Global Journal of Environmental Research 6 (3): 84-90, 2012
ISSN 1990-925X

DOI: 10.5829/idosi.gjer.2012.6.3.390

Antioxidant Activity of Some Selected Wild Edible Fruits of North-Eastern Region in India and Effect of Solvent Extraction System

[1]Tapan Seal, [2]Kaushik Chaudhuri and [3]Basundhara Pillai

[1]Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, India
[2]Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, I India
[3]Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, India

Abstract: The present communication deals with the antioxidant activity of the benzene, chloroform, acetone and methanol extracts of five different wild edible fruits *viz, Zanthoxylum armatum*, *Gomphogyne cissiformis*, *Gymnopetalum cochinensis*, *Artocarpus gomeziana* and *Baccaurea sapida* collected from different places of North-eastern region in India. The total phenolic content varied from 0.72±0.34 to 4.30±0.53 mg/g, 0.19±0.11 to 4.89±0.86 mg/g, 1.58 ±0.21 to 34.24±0.25 mg/g and 21.14 ± 0.23 to 96.19 ± 1.18 mg/g dry material in the benzene, chloroform, acetone and methanol extracts of the fruits respectively. Flavonoids and flavonols content were found highest in the acetone extract of *Gymnopetalum cochinensis* whereas least amount of flavonoids present in the acetone of *Baccaurea sapida*. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging was observed in the methanol extract of *Artocarpus gomeziana* with IC_{50} = 0.19±0.0005 mg dry material. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by the methanol extract of *Artocarpus gomeziana*. 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.

Key words: Wild edible fruits · Meghalaya State · Antioxidant activity · Different solvent extracts

INTRODUCTION

Antioxidants are substances that may protect the cells against the effects of free radicals. Oxidation reactions can produce free radicals and these radicals are responsible to vast variety of human diseases including atherosclerosis, arthritis, ischemia diabetic mellitus, hypertension, aging, cancer and AIDS [1]. It is established that consumption of antioxidant substances has been linked to the reduction in the incidence of oxidative-stress related diseases [2]. The use of currently available synthetic antioxdants 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 [3].

Plant materials are rich source of active constituents of varied chemical characteristics and polarities and complete extraction of active components, responsible for antioxidant activities, are strongly dependant on the nature of solvents and plant parts used. During the extraction of plant material, the selection of solvents and plant parts is very much important to minimize interference from compounds that may co-extract with the chemicals and to avoid the contamination of the extract. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix. Solvents, such as methanol, ethanol, acetone, chloroform and ethyl acetate have been widely used for the extraction of antioxidant compounds from various plants and plant based foods and medicines. Results of previous studies showed that the extraction yield of phenolic and flavonoid content is greatly depending on the polarity of the solvent [4, 5]. Bonoli *et al*, 2004 [6] reported that maximum phenolic compounds were obtained from barley flour with the

Corresponding Author: T. Seal, Botanical Survey of India, A. J. C. Bose Indian Botanic Garden, Shibpur, Howrah, India.

mixture of ethanol and acetone. The aqueous methanol was found to be more effective solvent to extract the phenolic compounds from rice brain and *Moringa oleifera* leaves [7, 8]. The extraction of high content of antioxidant compounds with 80% aqueous methanol (methanol: water 80 : 20) were found from various plant materials like rice bran, wheat bran, oat groats and hull, coffee beans, citrus peel and guava leaves as reported by Anwar *et al*, 2006 [9]. The highest extract yields were obtained from polar alcohol based solvents. Addition of water to ethanol improves the extraction rate but too high water content may leads to the extraction of other compounds.The highest level of phenolic compounds was found with 50% acetone from wheat, whereas ethanol is the least effective solvent to isolate phenolics from wheat bran [10]. It can be concluded that it is not clear which type of solvent is more effective for extracting the antioxidant components from plant.

Zanthoxylum armatumb DC of the rutaceae family is an important medicinal plant. The bark, fruits and seeds of which are extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The fruit and seeds are employed as an aromatic tonic in fever and dyspepsia. The extract of the fruits are reported to be used in the treatment of toothache. The dried fruits are used as spice. It possesses antilarvicidal, antifungal, hepatoprotective and alleopathic properties [11].

