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Original Article

ANTIOXIDANT ACTIVITIES OF SOME WILD VEGETABLES OF NORTH-EASTERN REGION IN INDIA AND EFFECT OF SOLVENT EXTRACTION SYSTEM

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ABSTRACT

The antioxidant activities of seven wild edible plants e.g. *Allium schoenoprasum*, *Carica papaya*, *Neptunia oleracea*, *Eurya acuminata*, *Hodgsonia heteroclita*, *Brassica nigra* and *Flacourtia jangomas* collected from Meghalaya state in India were studied by assessing 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, reducing power ability, estimation of total phenolic content, flavonoid content and flavonol content, using different solvent extraction system. The solvents used for extracting active components from plant 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 vegetables can be utilized as natural antioxidant. These findings are also important for providing evidence that the studied plants might be the potential sources of nutritional and medicinal agents.

Keywords: Wild edible vegetables, Meghalaya, Antioxidant activity, Different solvent extracts.

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 to many chronic diseases in humans such as atherosclerosis, parkinson's disease, arthritis, alzheimer's disease, stroke, chronic inflammatory diseases, cancers, and other degenerative diseases **[2]**. Reactive oxygen species (ROS) like hydroxyl radical, superoxide anion, hydrogen peroxide can cause cellular injuries which in term reponsible for DNA and protein damage and oxidation of important enzymes in human body **[3]**.

Therefore, antioxidants with free radical scavenging activities of medicinal plants may have great relevance in the prevention diseases and therapeutic properties. Phytoconstituents like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity **[4-5]**. Generally, medicinal plants could be a potential source of natural antioxidants

The antioxidant activities of plant materials are strongly dependant on the nature of solvents used for extraction of active constituents 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. 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. From literature survey it is reported that maximum phenolic compounds were obtained from barley flour with the mixture of ethanol and acetone **[4]**.

The aq. methanol was found to be more effective solvent to extract the phenolic compounds from rice brain and *Moringa oleifera* leaves **[5-6]**. The extraction of high content of antioxidant compounds with 80 % aq. 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 **[7]**. 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 **[8]**. It can be concluded that it is not clear which type of solvent is more effective for extracting the antioxidant components from plant.

Therefore, it is our intention in this work to evaluate the effect of different extracting solvents with different polarity on the antioxidant activities of edible parts of seven wild edible plants collected from North-East India *viz Allium schoenoprasum*, *Carica papaya*, *Neptunia oleracea*, *Eurya acuminata*, *Hodgsonia heteroclita*, *Brassica nigra* and *Flacourtia jangomas* Thus the results from this preliminary study will provide a better understanding of the antioxidant properties of these plants and would be enabling to develop potent and safe antioxidant compounds.

MATERIALS AND METHODS

Plant materials

The seven plant materials e.g *Allium schoenoprasum* (bulb), *Carica papaya* (flower), *Neptunia oleracea* (leaves & stem), *Eurya acuminata* (leaves), *Hodgsonia heteroclita* (fruits), *Brassica nigra* (leaves) and *Flacourtia jangomas* (fruits) 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 34, BSITS 35, BSITS 41, BSITS 42, BSITS 44, BSITS 46 and BSITS 47 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 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

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard **[9]**. 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 equivalent (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

The amount of total flavonoids were estimated using the method of Ordonez et al., 2006 **[10]**. 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 content was calculated as rutin equivalent (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

The amount of total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran, 2006 **[11]**. 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 equivalent (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 **[12]**. Plant extracts (100 µl) 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 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) **[13]**. 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 plants with four different solvents are depicted in Table 1. The yield was generally observed to be high in the methanol extracts of all leafy vegetables and fruits. The maximum yield was obtained in the methanol extract of the fruits of *H. heteroclita* (35.40±0.17g/100g dry material) whereas lowest yield was seen in the chloroform extract of the leaves of *E. acuminata* (1.20±0.18 g/100g dry material). The methanol extract of the fruits of *F. jangomas* and leaves of *B. nigra* also showed very good extractive value (10.25±0.32 and 21.35±0.21 g/100g dry material respectively).

