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Evaluation of phytochemicals and antioxidant activity in underutilised wild edible plants of Meghalaya state, India

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Abstract : Free radicals are generated in the living cells during cell metabolism. Excess of free radicals are harmful as they can cause oxidative damage to the living cells. Antioxidants can interfere by donating electrons to stabilise and neutralise the harmful effects of free radicals. Natural antioxidants are easily available in many wild edible plants. Consumption of such plants has been associated with reducing the risk of developing chronic diseases such as cardiovascular disease and cancer in humans. These protective effects have been attributed partially to the presence of phytochemicals in plants particularly flavonoids and phenolic compounds. The overall objectives of the present study were to investigate vitamin C, total phenolic content, flavonoid content and free radical scavenging activity in methanolic extracts of eleven underutilised wild edible plants available in Meghalaya state of India. From the study it was found that vitamin C, total phenol and flavonoids in vegetables range from moderate to high concentration. The plant samples showed high radical scavenging activity which can be concluded that these plant samples provide good sources of antioxidants and can be useful natural remedies in the treatment of various types of human related diseases, malnutrition associated problems as well as increasing the health status of the rural population.
Keywords : Vitamin C, total phenolic content, flavonoids, radical scavenging activity.

Introduction:

Meghalaya is one of the states in India that is very rich in vegetation due to variable climatic conditions and ecological diversity. Wild edible fruits and vegetables are consumed mostly by the rural people and are even sold in the village markets to generate income in poor rural families. Wild edible plants play a major role in the diet of rural people and their nutritional values are in some cases superior to those of domestic foods[1]. Wild edible plants contain higher amount of nutrients and bioactive compounds than many cultivated species, especially those that have been under cultivation for many generations[2,3,4]. Various plant species contain phytochemicals which may be effective for the treatment of certain chronic diseases because they possess antioxidant, antibacterial, antiviral, anti-carcinogenic activities. The investigation of phytochemical components of fruits and vegetables being beneficial in preventing a number of these chronic diseases was reported by Liu[5]. According to the World Health Organisation more than 80% population of the developing countries depend on traditional medicinal plants for primary health care, livelihood improvement and income generation[6, 7].

In Meghalaya, 42% of the total population is under malnutrition and the effect is more remarkable in rural areas. Some studies[8] reported that the underutilized plants contribute immensely to family food security and serve as means of survival during times of drought, famine, shocks and risks.Consumption of these plants might be helpful to fight malnutrition associated problems and increasing the health status of the rural population. Natural antioxidants occurring in such plants have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural

antioxidants is associated with a lower risk of cardiovascular disease and cancer[9,10]. Antioxidants are compounds which retard or prevent the oxidation process in general and prolong the life of an oxidizable matter. Free radicals are highly reactive species capable of independent existence and contain one or more unpaired electrons. They play an important role in biological process such as metabolic pathways, cell signalling and immune response. However the steady increase of free radicals in cells creates the condition so-called oxidative stress, where free radicals oxidize blood vessel walls, protein molecules, DNA, and lipids. Excess free radicals are harmful as they can damage many tissues, lipids, proteins, DNA thereby causing central nervous system injury, gastritis, cancer and many other types of diseases. Antioxidants can interfere by donating electrons to stabilise and neutralise the harmful effects of free radicals. The defensive action of natural antioxidants in plants is mainly related to the presence of vitamins, phenolic compounds and carotenoids. Epidemiological studies also show that there is a positive association between intake of vegetables and reduced mortality rate from heart diseases, common cancers, and other degenerative diseases[11,12,13].Low fruits and vegetables intake is considered as the sixth main risk factor for mortality in the world[14,15,16]. Consumption of these wild edible plants may be helpful to reduce the overproduction of harmful free radicals that can cause several types of chronic diseases like common cancer. Despite their significant role, only a little attention has been paid to explore the health beneficial effects of the underutilised and unexplored wild edible plants. Therefore large scale exploitation of these plants is needed which will facilitate research in pharmaceutical industry to identify the bioactive components that possess health promoting antioxidant properties.

Hence the present study aimed at determining the amount of vitamin C, total phenolic content, flavonoids as well as free radical scavenging activity present in these wild edible plants and to create nutritional awareness among the rural communities on the health beneficial effects of these traditionally important wild edible plants in terms of their anti free radical activities.

Experimental:

Materials and methods

i. Instruments and chemicals

UV-1800 (Shimadzu-UV Visible spectrophotometer), Centrifuge (sciencetech medico centrifuge, 3500rpm)

ii. Chemicals

Methanol, Ascorbic acid, Gallic acid, FolinCioalteau reagent, Sodium carbonate, Rutintrihydrate, $AlCl_3$, $NaNO_2$, NaOH, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH)

All chemicals were of analytical grade. Deionised water was used throughout the experiment.

iii. Plant materials

Eleven plant samples (Table 1) were bought from different local markets and then stored in a refrigerator at below 0^oC in the laboratory before analysis.

iv. Sample preparation for determination of vitamin C.

