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Full Length Research Paper

Antioxidant activity of some wild edible plants of Meghalaya state of India: A comparison using two solvent extraction systems

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The objective of the present study was to find out the antioxidant potential of some wild edible plants, traditionally used by the local people of Meghalaya state in India and also to investigate the effect of solvent extraction system (aq. methanol and acetone) on the total phenolic, flavonoids and flavonols content, reducing power and antioxidant activity of the plants. The total phenol content varied from 3.31±0.10 to 27.67±0.16 mg/g in the aqueous methanol extract and 2.61±0.13 to 6.85±0.13 mg/g in the acetone extract of the plants. Flavonoids content were between 8.11±0.071 and 52.14±0.004 mg/g in aqueous methanol extract and varied from 1.22± 0.01 to 52.17± 0.01 mg/g in the acetone extract. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts were determined spectrophotometrically. The highest radical scavenging was observed in the aq. methanol extract of *Gentiana pedicellata* with IC_{50} = 0.23±0.0007 mg dry material. The greater amount of phenolic compounds, flavonoids and flavonol content leads to more potent radical scavenging effect as shown by the aq. methanol extract of *G. pedicellata*. Flavonol content was observed highest in the aq. methanol extract of *G. pedicellata* (23.12 ±0.006 mg/g) and least in the acetone extract of *Gynocardia odorata* (0.09±0.008 mg/g). The reducing power of the extracts of the plants were also evaluated as mg AAE (ascorbic acid equivalent)/g dry material and highest reducing power (16.11 ± 0.03) observed in the aq. methanol extract of *Bauhinia purpurea*, which contain maximum amount of phenolic compounds (27.67±0.16 mg/g GAE). The results indicate that the type of extragent significantly influenced the antioxidant activity of these wild edible plants and could be utilized as potential source of natural antioxidant in the food or in pharmaceutical industry.

Key words: Wild edible plants, Meghalaya, phenolic, antioxidant activity, two different solvent extraction system.

INTRODUCTION

The main characteristic of an antioxidant is to inhibit the oxidation of lipids or other molecules and hence provides a protective effect against ROS (Reactive oxygen species) such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) and free radicals, such as the hydroxyl radical (·OH) and superoxide anion (O_2^-) (Ghimire et al., 2011). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms which are responsible for many disorders and diseases in humans such as infections, diabetes, arthritis, cardiovascular diseases, cancer, Alzheimer's diseases, AIDS etc. (Patel et al., 2010).

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude plant drugs. Fruits and vegetables have long been viewed as a rich source of natural antioxidant compounds. Natural antioxidants are used to improve food quality and stability and also act as nutraceuticals to terminate free radical

chain reaction in biological systems and thus may provide additional health benefits to consumers (Nahak and Sahu, 2010).

The use of synthetic antioxdants like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) has been limited due to their toxicity and side effects and therefore search for the novel sources of natural oxidants is important (Pourmorad et al., 2006).

Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (Uddin et al., 2008). It has been observed that the antioxidant activity of plant materials are strongly dependent on the nature of the different solvent extraction system due to the presence of different antioxidant components of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Water, methanol, mixture of water-methanol, acetone have been widely used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables etc.) (Sultana et al., 2009).

Though many other plant species have been investigated in the search for novel antioxidants but generally there is still a demand to collect more information regarding the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, much attention has been given towards the identification of plants with antioxidant ability.

The forests of Meghalaya (Northeastern region in India) provide a large number of plants whose leaves, fruits, seeds, tubers, shoots etc make an important contribution to the diet of the local people. These plants also provide some useful products like medicine, fibre, fodder, dyes etc (Kayang, 2007).

The present study was undertaken to evaluate the antioxidant potential of some wild edible plants, collected from different places of Meghalaya state, India. These plants are used by the tribals of Meghalaya for their day-to -day needs. 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 and reducing power of these nine wild edible plants. The objective of the present study was also to investigate the most effective solvent extraction system to extract potent antioxidant compounds from different wild edible plants which will guide us to obtain the best sources of dietary antioxidants.

MATERIALS AND METHODS

Plant materials

The nine plant materials e.g the leaves of *Bauhinia purpurea*, *Diplazium esculentum*, *Fagopyrum cymosum*, *Ficus clavata*, *Ficus geniculata*, *Ficus pomifera* , *Gentiana pedicellata*, flower of *Dillenia pentagyna* and seeds of *Gynocardia odorata* were collected from different tribal 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 15, BSITS 16, BSITS 17, BSITS 20, BSITS 21, BSITS 22, BSITS 23, BSITS 24 and BSITS 25 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, with agitation for 18 - 24 h at ambient temperature. The extracts were filtered and diluted to 50 ml and aliquot were analyzed for their total phenolic, flavonoid 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 phenolics

The amount of total phenolic content of crude extracts was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). 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) of extract.