The benzene, chloroform and acetone extract of the fruits of *H. heteroclita* showed good extractive value. 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 **[14]**.

Table 1: Extractive value of wild vegetables collected from Meghalaya using different solvents

S. No.	Name of the plant	Parts used	Extractive value (g / 100g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Allium schoenoprasum*	Bulb	6.95±0.03	6.90±0.09	7.65±0.01	8.40±0.06
2	*Carica papaya*	Flower	8.13±0.03	10.32±0.06	8.45±0.08	11.10±0.06
3	*Neptunia olearacea*	Leaves	9.27±0.01	10.80±0.01	7.53±0.01	8.28±0.03
4	*Neptunia olearacea*	Stem	6.30±0.06	8.07±0.03	7.30±0.03	7.98±0.06
5	*Eurya acuminata*	Leaves	2.35±0.08	1.20±0.18	4.75±0.07	6.25±0.19
6	*Hodgsonia heteroclita*	Fruits	19.25±0.15	20.05±0.09	28.45±0.16	35.40±0.17
7	*Brassica nigra*	Leaves	4.65±0.10	3.00±0.11	4.45±0.09	21.35±0.21
8	*Flacourtia jangomas*	Fruits	3.85±0.12	4.70±0.14	8.00±0.05	10.25±0.32

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 eight wild plants revealed that there was a wide variation in the amount of total phenolics ranging from 7.69± 0.48 to 487.13± 0.52 mg GAE/g dry material (Table 2).

The highest amount of phenolic content was found in the methanol extract of *E. acuminata* (487.13± 0.52 mg GAE/g dry material). The another three extracts i.e benzene, chloroform and acetone extracts of this plant were found to contain a very good amount of phenolic compounds (84.99 ±0.72, 188.88±2.82 and 360.94±1.98 mg GAE/g dry material respectively). An appreciable amount of phenolic

compounds were also present in the methanol extracts of the bulb of *A. schoenoprasum* (62.46 ±0.92 GAE), leaves and stem of *N. olearacea* (85.02 ±0.38 & 58.40 ±0.43 GAE) and fruits of *F. jangomas* (86.77±0.31 GAE). The chloroform and acetone extracts of the leaves of *B. nigra* and benzene and chloroform extract of the fruits of *F. jangomas* contain significant amount of phenolic compounds. While the lowest amount of phenolics was observed in the acetone extract of *H. heteroclita* (7.69± 0.48 GAE). The flavonoid contents of the extracts in terms of rutin equivalent were between 0.57±0.02 to 144.32±1.40 mg/g dry material (Table 3)

Table 2: Total phenolic content in the wild edible plants collected from meghalaya using different solvents

S. No.	Name of the plant	Parts used	Total phenolic content (GAE mg / g dry material)			
			Benzene	**Chloroform**	**Acetone**	**Methanol**
1	*Allium schoenoprasum*	Bulb	24.03 ±0.66	20.49 ±0.56	26.27 ±0.85	62.46 ±0.92
2	*Carica papaya*	Flower	15.58 ±0.47	13.13 ±0.32	19.46 ±0.30	19.26 ±0.60
3	*Neptunia olearacea*	Leaves	13.51 ±0.47	10.42 ±0.25	25.35 ±0.53	85.02 ±0.38
4	*Neptunia olearacea*	Stem	26.61 ±0.63	14.81 ±0.99	18.84 ±0.23	58.40 ±0.43
5	*Eurya acuminata*	Leaves	84.99 ±0.72	188.88±2.82	360.94±1.98	487.13± 0.52
6	*Hodgsonia heteroclita*	Fruits	21.06 ±1.15	19.71± 1.42	7.69± 0.48	19.45±0.39
7	*Brassica nigra*	Leaves	29.58± 1.22	59.10±1.33	53.81± 0.38	18.81± 0.28
8	*Flacourtia jangomas*	Fruits	51.38±1.45	40.18± 0.62	32.41±0.76	86.77±0.31

