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Phytochemicals and antioxidant properties of eleven wild edible plants from Assam, India

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Abstract. The aim of this study was to examine the phytochemicals and antioxidant properties of eleven wild edible plants from Assam of North-East India. The phytochemical study indicated the presence of several medicinally active compounds in the methanolic extracts of plants. Evaluation of antioxidant activities were done by DPPH, ABTS, H_2O_2 and FRAP assays. The investigation revealed antioxidant activities with DPPH IC_{50} value ranging from 135.0 ± 1.49 μg/mL (*L. javanica*) to 516.34 ± 2.52 μg/mL (*B. lanceolaria*), ABTS IC_{50} value from 74.3 ± 0.29 μg/mL (*T. angustifolium*) to 437.77 ± 3.93 μg/mL (*D. cordata*), H_2O_2 IC_{50} value from 20.37 ± 0.01 μg/mL (*B. lanceolaria*) to 376.75 ± 14.12 μg/mL (*P. perfoliatum*), and the FRAP value from 64.76 ± 7.43 μM TE/g (*D. cordata*) to 799.28 ± 7.14 μM TE/g (*L. javanica*). The maximum total phenolic content (TPC) was obtained in the extract of *E. fluctuans* (269.49 ± 2.96 mg GAE/g dry extract) and the lowest being in *C. sinensis* (26.96 ± 9.81 mg GAE/g dry extract). *S. media* extract had the lowest (0.23 ± 0.10 mg QE/g dry extract) total flavonoid content (TFC) and the maximum being in *P. perfoliatum* (4.34 ± 1.03 mg QE/g dry extract). Pearson's correlation study of the plants indicated a strong positive correlation of DPPH assay with ABTS assay. A positive correlation of FRAP with TFC, H_2O_2 with FRAP, TPC and TFC, and TPC with TFC were also seen in this study. These plants could be supportive in stopping or slowing the growth of oxidative stress related diseases.

Keywords: Wild plants, phytochemicals, antioxidant activities, phenolic, flavonoid

1. Introduction

Plants contain many phytochemical constituents which have various activities like antioxidant, antidiabetic, anthelmintic and many more [1, 2]. Antioxidant compounds are the group of compounds which prevent the oxidation of certain molecules present in the living system as well as in the food stuff or in the industrial products. These compounds help in the inhibition of generating reactive oxygen species (ROS) in the living systems including oxygen free radical species *viz*. superoxide anion ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), peroxynitrite and nitric oxide ($NO^{\cdot}$) radicals as well as non-free radicals *viz*. H_2O_2, HNO_2 and singlet oxygen ($^1O_2$). Generation of such species in the body leads to oxidative stress which ultimately damage the cells by

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reacting with biomolecules leading to a number of diseases *viz*. stroke, diabetes, cancer, heart disease, cataracts, rheumatoid arthritis, Alzheimer's disease, and also premature aging [3–5]. The antioxidants protect the cells in our body against reactive oxygen species and hence, antioxidant supplements are essential to fight oxidative cellular damage [6, 7].

Wild edible plants have played an important role in human lives from ancient times. They are consumed by ethnic people as traditional vegetables and also used for medicinal purposes. Plant food contains many phytochemicals including phenolic compounds along with nutrients such as proteins, fats, carbohydrates, vitamins, and minerals. Phytochemicals are potent antioxidants against ROS and have several potential health benefits. Many phytochemicals have been identified in plant foods and just one plant may contain more than 100 different phytochemicals [8, 9]. The study on less-utilized vegetables in different areas exposed that most of the wild plant species contain rich nutritional and strong antioxidant properties which are even analogous to those vegetables produced commercially [10–13]. Hence, the recent research should be emphasized on wild plant species for their potential food and medicinal properties to widen the variety of foodstuff for human consumption.