Vitamin C (ascorbic acid) was determined according to the volumetric method[17]. 10ml of 4% oxalic acid was added to standard solution of vitamin C (100µg/ml) and the resulting solution was titrated against 2, 6-dichloroindophenol dye until a pink colour end point was obtained and the titre value was noted as V_1. Again the samples (5gm) were extracted with 4% oxalic acid and volume was made to 100 ml and centrifuged. 5 ml of the supernatant was mixed with 10 ml of 4% oxalic acid and titrated against 2, 6-dichloroindophenol dye until a pink colour end point was obtained and the titre value was noted as V_2. Vitamin C content was calculated based on the following equation:

Amount of vitamin C (mg/100 g extract) = $[(0.5mg \times V_2 \times 100ml) / (V_1 \times 5\ ml \times Wt.\ of\ samples)] \times 100$, where V_1 is and V_2 were the volume of the dye used to titrate vitamin C and sample extract respectively. The result was expressed as mg ascorbic acid /100g extract.

v. Sample preparation for total phenolic content, flavonoid and free radical scavenging activity.

All samples were cleaned with deionised water before analysis. Sample extraction was done according to the method described by Medina[18] with slight modifications. The samples (25gm) were cut into small pieces and homogenised in a grinder until the sample was smooth. Then each sample (5gm) were mixed with 10ml 0f 70% methanol, homogenised and stirred for 20mins using a mechanical shaker and then centrifuge at 2000rpm for 10mins. The clear supernatant is decanted off in calibrated glass tubes and the volume was measured. The samples were extracted again with another 5ml of methanol, homogenised, stirred for 20mins and centrifuged at 2000rpm for 10mins. The supernatant was added to the first extract and the total volume was measured. The extracts were diluted (1:5) with deionised water and analysed for total phenol, flavonoid and free radical scavenging activity by DPPH

vi. Determination of Total phenol content

Total phenolic content of crude extracts was determined by calorimetric assay based on procedures described by Thimmaiah[17] with slight modifications. 0.5ml of the methanolic extract was mixed with 1.0 ml of Folin-Ciocalteu reagent (1:2) and allowed to stand for 3mins. Then 2.5ml of sodium carbonate (20%) was added. The mixtures were allowed to stand for 30 min. Absorption at 650 nm was measured against a reagent blank by UV-visible spectrophotometer). Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligram Gallic acid equivalents (GAE) per 100gram (mg/100g) fresh weight (fw) of the extract.

vii. Determination of Total flavonoids

The total flavonoids assay was done as described by Zhishen[19] with slight modifications. A volume of 1 ml of diluted extracts was placed in a 10-mL volumetric flask, 0.3 ml of $NaNO_2$ (5%) and 1.5 ml of $AlCl_3$ (2%) were added. The mixture was shaken and 5 min later 2 ml of 1 M solution of NaOH were added, again well shaken. The absorbance was measured at 510 nm against a reagent blank. Rutin was used as a standard for the calibration curve. The total flavonoid content was expressed as milligram rutin equivalents (RE) per 100gram (mg/100g) fresh weight (fw) of the extract.

viii. 2, 2- Diphenyl 1-picrylhydrazyl (DPPH) method-scavenging assay

DPPH is widely used to determine antioxidant activity in plant extracts. The DPPH radical scavenging assay depends on the capacity of antioxidants to scavenge DPPH radicals. DPPH is a stable free radical. It is deep purple in colour and decolorize when reduced into non-radical form by antioxidants (AH).

$DPPH\cdot + AH \rightarrow DPPH\text{-}H + A\cdot$

The deep purple DPPH solution absorbs optimally at 517 nm. Thus, a measurement of the decrease in absorbance due to the reaction is used to determine the level of antioxidants in the samples. The radical scavenging acitivity[20] was tested on the basis of the radical scavenging effect on the DPPH free radical. 2 ml of DPPH solution (0.002% in methanol) was mixed with 2 ml of the extracts. The tubes were thoroughly vortex, kept in the dark for 30 minutes and the absorbance was measured at 517 nm using UV-Vis Spectrophotometer. The scavenging activity of the plant extracts was calculated using the formula:

Scavenging activity (%) = [(A – B) / A] x 100, where A is the absorbance of DPPH and B is absorbance of DPPH and sample.

