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ANTIOXIDANT ACTIVITIES OF FIVE WILD EDIBLE FRUITS OF MEGHALAYA STATE IN INDIA AND EFFECT OF SOLVENT EXTRACTION SYSTEM

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Keywords:

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ABSTRACT: The antioxidant activities of five wild edible fruits e.g. e.g. *Debregeasia longifolia, Helicia erratica, Ilex venulosa, Rhus semialata* and *Spondias axillaris* collected from Meghalaya state in India were determined by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, ABTS radical scavenging ability, reducing power capacity, estimation of total phenolic content, flavonoid content and flavonol content. The solvent systems used were benzene, chloroform, acetone and methanol. The different levels of antioxidant activities were found in the solvent systems used. The results indicate that these wild edible fruits could be utilized as natural antioxidant.

INTRODUCTION: An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. 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 [1].

Reactive oxygen species affect living cells and these radicals are responsible for many chronic diseases in human being such as atherosclerosis, parkinson's disease, arthritis, alzheimer's disease, stroke, chronic inflammatory diseases, cancers, and other degenerative diseases [2]. Plant materials are rich sources of active constituents of varied chemical characteristics.

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Studies on herbal plants, vegetables, and fruits have indicated the presence of active components *viz.* Phenolic compounds, flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins and they have been reported to have multiple biological effects, including antioxidant activity [3]. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases. The antioxidant activities of plants are strongly dependant on the polarity of the solvents and plant parts used for the complete extraction of active components [4-5]. Solvents, such as methanol, ethanol, acetone, chloroform, ethyl acetate and water have been widely used for the extraction of antioxidant compounds from various plants and plant based foods and medicines.

Therefore, the objective of present study was to investigate the effect of different extracting solvents with different polarity on the antioxidant activities of five wild fruits *viz*. *Debregeasia longifolia*, *Helicia erratica*, *Ilex venulosa*, *Rhus*

semialata and *Spondias axillaris* collected from North-East India.

MATERIALS AND METHODS:

Plant Materials: The five plant materials e.g the fruits of *Debregeasia longifolia*, *Helicia erratica*, *Ilex venulosa*, *Rhus semialata* and *Spondias axillaris* were collected from different market of Meghalaya state, India on December 2012 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under registry no BSITS 55, BSITS 62, BSITS 59, BSITS 57, BSITS 52 respectively. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

Chemicals:

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 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, potassium per sulphate, 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 coarse powdered fruits were extracted with 20 ml each of benzene, chloroform, acetone and methanol 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.

Estimation of total phenolic content:

The amount of total phenolics in the four different solvent extracts of the fruit samples was measured according to Folin-Ciocalteu procedure [6]. 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 Shimadzu UV 1800). 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 [7]. 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 420nm (UV-visible spectrophotometer Shimadzu UV 1800). 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 [8]. To 2.0 ml of extract, 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440nm (UV-visible spectrophotometer Shimadzu UV 1800) 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 [9]. Extracts (100 µl) of plant extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 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 DPPH 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) [10]. 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 Shimadzu UV 1800). 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.

Scavenging activity of ABTS radical cation:

The 2, 2'- azino – bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Re *et al.* [11]. ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium persulphate (final concentration). The completion of radical generation was obtained in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. To determine the scavenging activity, 1 ml of diluted $ABTS^{\cdot+}$ solution was added to 10 µl of plant extract, and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

ABTS scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$

where A_c and A_s are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the IC_{50} value of the sample. 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 result shows that, methanol is the most suitable solvent to obtain the maximum extract from all the plants under investigation in comparison to the other solvents like benzene, chloroform and acetone used for extraction. The fruits of *I. venulosa* give maximum yield (23.85±0.05 g/100g) when it is extracted with methanol and the least amount is observed with benzene. Likewise, the fruit extract of other plant materials also followed the same order of *R. semialata* extracts. The differences in the extractive value of the plant materials may be due to the varying nature of the chemical components present and the polarities of the solvent used for extraction [12].

TABLE 1: EXTRACTIVE VALUE OF FRUITS COLLECTED FROM MEGHALAYA USING DIFFERENT SOLVENTS

Sl No	Name of the plant	Parts used	Extractive value (g / 100g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*D. longifolia*	Fruits	1.30±0.01	1.67±0.06	1.47±0.02	2 .12±0.02
2	*H. erratica*	Fruits	1.15±0.02	1.67±0.04	1.20±0.03	9.87±0.03
3	*I. venulosa*	Fruits	4.07±0.04	9.35±0.01	8.12±0.01	15.85±0.03
4	*R. semialata*	Fruits	5.87±0.03	6.92±0.02	5.07±0.05	13.85±0.05
5	*S. axillaris*	Fruits	4.55±0.05	6.70±0.03	5.35±0.02	9.80±0.04

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

Total phenol, flavonoid and flavonol content of the extracts: The screening of the benzene, chloroform, acetone and methanol extracts of five wild fruits revealed that there is a wide variation in the amount of total phenolics ranging from 26.36 ±1.05 to 582.91 ±3.84 mg GAE/g dry extract (**Table 2**).