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 wild edible plants collected from Meghalaya using different solvents**

S. No.	Name of the plant	Parts used	Total flavonoid content (Rutin equivalent mg / g dry material)			
			Benzene	**Chloroform**	**Acetone**	**Methanol**
1	*Allium schoenoprasum*	Bulb	0.57±0.02	6.86±0.07	1.83±0.29	3.86±0.05
2	*Carica papaya*	Flower	1.24±0.18	1.62±0.10	1.02±0.01	1.36±0.03
3	*Neptunia olearacea*	Leaves	1.87±0.02	5.41±0.04	3.69±0.29	3.39±0.02
4	*Neptunia olearacea*	Stem	9.97±0.21	25.24±0.10	9.96±0.27	26.86±0.12
5	*Eurya acuminata*	Leaves	39.62±0.77	**144.32**±1.40	56.25 ±0.13	45.30 ±0.01
6	*Hodgsonia heteroclita*	Fruits	1.71± 0.04	1.74 ± 0.10	2.26± 0.02	1.10± 0.01
7	*Brassica nigra*	Leaves	13.56± 0.41	40.14 ±0.66	31.85 ±0.10	5.51±0.008
8	*Flacourtia jangomas*	Fruits	5.20± 0.11	4.24± 0.03	3.00± 0.01	10.32 ±0.003

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 was found in the chloroform extract of the leaves of *E. acuminata* (144.32±1.40 mg/g dry material) and the benzene, acetone and methanol extracts of this plant also contain a very good amount of flavonoids (39.62±0.77, 56.25 ±0.13 and 45.30 ±0.01 mg/g dry material respectively).

The chloroform and acetone extract of *B. nigra* and chloroform extracts of the stem of *N. olearacea* were also found to contain a very good amount of flavonoids. The flavonol contents in the different extracts of plant materials were evaluated in terms of quercetin equivalent (Table 4).

Table 4: Total flavonol content in the wild edible plants collected from Meghalaya using different solvents

S. No.	Name of the plant	Parts used	Total flavonol content (Quercetin equivalent mg / g dry material)			
			Benzene	**Chloroform**	**Acetone**	**Methanol**
1	*Allium schoenoprasum*	Bulb	3.08±0.50	3.39±0.06	5.19±0.98	16.51±0.23
2	*Carica papaya*	Flower	5.52±0.18	2.51±0.15	3.64±0.18	12.85±1.24
3	*Neptunia olearacea*	Leaves	3.81± 0.37	2.02±0.10	5.30±0.04	29.51±1.29
4	*Neptunia olearacea*	Stem	3.18±0.16	4.48±0.09	3.93±0.10	13.34±0.18
5	*Eurya acuminata*	Leaves	80.82±0.78	219.09±1.22	23.94±0.50	160.56±0.76
6	*Hodgsonia heteroclita*	Fruits	5.05±0.09	3.45 ±0.08	1.32±0.10	2.13±0.04
7	*Brassica nigra*	Leaves	24.24±0.78	54.01±0.34	25.64±0.60	10.07±0.04
8	*Flacourtia jangomas*	Fruits	43.02±1.92	62.92±0.63	4.48 ±0.09	24.83±0.15

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 was observed in the chloroform extract of *E. acuminata* (219.09±1.22 mg/g dry material).

The other extracts of this plant also contain an appreciable amount of flavonol. A very good amount of flavonols were also found in the chloroform extract of the leaves of *B. nigra* and fruits of *F. jangomas*.

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 **[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 **[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, 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 methanolic extracts of *A. schoenoprasum*, *N. olearacea, E. acuminata* and *F.jangomas* can explain their high radical scavenging activity.

Reducing power assay

The reducing powers of wild edible plants were evaluated as mg AAE/g dry material as shown in Table 5.