Results and Discussion:

In this study, standard calibration method was used to assess and determine the total phenolic and flavonoid content in the samples. Total phenolioc content was calculated using the equation based on the calibration curve: $y = 0.013x + 0.006$, $R^2 = 0.997$, where y is the absorbance and x is the gallic acid equivalent. Total flavonoids were calculated based on the calibration curve: $y = 0.001x – 0.021$, $R^2 = 0.994$, where y is the absorbance and x is the rutin equivalent.

Table 1: Types of plant samples used in the study.

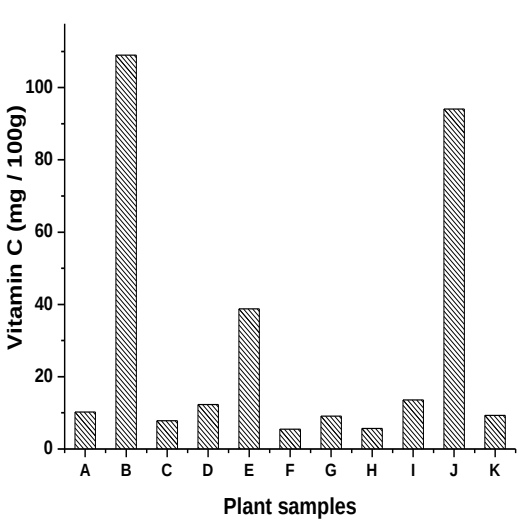
Scientific name	Local name	Sample code	Parts used
Sonchusasper	Jangew	A	leaves
Brassica nigra	Jaiing	B	leaves
Lactucagracilis	Jalynshir	C	leaves
Sonchusarvensis	Jakhain	D	leaves
Allium hookeri	Jaut	E	leaves
Centellaasiatica	Khliangsyiar	F	leaves
Ipomoea batatas	Sla phankaro	G	leaves
Ficusclavata	Sla sohshit	H	leaves
Begonia palmata	Sla jajew	I	leaves
Cucurbitapepo	Sla pathaw	J	leaves
Eryngiumfoetidum	Dhaniakhasi	K	leaves

Vitamin C, Total Phenol and Flavonoid content of the extracts:

The results in figure 1 showed that the vitamin C concentration varies appreciably from one sample to another. The highest concentration of vitamin C was observed in the plant extract of *Brassica nigra* at 108.95mg/100g and the lowest concentration was observed in *Centellaasiatica*at 5.48mg/100g and*Ficusclavata at* 5.70mg/100g.

Plant phenols represent one of the major groups of compounds acting as primary antioxidants or free radical terminators. The results in figure 2 showed that there is a wide variation in the total phenolic content of the fourteen vegetables ranging from 151.46mg GAE/100g in *Brassica nigra* to 35.15mg GAE/100g in *Sonchusasper*.

Among the studied plant extracts, the highest flavoniod content was found in *Begonia palmata* at 560.44mg RE/100g and *Allium hookeri* has the lowest at 53.50mg RE/100g (figure3).



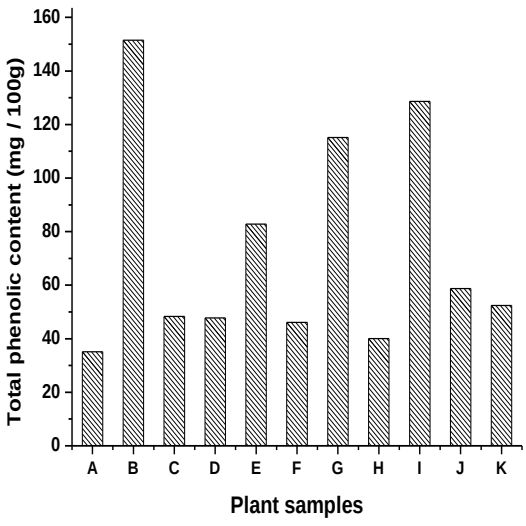


Fig 1: Vitamin C in plant samples. Fig 2: Total phenolic content in plant

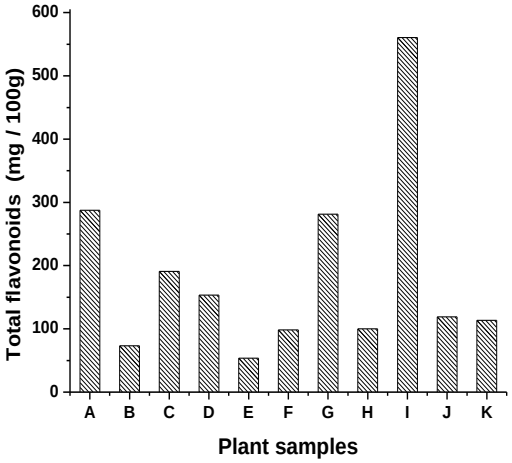


Fig 3: Total flavonoids in plant samples.