Estimation of total flavonoids

Total flavonoids were estimated using the method of Ordonez et al. (2006). 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, 2006. 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:

$y = 0.0513x + 0.1658$, $R^2 = 0.9995$,

Table 1. Total phenolics content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total phenolics content (GAE mg / g of dry material) (Mean ± SEM)	
			Aq methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	27.67±0.16	3.47±0.48
Dillenia pentagyna	Agachi	Flower	9.33±0.15	2.61±0.13
Diplazium esculentum	Jhur- Tyrkhang	Leaves	15.01±0.32	3.77±0.05
Fagopyrum cymosum	Jarain	Leaves	9.22±0.08	6.85±0.13
Ficus clavata	Slachit	Leaves	14.47±0.32	5.23±0.53
Ficus geniculata	Mong lor	Leaves	12.07±0.20	6.04±0.10
Ficus pomifera	Jhu jri	Leaves	7.50±0.26	3.17±0.18
Gentiana pedicellata	Jamiaw	Leaves	23.46±0.32	3.07±0.22
Gynocardia odorata	So liang	Seeds	3.31±0.10	4.43±0.36

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

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). 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 (mg/g) 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) (Blois, 1958). 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:

DPPH scavenged (%) = {(Ac – At)/Ac} x 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) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.
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.

RESULTS AND DISCUSSION

Total phenol, flavonoid and flavonol content of the extracts

Phenolic components are very important plant constituents with scavenging ability because of its hydroxyl group. It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals (Florence et al., 2011). 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 (Pourmorad et al., 2006).

Total phenolic contents of different plant materials, using two different solvent systems are presented in Table 1. The screening of the aq methanol and acetone extracts of nine wild plants revealed that there was a wide variation in the amount of total phenolics ranging from 2.61±0.13 to 27.67±0.16 mg GAE/g dry material (Table 1). The highest amount of phenolic content was found in the aq. methanol extract of *B. purpurea* (27.67±0.16 mg GAE/g dry material), while least amount was observed in the acetone extract of *D. pentagyna* (2.61±0.13 GAE). The aq methanol extract of *G. pedicellata* (23.46±0.32 GAE), *D. esculentum* (15.01±0.32 GAE), *F. clavata* (14.47±0.32 GAE) and *F. geniculata* (12.07±0.20 GAE) were also found to contain a very good amount of phenolic compounds and the phenolic content of the plants are very much comparable with some other wild edible plants e. g. *Morus indica* (24.94 ±0.58 GAE), *Parkia roxburghii* (49.39 ±0.25 GAE), *Prunus nepalensis* (10.49 ±0.14 GAE), *Terminalia bellirica* (95.40 ±0.74 GAE), collected from Meghalaya state, India (Seal, 2011). In this study the content of phenolic components extracted by aq methanol was

Table 2. Total flavonoids content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total flavonoids content (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	23.19±0.009	5.39 ±0.04
Dillenia pentagyna	Agachi	Flower	52.14±0.004	2.74 ±0.07
Diplazium esculentum	Jhur- Tyrkhang	Leaves	34.81±0.003	2.49± 0.08
Fagopyrum cymosum	Jarain	Leaves	20.89±0.009	52.17± 0.01
Ficus clavata	Slachit	Leaves	34.81±0.003	10.30 ±0.08
Ficus geniculata	Mong lor	Leaves	41.73±0.011	7.35 ±0.03
Ficus pomifera	Jhu jri	Leaves	30.50±0.210	5.04 ±0.03
Gentiana pedicellata	Jamiaw	Leaves	34.79±0.013	19.76 ±0.17
Gynocardia odorata	So liang	Seeds	8.11±0.071	1.22± 0.01

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

much higher than that extracted by acetone. This may be due to the fact that phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ ethanol or acetone (Sultana et al., 2009; Ghasemzadeh et al., 2011).

Total flavonoids content of different plant materials, using two different solvent systems are presented in Table 2. The flavonoid contents of the extracts in terms of quercetin equivalent were between 1.22 ± 0.01 to 52.17± 0.01 mg/g dry material. Highest amount of flavonoid content was observed in the acetone extract of *F. cymosum* (52.17± 0.01 mg/g). The aq. methanol extracts of all wild edible plants under investigation were found to contain greater amount of flavonoid than that of acetone extract except in case of *F. cymosum*. Results of the present study showed that the aq. methanolic extracts were better for flavonoid extraction.

In case of flavonol, the highest amount was observed in the aq. methanol extract of *D. esculentum* (23.20 ±0.03 mg/g) followed by *G. pedicellata* (23.12 ±0.006 mg/g) and *F. clavata* (23.10 ±0.005 mg/g) (Table 3). Appreciable quantities of flavonol were found in the aq. methanol extract of *B. purpurea* (15.50 ±0.004 mg/g) and *F. cymosum* (13.87± 0.005 mg/g) (Table 3).

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 the aq. methanol extract of *G. pedicellata*, *F. clavata*, *B. purpurea*, *F. geniculata, D. pentagyna* and in the acetone extract of *F. cymosum* can explain their high radical scavenging activity.