Table 5: Reducing power (Ascorbic acid equivalent) in the wild edible plants collected from Meghalaya using different solvents

S. No.	Name of the plant	Parts used	Reducing power (Ascorbic acid equivalent mg/g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Allium schoenoprasum*	Bulb	9.22±0.81	6.87±0.74	11.74±0.17	13.41±0.81
2	*Carica papaya*	Flower	13.01±1.22	8.13±0.61	8.35±1.66	10.14±0.39
3	*Neptunia olearacea*	Leaves	12.06±1.21	5.46±0.95	13.76±0.94	23.24±0.30
4	*Neptunia olearacea*	Stem	14.94±1.06	4.97±1.43	6.64±0.68	8.12±0.55
5	*Eurya acuminata*	Leaves	100.04±0.96	170.86±1.08	168.18±2.01	193.96±0.65
6	*Hodgsonia heteroclita*	Fruits	17.74±0.25	16.28±0.24	16.47±0.12	11.39±0.17
7	*Brassica nigra*	Leaves	45.03± 0.69	88.15±0.83	59.18±1.76	15.56±0.07
8	*Flacourtia jangomas*	Fruits	60.87±2.47	70.99±0.94	32.69±0.39	52.13±0.04

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 *E. acuminata* (193.96±0.65 mg/g AAE) which also contain a very good amount of flavonoids and flavonols. The chloroform extract of the stem of *N. olearacea* showed lowest activity in terms of ascorbic acid equivalent (4.97±1.43 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 **[17]**.

DPPH radical scavenging activity: The evaluation of anti-radical properties of the wild edible plants were 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 **[18]**.

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.

Table 6: Free radical scavenging ability of the wild edible plants collected from Meghalaya by the use of a stable DPPH radical (antioxidant activity expressed as IC_{50})

S. No.	Name of the plant	Parts used	Free radical scavenging ability (IC_{50} mg / g dry material)			
			Benzene	Chloroform	Acetone	Methanol
1	*Allium schoenoprasum*	Bulb	0.36±0.004	0.38±0.002	0.41±0.003	0.28±0.001
2	*Carica papaya*	Flower	0.45±0.004	0.56±0.003	0.45±0.004	0.56±0.002
3	*Neptunia olearacea*	Leaves	0.49±0.007	0.68±0.012	0.38±0.002	0.20±0.0004
4	*Neptunia olearacea*	Stem	0.44±0.002	0.55±0.004	0.47±0.005	0.30±0.002
5	*Eurya acuminata*	Leaves	0.38±0.01	0.24±0.01	0.11±0.0002	0.14±0.002
6	*Hodgsonia heteroclita*	Fruits	13.89±0.93	30.02±2.46	17.28±2.25	7.32±0.55
7	*Brassica nigra*	Leaves	5.48 ±0.24	2.07±0.16	1.37±0.08	3.99±0.25
8	*Flacourtia jangomas*	Fruits	2.08±0.27	3.45±0.26	1.23±0.09	0.39±0.01

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

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 *E. acuminata* (IC_{50} = 0.11±0.0002 mg dry material), whereas the chloroform extract of *H. heteroclita* showed lowest activity (IC_{50} = 30.02±2.46 mg dry material). Strong inhibition was also observed for the methanol extract of *E. acuminata* (IC_{50} = 0.14±0.002mg dry material, *N. olearacea* (0.20±0.0004 mg dry material). The high radical scavenging property of *E. acuminata* 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 leafy vegetables under investigation exhibited different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements.

CONCLUSION

The result of present study showed that the methanol extract of *E. acuminata*, which contain highest amount of phenolic compounds exhibited potent radical scavenging activity and also found to contain a very good amount of flavonoid, flavonol and showed very good reducing power. The benzene, chloroform and acetone extract of *E. acuminata, B. nigra* and methanol extract of *A. schoenoprasum, N. olearacea* and *F. jangomas* contain a very good amount of phenolics also showed strong radical scavenging activity. The benzene and chloroform extract of *B. nigra* and *F. jangomas* which contain a very good amount of flavonol exhibited potent reducing power. 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 plants could be exploited as antioxidant additives or as nutritional supplements.

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