Gomphogyne cissiformis Griff belongs to the family cucurbitaceae. The leaves and fruits of this plant are used by the local people of Meghalaya State as vegetable [12].

Gymnopetalum cochinensis (Lour.) Kurz belongs to the family cucurbitaceae. The whole plant is used by the tribal people of North-east for the treatment of high blood pressure, fever, jaundice, gastritis, killing maggots and wound healing in cattle [13].

Baccaurea sapida (Roxb.) belongs to the family euphorbiaceae. The yellowish fruits of this plant are edible when ripe and are available during May–July. In fact, the flesh or aril around the seed coat can be eaten and tastes are delicious. The rind of the fruits is occasionally used for making chutney. It is sold in the market at Rs: 16–20 per kg and the fruit yield is 21–156 kg/tree. Squash-makinghas increased the value of the fruits up to Rs: 17.4 per kg. *B. sapida* can be a good source of vitamin C (273 mg/100 g), as recorded in this investigation [14].

However, the objective of the present study was to investigate the effect of different extracting solvents with different polarity on the antioxidant activities of these five wild edible fruits collected from the different parts of North-East India *viz, Zanthoxylum armatum*, *Gomphogyne cissiformis*, *Gymnopetalum cochinensis*, *Artocarpus gomeziana* and *Baccaurea sapida* which has not been deliberated till date. Hence present research would be enabling us to develop natural antioxidant which could be used as nutritional supplements.

MATERIALS AND METHODS

Plant Materials: The five plant materials e.g. the fruits of *Zanthoxylum armatum*, *Gomphogyne cissiformis*, *Gymnopetalum cochinensis*, *Artocarpus gomeziana* and *Baccaurea sapida* were collected from different market of Meghalaya state, India on March 2011 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under registry no BSITS 32, BSITS 33, BSITS 43, BSITS 45, BSITS 48 respectively. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

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.

Extraction of Plant Material (Benzene, Chloroform, Acetone and Methanol): One gram of each plant material were extracted with 20 ml each of benzene, chloroform, acetone and methanol with agitation for 18-24 hours 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.

Estimation of Total Phenolic Contents: The amount of total phenolic contents of crude extracts was determined according to Folin-Ciocalteu procedure [15]. 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

using the following equation based on the calibration curve: y = 0.0013x + 0.0498, R^2 = 0.999 where y was the absorbance and x was the Gallic acid equivalent (mg/g).

Estimation of Total Flavonoids: Total flavonoids were estimated using the method of Ordonez *et al*, 2006 [16]. To 0.5 ml of sample, 0.5 ml (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 rutin (mg/g) using the following equation based on the calibration curve: y = 0.0182x - 0.0222, R^2 = 0.9962, where y was the absorbance and x was the Rutin equivalent (mg/g).

Estimation of Total Flavonols: Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran, 2006 [17]. To 2.0 ml of sample (standard), 2.0 ml (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 = 0.0049x + 0.0047, R^2= 0.9935, 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 [18]. Extracts (100 µl) of plant 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 using the following equation based on the calibration curve: y = 0.0023x - 0.0063, R^2= 0.9955 where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

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) [19]. 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) 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

Extractive Value: The extractive value of the tested wild edible fruits with four different solvents are depicted in Table 1. The yield of extracts using benzene, chloroform, acetone and methanol in case of fruits of *Zanthoxylum armatum* were 10.8±0.06, 12.23±0.09, 13.20±0.12, 16.77±0.15 g/100g dry material respectively. Likewise the fruit extract of other plant materials also followed the same order as *Zanthoxylum armatum* extracts. The differences in the extractive value of the plants may be due to the varying nature of the components present and the polarities of the solvent used for extraction [20].

Total Phenol, Flavonoid and Flavonol Content of the Extracts: The screening of the benzene, chloroform, acetone and methanol extracts of fiver wild fruits revealed that there was a wide variation in the amount of total phenolics ranging from 0.72±0.34 to 96.19±1.18 mg GAE/g dry material (Table 2). The highest amount of phenolic content was found in the methanol extract of *A. gomeziana* (96.19±1.18 mg GAE/g dry material) followed by the methanol extract of *Z. armatum* (59.34±0.13GAE). While lower amounts was observed in the benzene extract of *G. cochinensis* (0.72±0.34 GAE). The acetone extracts

of *Z. armatum* (34.24±0.25 GAE) and *A. gomeziana* (33.21±0.45 GAE) were also found to contain a very good amount of phenolic compounds.