TABLE 2: TOTAL PHENOLIC CONTENT IN THE FRUITS COLLECTED FROM MEGHALAYA USING DIFFERENT SOLVENTS

Sl No	Name of the plant	Parts used	Total phenolic content (GAE mg / g dry extract)			
			Benzene	Chloroform	Acetone	Methanol
1	*D. longifolia*	Fruits	59.46 ±3.07	60.69 ±0.33	53.28 ±1.14	117.52 ±4.25
2	*H. erratica*	Fruits	63.32 ±2.94	63.94 ±1.83	145.61 ±3.73	101.62 ±0.36
3	*I. venulosa*	Fruits	49.33 ±1.28	26.36 ±1.05	132.74 ±1.52	357.94 ±7.00
4	*R. semialata*	Fruits	48.72 ±0.39	47.72 ±0.51	340.03 ±2.25	582.91 ±3.84
5	*S. axillaris*	Fruits	136.32 ±0.70	64.54 ±0.09	77.47 ±0.23	153.29 ±1.24

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

The highest amount of phenolic content is found in the methanol extract of *R. semialata* (582.91 ±3.84 mg GAE/g dry material) followed by the methanol extract of *I. venulosa* (357.94 ±1.82 GAE**).** The least amount of phenolics is observed in the chloroform extract of *I. venulosa* (26.36 ±1.05 GAE). The acetone extracts of *H. erratica*, *R. Semialata* and benzene extract of *S. axillaris* and *H. erratica* are found to contain a very good amount of phenolic compounds. The flavonoid contents of the fruit extracts in terms of rutin equivalent are between 01.22±0.02 to 54.37±0.17 mg/g dry material (**Table 3**).

TABLE 3: TOTAL FLAVONOID CONTENT IN THE FRUITS COLLECTED FROM MEGHALAYA USING DIFFERENT SOLVENTS

Sl No	Name of the plant	Parts used	Total flavonoid content (Rutin equivalent mg / g dry extracts)			
			Benzene	Chloroform	Acetone	Methanol
1	*D. longifolia*	Fruits	44.25±0.68	43.01±0.21	35.96±0.42	18.97±0.04
2	*H. erratica*	Fruits	5.99±0.06	13.72±0.32	23.33±0.37	6.22±0.05
3	*I. venulosa*	Fruits	7.69±0.08	3.96±0.02	11.73±0.10	7.68±0.01
4	*R. semialata*	Fruits	01.22±0.02	1.26±0.01	33.35±0.33	54.37±0.17
5	*S. axillaris*	Fruits	22.80±0.06	12.52±0.15	11.66±0.11	23.64±0.10

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

The highest amount of flavonoid (54.37±0.17 mg/g dry material) is found in the methanol extract of *R. Semialata* and acetone extract of this plant also contain a very good amount of flavonoids. The benzene, chloroform, acetone and methanol extract of *D. longifolia* also contain a very good amount of flavonoids. The flavonol contents in the different extracts of fruits are evaluated in terms of quercetin equivalent (**Table 4**).

TABLE 4: TOTAL FLAVONOL CONTENT IN THE FRUITS COLLECTED FROM MEGHALAYA USING DIFFERENT SOLVENTS

Sl No	Name of the plant	Parts used	Total flavonol content (Quercetin equivalent mg / g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*D. longifolia*	Fruits	23.14±0.94	18.36±1.53	24.66±1.70	23.28±1.04
2	*H. erratica*	Fruits	26.60±0.39	23.79±1.19	44.20±0.92	12.60±0.10
3	*I. venulosa*	Fruits	5.29±0.61	6.32±0.40	9.22±1.26	7.98±0.66
4	*R. semialata*	Fruits	4.71±0.22	3.28±0.23	29.11±0.33	12.36±0.32
5	*S. axillaris*	Fruits	27.95±0.48	5.27±1.00	23.29±3.57	22.80±0.52

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

The highest amount of flavonol is observed in the acetone extract of *R. semialata* (29.11±0.33 mg/g). A very good amounts of flavonol are also found in the benzene, chloroform, acetone and methanol extract of *D. longifolia*, *H. erratica* and *S. axillaris*. 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 absorb and neutralize the free radicals [13]. 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 [14]. 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 and benzene, chloroform and acetone are the best solvent to isolate the flavonoids and flavonols from the plant materials. The high content of the phenolic compounds in the fruits of *R. semialata*, *I. venulosa* and *S. axillaris* can explain their high radical scavenging activity.

Reducing power assay: The reducing powers of the five edible fruits are evaluated as mg AAE/g dry material as shown in **Table 5**.

