as antioxidant additives or as nutritional supplements.

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REFERENCES

1. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 2006 ; 5 (11) : 1142-1145.
2. Patel VR,. Patel PR, Kajal SS. Antioxidant activity of some selected medicinal plants in western region of India. Advances in Biological Research 2010 ; 4 (1) : 23-26.
3. Jamuna KS, Ramesh CK, Srinivasa TR, Raghu KI. In vitro antioxidant studies in some common fruits. International Journal of Pharmacy and Pharmaceutical Sciences 2011 ; 3 (1) : 60-63.
4. Torane RC, Kamble GS, Khatiwora E1, Ghayal NA, Deshpande NR. Antioxidant capacity of leaves and stem of *Ehretia laevis*. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3 (2) : 149-151.
5. Mishra J, Srivastava RK, Shukla SV, Raghav C S. Antioxidants in aromatic & medicinal plants. Science Tech Entrepreneur 2007; 1-16.
6. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 1999; 47 : 3954-3962.
7. Chopra RN, Nayar SL Chopra IC. Glossary of Indian medicinal plants. Seventh reprint. p. 170-241 (2006)
8. Bhuyan TC. Parkia roxburghii the tree bean. Rain Forest News 1996 ; 1 : 1-2.
9. Agrahar-Murugkar D, Subbulakshmi G. Nutritive values of wild edible fruits, berries, nuts, roots and spices consumed by the Khasi tribes of India. Ecol. Food Nutr. 2005 ; 44: 207-223.
10. Singleton VL, Rossi JA. Colorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965 ; 16 : 144-158.
11. Ordonez AAL, Gomez JG, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem. 2006; 97: 452-458.
12. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus,* Food Chem. 2006 ; 97 : 109-114.
13. Oyaizu M. Studies on product on browning reaction prepared from glucose amine. Jpn. J. Nutr. 1986 ; 44 : 307-315.
14. Blois MS. Antioxidant determination by the use of of a stable free radical. Nature 1958 ; 181 : 1199-1200.
15. Florence OJ, Adeolu AA, Anthony JA. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus* Rec. Nat. Prod. 2011 ; 5 (1) : 29-42.
16. Karadeniz F, Burdurlu HS, Koca N, Soyer Y. Antioxidant activity of selected fruits and vegetables grown in Turkey. Turk J Agric For. 2005; 29: 297-303.
17. Koleva I I, Van Beek TA, Linssen JPH, deGroot A, Evstatieva LN. Screening of plant extracts for antioxidant activity : a comparative study on three testing methods. Phytochemical Analysis 2002 ; 13: 8-17.