The flavonoid contents of the extracts in terms of rutin equivalent were between 0.82±0.03 to 22.14±0.03 mg/g dry material (Table 3). The highest amount of flavonoid was found in the acetone extract of *G. cochinensis* and the benzene, chloroform and methanol extract of this plant also contain a very good amount of flavonoids. The methanol extract of *G. cissiformis* and *A. gomeziana* also contain a considerable amount of flavonoids.

The flavonol contents in the different extracts of plant materials were evaluated in terms of querceitin equivalent (Table 4). The highest amount of flavonol was observed in the acetone extract of *G. cochinensis* (42.44±0.15 mg/g). The other extracts of this plant also contain a very good amount of flavonol. Appreciable quantities of flavonol were found in the methanol extract of *G. cissiformis* (31.01±0.10 mg/g) and *A. gomeziana* (18.26±0.05 mg/g).

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 [21]. 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]. 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, methanol was the most suitable solvent to isolate the phenolic compounds from the plant materials and the high content of these phenolic compounds in *A. gomeziana*, *Z. armatum* and *G. cissiformis* can explain their high radical scavenging activity.

Reducing Power Assay: The reducing powers of the five wild fruits were evaluated as mg AAE/g dry material as shown in Table 5. The highest reducing power was exhibited by the methanol extract of *A. gomeziana* (32.12±0.26 mg/g AAE) which is also high in phenolic content (96.19±1.18 mg GAE/g dry material) and benzene extract of *B. sapida* showed lowest activity in terms of ascorbic acid equivalent (6.23±0.29 mg/g AAE). 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 [22].

Table 1: Extractive value of fruits collected from Meghalaya using different solvents.

			Extractive value (g/100g dry material)			
Sl No	Name of the plant	Parts used	Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	10.8±0.06	12.23±0.09	13.20±0.12	16.77±0.15
2	*Gomphogyne cissiformis*	Fruits	8.4±0.06	9.20±0.12	10.20±0.12	11.07±0.12
3	*Gymnopetalum cochinensis*	Fruits	6.43±0.09	8.03±0.09	9.33±0.12	10.77±0.09
4	*Artocarpus gomeziana*	Fruits	10.9±0.06	10.67±0.09	15.03±0.15	16.73±0.12
5	*Baccaurea sapida*	Fruits	6.47±0.09	7.60±0.17	12.07±0.12	33.53±0.15

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

Table 2: Total phenolic content in the fruits collected from Meghalaya using different solvent extracts

			Total phenolic content (GAE mg/g dry material)			
Sl No	Name of the plant	Parts used	Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	4.30±0.53	4.89±0.86	34.24±0.25	59.34±0.13
2	*Gomphogyne cissiformis*	Fruits	2.68±0.80	1.75±0.24	1.58±0.21	23.03±0.64
3	*Gymnopetalum cochinensis*	Fruits	0.72±0.34	4.58±0.42	3.90±0.36	13.84±0.31
4	*Artocarpus gomeziana*	Fruits	3.71±0.11	0.19±0.11	33.21±0.45	96.19±1.18
5	*Baccaurea sapida*	Fruits	2.09±0.19	0.77±0.16	3.80±0.28	21.14±0.23

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

Table 3: Total flavonoid content in the fruits collected from Meghalaya using different solvent extracts

Sl No	Name of the plant	Parts used	Total flavonoid content (Rutin equivalent mg/g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	2.30±0.08	3.31±0.03	3.60±0.02	5.27±0.03
2	*Gomphogyne cissiformis*	Fruits	8.07±0.03	11.05±0.03	6.93±0.02	17.14±0.05
3	*Gymnopetalum cochinensis*	Fruits	16.17±0.12	21.89±0.05	22.14±0.03	21.74±0.14
4	*Artocarpus gomeziana*	Fruits	5.16±0.03	4.12±0.02	2.59±0.07	10.28±0.02
5	*Baccaurea sapida*	Fruits	1.51±0.03	2.00±0.03	0.82±0.03	5.02±0.02

