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Qualitative and quantitative phytochemical assessment and antioxidant activity of selected green leafy vegetables of Assam

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Abstract
Plant foods contain many bioactive compounds in addition to those which are traditionally considered as nutrients, also known as phytochemicals. North-East India is blessed with varying climates and natural surroundings and home of numerous herbs and medicinal plants. Present study was conducted to assess phytochemical and antioxidant activity of 12 commonly grown plants of Assam. Preliminary phytochemical screening reveal that *Chenopodium album, Hibiscus sabdariffa, Phyllanthus amarus*, *Polygonum microcephalum* and *Pogostemon benghalensis* showed positive results for the presence of alkaloids, flavonoids, phenols, saponin, tannin and glycosides. Quantitative assessment revealed that these plants are rich in phenols and flavonoids. The DPPH free radical scavenging activities (% inhibition) of the selected green leafy vegetables were in the range of 18.64-69.34%. *Hibiscus sabdariffa* was having the highest inhibition percentage (69.34%) whereas *Spinacia oleracea* having the lowest value (18.64%). *Amaranthus spinosus* (62.64%), *Chenopodium album* (61.04%), *Diplazium esculentum* (58.43%) and *Pogostemon benghalensis* (64.27%) also showed higher DPPH free radical scavenging activities.

Keywords: Phytochemicals, alkaloid, flavonoid, phenolics, antioxidants

Introduction
Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [1]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [2]. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world [3]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [4]. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product.

Materials and Methods
Collection of Plant Material
On the bases of ethnobotanical knowledge of available literature and visual observations of plants that were relatively free from diseases and insect damages, 12 plant species have been selected for the present study. Collected plant material were thoroughly washed and then dried under shade at 25 ± 2 ºC for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder. The powdered samples were then stored in airtight containers at room temperature.

Phytochemical Screening
Phytochemical screening has been carried out following the procedures reported in literature [5, 6].

Qualitative analysis on phytochemical constituents

Test for tannins: Powdered sample (0.5 g) of each plant is boiled in 20 mL of distilled water in a test tube and then filtered. 0.1 % $FeCl_3$ is added tothe filtered samples and observed for brownish green or a blue-black colouration, which shows the presence of tannins.

Test for phlobatannins: In a test tube take 10 mL ofaqueous extract of each plant sample and boiled it with 1 % HCl. If the sample of plant carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence of phlobatannins.

Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Test for saponins: Two g of powdered samples of each plant is boiled together with 20 mL of distilled water in a water bath and filtered. 10 mL of the filtered sample is mixed with 5 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for flavonoids: A few drops of 1 % NH_3 solution isadded to the aqueous extract of each plant sample in a test tube. A yellow colouration is observed if flavonoid compounds are present.

Test for terpenoids: Five mL of aqueous extract of each plant sample is mixed with 2 mL of $CHCl_3$ in a test tube. Three mL of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed if terpenoids constituent is present.

Test for cardiac glycosides: One mL of concentrated H_2SO_4 is prepared in a test tube. 5 mL of aqueous extract from each plant sample is mixed with 2 mL of glacial CH_3COOH containing 1 drop of $FeCl_3$. The above mixture is carefully added to the 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycosideis present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Quantitative analysis on phytochemical constituents

Alkaloids: Five g of the plant sample is prepared in a beaker and 200 mL of 10 % CH_3COOH in C_2H_5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture then filtered and the extract is allowed to become concentrated in a water bath until it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed.

Flavonoids: Ten g of plant sample is repeatedly extractedwith 100 mL of 80 % aqueous methanol at room temperature.The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until aconstant weight.

Tannin: 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [7].

Phenol: 100 mg of the extract of the sample was weighed and dissolved in 100 ml of distilled water. 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid [8].