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones which breaks the free radical chain by donating a hydrogen atom (Subhasini et al., 2011). The reducing powers of the nine wild plants were evaluated as mg AAE/g dry material as shown in Table 4. The reducing abilty of the aq methanol extract of the nine wild edible plants in descending order was *B. purpurea* > *D. pentagyna* > *G. pedicellata* > *F. geniculata* > *F. pomifera* > *F. clavata.* The highest reducing power was exhibited by the aq methanol extract of *B. purpurea* (16.11 ± 0.03 mg/g AAE) which is also high in phenolic content (27.67±0.16 mg GAE/g dry material) and acetone extract of *G. odorata* showed lowest activity in terms of ascorbic acid equivalent. In general, the aqueous methanol extracts of the tested plant materials, exhibiting greater phenol, flavonoids and flavonol content, also depicted good reducing power in the present analysis. 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 (Jamuna et al., 2011).

DPPH radical scavenging activity

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002). The antioxidant effect is proportional to the dis-appearance 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

Table 3. Total flavonols content in the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Total flavonols content (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	15.50 ± 0.004	2.99 ± 0.14
Dillenia pentagyna	Agachi	Flower	6.73 ± 0.03	0.19± 0.02
Diplazium esculentum	Jhur- Tyrkhang	Leaves	23.20 ± 0.03	1.96± 0.006
Fagopyrum cymosum	Jarain	Leaves	13.87± 0.005	6.56± 0.02
Ficus clavata	Slachit	Leaves	23.10 ± 0.005	2.28± 0.11
Ficus geniculata	Mong lor	Leaves	5.31 ± 0.02	3.53± 0.02
Ficus pomifera	Jhu jri	Leaves	2.77 ± 0.02	7.50± 0.12
Gentiana pedicellata	Jamiaw	Leaves	23.12 ± 0.006	7.43± 0.02
Gynocardia odorata	So liang	Seeds	2.21± 0.07	0.09± 0.008

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Table 4. Reducing power (Ascorbic acid equivalent) of the plants extracted by two different solvent.

Name of the plant	Local name at Meghalaya	Parts used	Ascorbic acid equivalent (AAE) (mg / g of dry material) (Mean ± SEM)	
			Aq. methanol extract	Acetone extract
Bauhinia purpurea	Megong	Leaves	16.11 ± 0.03	4.63±0.09
Dillenia pentagyna	Agachi	Flower	13.19±0.09	5.03±0.09
Diplazium esculentum	Jhur- Tyrkhang	Leaves	8.78±0.03	4.99±0.15
Fagopyrum cymosum	Jarain	Leaves	6.11±0.03	4.99±0.10
Ficus clavata	Slachit	Leaves	9.98±0.07	6.45±0.18
Ficus geniculata	Mong lor	Leaves	10.56±0.08	7.14±0.18
Ficus pomifera	Jhu jri	Leaves	10.33±0.05	4.75±0.10
Gentiana pedicellata	Jamiaw	Leaves	13.07±0.04	5.79±0.10
Gynocardia odorata	So liang	Seeds	8.46±0.25	3.19±0.09

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

be minimum.

The evaluation of anti-radical properties of nine wild edible plants 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. In the present study the highest radical scavenging activity was shown by the aq. methanol extract of *G. pedicellata* (IC_{50} = 0.23±0.0007 mg dry material), whereas the acetone extract of *G. odorata* showed lowest activity (IC_{50} = 2.71±0.04 mg dry material).

Strong inhibition was also observed for the aq. methanol extract of *B. purpurea* (IC_{50} = 0.34±0.0004 mg dry material and *F. clavata* (IC_{50} = 0.31±0.0009 mg dry material). The high radical scavenging property of *G. pedicellata* 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.

Conclusion

The result of present study showed that the aq. methanol extract of *B. purpurea* which contain highest amount of phenolic compounds and appreciable amount of flavonoids and flavonols exhibited the greatest reducing power and also showed strong radical scavenging activity. The highest radical scavenging activity and very strong reducing power of the aq. methanol extract of *G. pedicellata* may be due to the presence of a very good amount of total phenolics, flavonoids and flavonols contents in this plant. The radical scavenging activities of

Table 5. Free radical scavenging ability of the plant samples extracted by two different solvent 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
Bauhinia purpurea	Megong	Leaves	0.34± 0.0004	1.02±0.02
Dillenia pentagyna	Agachi	Flower	0.51± 0.006	2.11±0.06
Diplazium esculentum	Jhur- Tyrkhang	Leaves	0.92± 0.01	3.60±0.04
Fagopyrum cymosum	Jarain	Leaves	0.55± 0.002	0.68±0.008
Ficus clavata	Slachit	Leaves	0.31±0.0009	0.81±0.02
Ficus geniculata	Mong lor	Leaves	0.39± 0.001	0.93±0.01
Ficus pomifera	Jhu jri	Leaves	0.64± 0.005	1.55±0.02
Gentiana pedicellata	Jamiaw	Leaves	0.23± 0.0007	1.22±0.02
Gynocardia odorata	So liang	Seeds	1.97± 0.02	2.71±0.04

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

the selected plant extracts are still less effective 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 plants could be exploited as antioxidant additives or as nutritional supplements. However, further investigation is required to isolate and characterize the individual components from these plants which are actually responsible for their antioxidant activities and develop their applications for food and pharmaceutical industries.

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