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

Table 4: Total flavonol content in the fruits collected from Meghalaya using different solvent extracts

Sl No	Name of the plant	Parts used	Total flavonol content (Quercetin equivalent mg/g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	2.77±0.16	4.79±0.12	5.48±0.09	8.76±0.09
2	*Gomphogyne cissiformis*	Fruits	13.27±0.18	18.22±0.12	11.23±0.05	31.01±0.10
3	*Gymnopetalum cochinensis*	Fruits	23.27±1.11	39.66±0.30	42.44±0.15	38.95±0.21
4	*Artocarpus gomeziana*	Fruits	8.11±0.08	6.32±0.11	3.69±0.07	18.26±0.05
5	*Baccaurea sapida*	Fruits	0.30±0.14	1.60±0.11	0.33±0.07	8.92±0.05

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

Table 5: Reducing power (Ascorbic acid equivalent) of the fruits collected from Meghalaya using different solvent extracts

Sl No	Name of the plant	Parts used	Reducing power (Ascorbic acid equivalent mg/g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	11.29±0.17	30.46±0.42	21.40±0.92	21.56±0.22
2	*Gomphogyne cissiformis*	Fruits	7.23±0.37	21.57±0.68	14.34±0.24	19.36±0.17
3	*Gymnopetalum cochinensis*	Fruits	8.14±0.29	6.24±0.23	17.26±0.40	21.59±0.23
4	*Artocarpus gomeziana*	Fruits	13.82±0.46	19.09±0.17	19.89±0.22	32.12±0.26
5	*Baccaurea sapida*	Fruits	6.23±0.29	10.47±0.25	10.44±0.15	15.61±0.17

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

Table 6: Free radical scavenging ability of the Fruits collected from Meghalaya by the use of a stable DPPH radical (Antioxidant activity expressed as IC_{50}).

Sl No	Name of the plant	Parts used	Free radical scavenging ability IC_{50} mg/g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Zanthoxylum armatum*	Fruits	2.05±0.14	1.38±0.04	1.37±0.04	0.63±0.004
2	*Gomphogyne cissiformis*	Fruits	0.98±0.08	0.96±0.01	1.14±0.02	0.69±0.02
3	*Gymnopetalum cochinensis*	Fruits	0.67±0.04	0.78±0.005	0.89±0.06	0.80±0.02
4	*Artocarpus gomeziana*	Fruits	1.42±0.10	1.13±0.007	0.70±0.006	0.19±0.0005
5	*Baccaurea sapida*	Fruits	0.62±0.03	0.59±0.009	0.79±0.01	0.94±0.01

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

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 (IC50) by the different plant materials was determined (Table 6), 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 [23]. 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 IC50 value will be minimum. In the present study the highest radical scavenging activity was shown by the methanol extract of *A. gomeziana* (IC_{50} = 0.19±0.0005 mg dry material), whereas the benzene extract of *Z. armatum* showed lowest activity (IC50 = 2.05±0.14 mg dry material).

Strong inhibition was also observed for the methanol extract of *Z. armatum* (IC50 = 0.63±0.004 mg dry material, *G. cissiformis* (0.69±0.02 mg dry material) and chloroform extract of *B. sapida* (IC50 = 0.59±0.009 mg dry material). The high radical scavenging property of *A. gomeziana* 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 benzene, chloroform, acetone and methanol extracts of all of the fruits 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 others.

CONCLUSIONS

The result of present study showed that the methanol extract of *A. gomeziana*, which contain highest amount of phenolic compounds exhibited the greatest reducing power and radical scavenging activity. The acetone extract of *G. cochinensis* contain highest amount of flavonoids 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 product 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.

ACKNOWLEDGEMENTS

The authors of this paper is highly grateful to Dr. P. Singh, Director, Botanical Survey of India, Kolkata, Dr. M. Sanjappa, Ex-Director, Botanical Survey of India, Kolkata for their encouragement and fecilities. I am also thankful to Mr. R. Shanpru, Scientist, Botanical Survey of India, Eastern Regional circle, Shillong, Meghalaya for identifying the plant specimens.