Determination of free radical scavenging activity [9]

Antioxidant activity was measured by using DPPH method (Vani *et al.*, 1997). Two grams of fresh sample was extracted with 20 ml of methanol (99.5%). The supernatant was filtered using whatman No. 1 filter paper after centrifuging the suspension at 10,000 rpm for 15 min. Till analysis, filtrated was stored at -20°C. 100 µl of aliquot of sample extract was taken in a test tube and then 2.9 ml of DPPH solution (0.05 Mm solution of 2, 2 diphenyl-1-picryl-hydrazyl prepared in 99.5% methanol) was added the solution was vortexed vigorously. The test tube was incubated in dark for half an hour. The discoloration of DPPH was measured against blank at 517nm. Methanol and DPPH methanolic solution was used as blank. Calculation: % inhibition = (Ab-Aa)/Ab ×100, where, Ab is the absorbance of blank, Aa is the absorbance of sample

Results and Discussion

Qualitative analysis on phytochemical constituents

In order to detect the presence of different phytochemical components in the studied samples various test were conducted and results are presented in the Table 1. The results reveal that the plants *Chenopodium album, Hibiscus sabdariffa, Phyllanthus amarus, Polygonum microcephalum* and *Pogostemon benghalensis* showed positive results indicating the presence of alkaloids, flavonoids, phenolic compounds, saponin, tannin and glycosides. Flavonoids were present in all of the green leafy vegetables. *Amaranthus spinosus, Spinacia oleracea* and *Rumex vesicarius* showed negative result indicating absence of alkaloid. *Spinacia oleracea* and *Ludwigia adscendens* showed negative result for saponin. Tannin was absent in *Spinacia oleracea, Diplazium esculentum* and *Malva verticillata*. *Spinacia oleracea, Diplazium esculentum, Talinum triangulare, Malva verticillata* and *Ludwigia adscendens* showed negative result indicating absence of glycosides. Chugh *et al.* (2012) carried

out phytochemical screening of 12 medicinal plant species namely *Ocimum sanctum, Psoralea corylifolia, Crotolarea juncea, Sansevieria ghiana, Gossypium herbaceum, Schleichera oleosa, Anethum sowa, Analrographis panicculata, Punica granatum, Argemone mexicana, Gmelina arborea* and *Prunella vulgaris.* They revealed that all the plant species except *Andrographis paniculata* and *Gmelina arborea* have shown the presence of alkaloids, while flavonoids have been found to be absent in *Gossypium herbaceum, Schleichera oleosa, Prunella vulgaris* and *Sansevieria ghiana*. Saponins have been found to be present in all species except *Crotolarea juncea, Andrographis paniculata, Prunella vulgaris, Anethum sowa* and *Psoralea corylifolia* [10].

Table 1: Preliminary phytochemical screening of selected green leafy vegetables

Botanical Name	Alkaloids	Flavonoids	Saponin	Tannin	Phloba-tannin	Phenols	Cardiac glycosides
Amaranthus spinosus	-	+	+	+	+	+	+
Chenopodium album	+	+	+	+	+	+	+
Rumex vesicarius	-	+	+	+	+	+	+
Spinacia oleracea	-	+	-	-	-	+	-
Hibiscus sabdariffa	+	+	+	+	+	+	+
Diplazium esculentum	+	+	+	-	-	+	-
Phyllanthus amarus	+	+	+	+	+	+	+
Polygonum microcephalum	+	+	+	+	+	+	+
Talinum triangulare	+	+	+	+	+	+	-
Malva verticillata	+	+	+	-	-	+	-
Pogostemon benghalensis	+	+	+	+	+	+	+
Ludwigia adscendens	+	+	-	+	+	+	-

Quantitative analysis on phytochemical constituents

Quantitative analysis on phytochemical constituents revealed that these greens contain a judicious amount of phytochemicals. Among the greens, the alkaloid content was found highest in *Talinum triangulare* (5.44 mg/100g). Flavonoid content was found highest in *Hibiscus sabdariffa* (18.78 mg/100g) and lowest in *Spinacia oleracea* (2.86 mg/100g). *Diplazium esculentum* contained highest saponin content of 31.63 mg per 100g of sample. Tannin was found highest in *Pogostemon benghalensis* which contained 14.38 mg per 100 g of sample. Phenol content was found highest in *Hibiscus sabdariffa* (36.23 mg/100g) and lowest in *Spinacia oleracea* (9.26mg/100g).