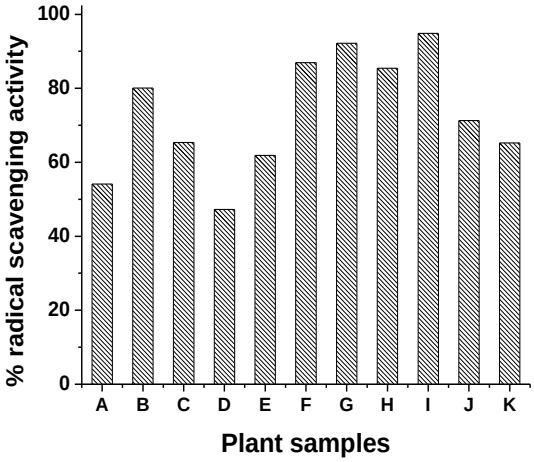


Fig 4: % radical scavenging activity in plant samples

Vitamin C concentration in the plant extracts vary from one sample to another and the variation of this micronutrient in the plant samples greatly depend on factors such as the variety, weather, and maturity[21]. More than 90% of the vitamin C in human diet is supplied by fruits and vegetables. It is required in our daily diet for the growth and development of healthy teeth, bones and gums. It is also important for the synthesis and maintenance of protein collagen. Humans cannot synthesize vitamin C in their bodies and therefore consumption of fruits and vegetables is one way to obtain this important nutrient. The antioxidant activity of plants might not be due to vitamin C alone but there are other compounds like phenolic compounds and flavonoids which have been reported[22] to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic action. The results obtained from the study shows that these wild edible plants are excellent sources of phenolic and flavonoid antioxidants. Phenolic compounds are a class of antioxidants which can adsorb and neutralize the free radicals mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and metal chelators[23].Generally, synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and gallic acid esters are commercially available but their usage has been limited due to their toxicity and side effects such as liver damage and carcinogenesis[24,25]and therefore consumption of wild edible plants may provide good source of harmless natural antioxidants.

Flavonoids are amongst the most important compounds in human diet due to their wide spread distribution in fruits and vegetables. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, antimicrobial, antiallergic activity, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[26]. 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, and Alzheimer's diseases etc[27].

DPPH radical scavenging activity:

The higher the antioxidant activity, the more DPPH radicals are able to be scavenged by the antioxidants. Hence, this antioxidant is excellent in preventing cell damage by reducing the oxidative damage caused by free radicals in human body. The plant extracts exhibit high free radical scavenging activities as seen in fig 4. The highest radical scavenging activity was reported in *Begonia palmata* at 94.83% and the lowest is recorded in *Sonchus arvensis* at 47.24%. The results also showed that *Ipomoea batatas* (92.2%) and *Solanum indicum* (89.14%) have high free radical scavenging activity.

The extracts of *Sonchus asper* and *Sonchus arvensis* contained appreciable amount of total phenol and high amount of flavonoids but could not show potent radical scavenging activity. The high contents of total phenol and flavonoid in the extracts of *Begonia palmata* and *Ipomoea batatas* can explain its high radical

scavenging activity. The high radical scavenging property may be due to the hydroxyl groups existing in the phenolic compounds which can provide the necessary component as a radical scavenger[28].

Correlation of radical scavenging activity with total phenolic content (TPC), total flavonoid content (TFV) and vitamin C was performed by linear regression analysis. But good correlation between radical scavenging activity and TPC and TFV could not been found and which has also been reported elsewhere[29, 30, 31]. This showed that the antioxidant activity of the plant extracts is influenced either by the total phenolic compounds and/or flavonoids. These differences in the antioxidant activities may be due to their differences in phenolic contents and compositions and also due to other non-phenolic antioxidants present in the samples. The results obtained in the study showed that these wild edible plants provide a significant source of natural antioxidants which are helpful in preventing the progress of various oxidative stresses and hence could be exploited as compulsory antioxidant additives or as nutritional supplements to check the formation of harmful free radicals generated in the body.

Conclusion

Determination of antioxidant activity and phytochemicals in plant extracts are necessary as this relates to the fact that antioxidants can prevent free radicals, primarily highly reactive oxygen and nitrogen species, from damaging human health. The findings of the study support this view that majority of the plant extracts especially *Begonia palmata, Brassica nigraand Ipomoea batatas* are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. The results of the present study suggest that tested plant materials have moderate to potent antioxidant activity and/or free radical scavenging activity. More investigation is needed to isolate and identify the active antioxidant compounds present in these plant as well as other *in vivo* assays are essential to characterize them as biological antioxidants which are beyond the scope of this study. Information obtained about the beneficial effects of antioxidants in these underutilised wild edible plants will facilitate research in pharmaceutical industry to find out the bioactive components that possess health promoting properties. The beneficial health effects of these plants also need public awareness to enhance their consumption as part of their daily diet.

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