REFERENCES

1. Pourmorad, F., S.J. Hosseinimehr and N. Shahabimajd, 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol., 5(11): 1142-1145.
2. Jamuna, K.S., C.K. Ramesh, T.R. Srinivasa and K.I. Raghu, 2011. In vitro antioxidant studies in some common fruits. International Journal of Pharmacy and Pharmaceutical Sciences, 3(1): 60-63.
3. Torane, R.C., G.S. Kamble, E.I. Khatiwora, N.A. Ghayal and N.R. Deshpand, 2011 Antioxidant capacity of leaves and stem of *Ehretia laevis*. International Journal of Pharmacy and Pharmaceutical Sciences, 3(2): 149-151.
4. Turkmen, N., F. Sari and Y.S. Velioglu, 2006. Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem, 99: 838-841.
5. Lapornik, B., M. Prosek and A.G. Wondra, 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. J. Food Eng., 71: 214-222.
6. Bonoli, M., V. Verardo, E. Marconi and M.F. Caboni, 2004. Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic acids. J. Agric. Food Chem., 52: 5195-5200.
7. Chatha, S.A.S., F. Anwar, M. Manzoor and J.R. Bajwa, 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas Aceites Sevilla, 57: 328-335.
8. Siddhuraju, P. and K. Becker, 2003. Antioxidant properties of various extracts of total phenolic constituents from three different agro climatic origins of drumstick tree (*Moringa oleifera lam*.) leaves. J. Agric. Food Chem., 51: 2144-2155.
9. Anwar, F., A. Jamil, S. Iqbal and M.A. Sheikh, 2006. Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grasas Aceites Sevilla, 57: 189-197.
10. Lolita, T., K. Zanda and G. Ruta, 2012. Comparison of different solvents and extraction methods for isolation of phenolic compounds from Horseradish roots (Armoracia rusticana). World Academy of Science, Engineering and Technology, 64: 903-908.
11. Singh, T.P. and O.M. Singh, 2011. Phytochemical and pharmacological profile of *Zanthoxylum armatumb* DC–An overview. Indian Journal of Natural Products and Resources, 2(3): 275-285.
12. Kayang, H., 2007. Tribal knowledge on wild edible plants of Meghalaya, Northeast India. Indian J. Traditional Knowledge, 6: 177-181.
13. Pfoze, N.L., Y. Kumar and B. Myrboh, 2012. Survey and assessment of ethnomedicinal plants used in Senapati District of Manipur state, Northeast India. Phytopharmacology, 2(2): 285-311.

14. Sundriyal, M. and R.C. Sundriyal, 2003. Underutilized edible plants of the Sikkim Himalaya: Need for domestication. Current Science. 85(6): 731-36.
15. Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic, 16: 144-158.
16. Ordonez, A.A.L., J.G. Gomez, M. A. Vattuone and M.I. Isla, 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem., 97: 452-458.
17. Kumaran, A. and R.J. Karunakaran, 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem., 97: 109-114.
18. Oyaizu, M., 1986. Studies on product on browning reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.
19. Blois, M.S., 1958. Antioxidant determination by the use of of a stable free radical", Nature, 181: 1199-1200.
20. Sarwar, S., F. Anwar, S. Raziq, M. Nadeem, Z. Zreen and F. Cecil, 2012. Antioxidant characteristics of different solvent extracts from almond (*Prunus dulcis* L.) shell. Journal of Medicinal Plants Research, 6(17): 3311-3316.
21. Florence, O.J., A.A. Adeolu and J.A. Anthony, 2011. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. Rec. Nat. Prod., 5(1): 29-42.
22. Jamuna, K.S., C.K. Ramesh, T.R. Srinivasa and K.I. Raghu, 2011. *In vitro* antioxidant studies in some common fruits. Int. J. Pharm. Pharm Sci., 3(1): 60-63.
23. Koleva, I.I., T.A.Van Beek, J.P.H. Linssen, A.D. Groot and L.N. Evstatieva, 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal, 13: 8-17.