TABLE 5: REDUCING POWER (ASCORBIC ACID EQUIVALENT) OF FRUITS COLLECTED FROM MEGHALAYA USING DIFFERENT SOLVENTS

Sl No	Name of the plant	Parts used	Reducing power (Ascorbic acid equivalent mg / g dry extracts)			
			Benzene	Chloroform	Acetone	Methanol
1	*D. longifolia*	Fruits	18.36±0.55	17.06±1.12	10.16±0.56	18.73±2.38
2	*H. erratica*	Fruits	15.09±0.63	19.90±1.03	34.69±1.04	5.64±0.16
3	*I. venulosa*	Fruits	10.66±0.72	7.51±1.07	25.41±1.76	28.77±1.40
4	*R. semialata*	Fruits	9.49±0.28	9.46±0.50	163.96±0.92	199.76±1.97
5	*S. axillaris*	Fruits	29.45±1.39	15.46±0.42	18.68±1.43	39.96±1.04

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

The highest reducing power was exhibited by the methanol extract of *R. semialata* (199.76±1.97 mg/g AAE) which also contain a very good amount of flavonoids and flavonols. The methanol extract of *H. erratica* showed lowest activity in terms of ascorbic acid equivalent (5.64±0.16 mg/g AAE). In this assay, the presence of antioxidants in the fruit 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 [15].

DPPH radical scavenging activity:

The evaluation of anti-radical properties of fiver 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 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 [16]. 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 is shown by the methanol extract of *R. semialata* (IC_{50} = 0.058±0.001 mg dry material), whereas the chloroform extract of *I. venulosa* shows the lowest activity (IC_{50} = 1.32±0.03 mg dry material). Strong inhibition is also observed for the methanol extract of *S. axillaris*, *I. venulosa H. erratica* with 90%, 86% and 59% of DPPH inhibition respectively. The high radical scavenging property of these plants may be due to the presene of hydroxyl groups that can provide the necessary component as a radical scavenger.

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 extracts			
			Benzene	Chloroform	Acetone	Methanol
1	D. longifolia	Fruits	0.42±0.01	0.46±0.03	0.32±0.003	0.35 ±0.05
2	H. erratica	Fruits	0.46±0.10	0.75±0.05	0.14±0.005	0.33±0.04
3	I. venulosa	Fruits	0.77±0.005	1.32±0.03	0.24±0.001	0.36±0.002
4	R. semialata	Fruits	0.89±0.008	0.96±0.01	0.11±0.001	0.058±0.001
5	S. axillaris	Fruits	0.44±0.001	0.33 ±0.004	0.23±0.004	0.21±0.002

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

ABTS radical scavenging activity:

ABTS scavenging activities in various extracts of five wild fruits using ABTS assay is shown in **Table 7**. The antioxidant effect is proportional to the disappearance of the colour of ABTS in test samples. Concentration of sample that could scavenge 50 % free radical (IC_{50}) is used to determine antioxidant capacity of sample compared to standard. Sample that had IC_{50} < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50} > 150 ppm [10]. In the present study the highest radical scavenging activity is shown by the acetone extract of *R. semialata* (IC_{50} = 0.10±0.0001 mg dry material), whereas the chloroform extract of *I. venulosa* shows lowest activity (IC_{50} = 7.87±0.09 mg dry material). Strong inhibition is also observed for the methanol extract of *R. semialata*, *I. venulosa S. axillaris* with 95 %, 94% and 91 % of ABTS cation inhibition respectively.

TABLE 7: FREE RADICAL SCAVENGING ABILITY OF THE FRUITS COLLECTED FROM MEGHALAYA BY THE USE OF A STABLE ABTS RADICAL CATION (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	*D. longifolia*	Fruits	0.17±0.001	0.17±0.002	0.13±0.005	0.15 ±0.0003
2	*H. erratica*	Fruits	1.69±0.07	0.84±0.02	0.66±0.02	1.01±0.006
3	*I. venulosa*	Fruits	1.19±0.02	7.87±0.09	0.23±0.005	0.34±0.0006
4	*R. semialata*	Fruits	2.17±0.24	5.05±0.67	0.10±0.0001	0.29±0.0002
5	*S. axillaris*	Fruits	0.22±0.007	0.29 ±0.009	0.24±0.0002	0.20±0.0001

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

The benzene, chloroform, acetone and methanol extracts of all of the fruits under investigation exhibits different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements.

CONCLUSION: The result of present study shows that the methanol extract of *H. aromatica*, which contain highest amount of phenolic compounds exhibited the greatest radical scavenging activity. The benzene, chloroform, acetone and methanol extract of all fruits under investigation contain a very good amount of flavonoids and flavonols also shows strong radical scavenging activity in both ABTS and DPPH method. The radical scavenging activities of the selected plants extracts are still less effective than the commercial available synthetic like BHT and trolox. 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 and supplements for the diseases associated with oxidative stress. In addition, naturally antioxidants have the capacity 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.

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