Table 2: Preliminary phytochemical screening of selected green leafy vegetables (mg/100g)

Botanical Name	Alkaloids (mg)	Flavonoids (mg)	Saponin (mg)	Tannin (mg)	Phenols (mg)
Amaranthus spinosus	-	11.48 ± 0.11	27.67 ± 0.23	5.14 ± 0.02	28.56 ± 0.13
Chenopodium album	5.21 ± 0.12	9.84 ± 0.13	18.52 ± 0.18	8.56 ± 0.09	26.11 ± 0.15
Rumex vesicarius	-	6.55 ± 0.10	6.11 ± 0.12	4.34 ±0.06	17.88 ± 0.17
Spinacia oleracea	-	2.86 ± 0.07	-	-	9.26 ± 0.09
Hibiscus sabdariffa	2.24 ± 0.09	18.76 ± 0.21	9.26 ± 0.09	6.86 ±0.09	36.23 ± 0.25
Diplazium esculentum	4.68 ± 0.36	8.68 ± 0.11	31.63 ± 0.13	-	23.89 ± 0.23
Phyllanthus amarus	3.77 ± 0.21	4.43 ± 0.02	16.32 ± 0.33	7.81 ± 0.11	11.38 ± 0.14
Polygonum microcephalum	2.86 ± 0.14	7.28 ± 0.08	18.23 ± 0.20	5.26 ± 0.02	21.45 ± 0.31
Talinum triangulare	5.44 ± 0.33	7.02 ± 0.06	14.31 ± 0.26	11.72 ± 0.14	19.67 ± 0.18
Malva verticillata	2.46 ± 0.17	5.23 ± 0.04	11.45 ± 0.22	-	15.25 ± 0.33
Pogostemon benghalensis	3.41 ± 0.26	16.08 ± 0.23	23.62 ± 0.26	14.38 ± 0.15	32.31 ± 0.13
Ludwigia adscendens	4.26 ± 0.38	5.08 ± 0.03	-	9.45 ± 0.12	13.26 ± 0.20

Free radical scavenging activity of the selected greens

The DPPH (2, 2 diphenyl-1-picryl-hydrazyl) scavenging activity (% inhibition) of the selected sample extracts of 100 µg/ml conc. is presented in Fig 1. In the present study, the DPPH free radical scavenging activites (% inhibition) were in the range of 18.64 - 69.34%. *Hibiscus sabdariffa* was having the highest inhibition percentage (69.34%) whereas *Spinacia oleracea* having the lowest value (18.64%). *Amaranthus spinosus* (62.64%), *Chenopodium album* (61.04%), *Diplazium esculentum* (58.43%) and *Pogostemon benghalensis* (64.27%) also showed higher DPPH free radical scavenging activites.

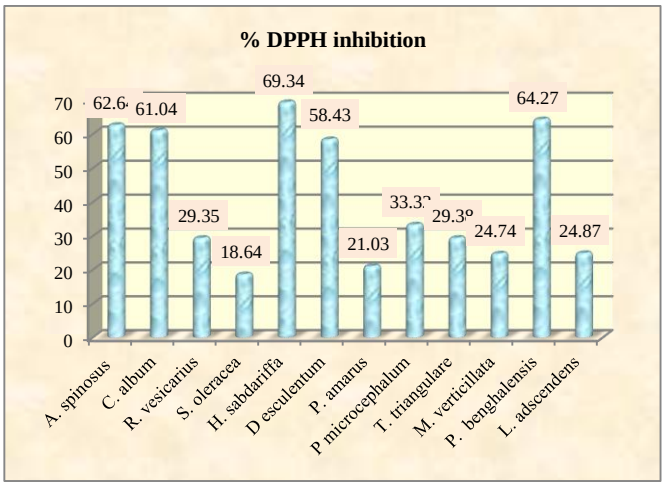

Fig 1: Free radical scavenging activity of the selected greens

Prasad and Ramakrishnan (2011) estimated DPPH scavenging percentage for *Rumex Vesicarius* as 31.60% which was nearer to the result of present finding (29.35%). Gacche *et al.* (2010) studied antioxidant potential of selected vegetables commonly used in diet in Asian subcontinent and reported DPPH scavenging activity (%) for spinach as 20.40% whereas in the present study inhibition percentage for spinach was 18.64% [11].

Conclusion

Green leafy vegetables are the treasure trove of micronutrients as well as good source of phytochemicals and antioxidants. The immense natural resources of this region can be safely harnessed by the pharmaceutical companies and food based industries. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

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