Assam (89°50′ E to 96°10′ E and 24°30′ N to 28°10′ N), one of the states of North-East (NE) India, is rich in biodiversity and the total area of Assam is 78,438 sq. km out of which 26,832 sq. km is outlined as forest area [14]. There is extensive study on antioxidant properties of cultivated vegetables and plants. Many researchers have studied and reported the functional properties of different wild edible plants from different areas. The same author reported nutritional value and vitamin C contents of some selected wild plants from Assam [15]. The nutritional, anti-nutritional and mineral compositions of eight locally available leafy vegetables of Sonitpur district of Assam were reported by Saha et al. [16]. Saikia et al. [17] reported mineral content of some wild green leafy vegetables of North-East India. Borah et al. [18] also reported mineral content in commonly consumed leafy vegetables used by the people of Assam. However, some of the wild plants consumed by the indigenous people of Assam of NE India are still not studied and very little informations are available about the functional properties of wild edible plants. The main objective of this study was to determine the antioxidant potentials of some commonly consumed wild plants growing in Assam of NE India. Therefore, eleven most prominently utilized wild plant species growing in Kokrajhar District of Assam of NE India *viz*. *Blumea lanceolaria* (Roxb.) Druce, *Tetrastigma angustifolium* (Roxb.), *Oenanthe javanica* (Blume) DC., *Drymaria cordata* (L.) Willd.ex Schult., *Cryptolepis sinensis* (Lour) Merr., *Stellaria media* (L.), *Antidesma acidum* Retz., *Eryngium foetidum* L., *Lippia javanica* (Burm.f.) Spreng., *Polygonum perfoliatum* L., and *Enhydra fluctuans* Lour were selected for the present study.

2. Materials and methods

2.1. Chemicals

Quercetin, 2, 2′-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Himedia Laboratories Pvt. Ltd., Nashik, Mumbai, India, ascorbic acid, hydrogen peroxide and Folin-Ciocalteu's reagent from Merck, Mumbai, India, gallic acid from Central Drug House Pvt. Ltd., Daryaganj, New Delhi, India, and trolox was obtained from Sigma Aldrich, Bangalore, India. Other solvents and chemicals were of analytical grade and used as obtained.

2.2. Collection of plants and sample preparation

A total of eleven fresh wild edible plants *viz*. *B. lanceolaria*, *T. angustifolium*, *O. javanica*, *D. cordata*, *C. sinensis*, *S. media*, *A. acidum*, *E. foetidum*, *L. javanica*, *P. perfoliatum*, and *E. fluctuans* were collected from Kokrajhar District of Assam during their seasonal availability in the year 2014. All these plants were identified by Botanical Survey of India (BSI), Shillong. The collected samples were washed properly with water, rinsed

with distilled water, dried in hot air oven at 55°C, crushed into powder by mixture grinder and stored in the air-tight plastic container for further use. For the preparation of extract, the powder material was mixed with methanol in 1 : 10 ratio (w/v), stirred, kept for 72 h, filtered with Whatman No. 1 filter paper and the solvent evaporated to dryness using Buchi Rotavapor R-215 (Switzerland) and the dry extract was kept in a container at 4°C for further analysis.

2.3. Phytochemical screening

The methanol extracts of the plants were analyzed for the detection of phytochemicals by using standard procedures [19, 20].

2.4. Determination of antioxidant property

2.4.1. DPPH free radical scavenging assay

The free radical scavenging activities of plant methanolic extracts were evaluated by DPPH method [21]. 1 mL of extract in different concentration (2, 5, 10, 50, 100, 200, 500 μg/mL) was added to 3 mL working DPPH solution (0.1 mM DPPH in methanol). The mixture was shaken and allowed to stand for 30 min in dark, and then the absorbance was read at 517 nm with UV-VIS spectrophotometer (Lambda 35, Perkin Elmer, USA) and it was compared with standard ascorbic acid using similar concentrations. 1 mL methanol and 3 mL working DPPH solution served as the blank. The percentage inhibition was calculated as:

$$\%\,\text{inhibition} = \left[(\text{A}_{control} - \text{A}_{sample})/\text{A}_{control} \times 100\right]$$

where, $\text{A}_{control}$ is the concentration of DPPH radical without the test sample, A_{sample} is the absorbance of the sample or standard. The concentration (μg/mL) of plant material extract was plotted against the percentage inhibition and IC_{50} was obtained from linear regression equation from the graph and the results were expressed in μg/mL of dry extract.

2.4.2. ABTS radical scavenging assay

Antioxidant activities of methanol extracts were investigated by ABTS method [22]. ABTS radical cation ($\text{ABTS}^{\cdot+}$) generated using 7 mM ABTS solution and 2.45 mM potassium persulphate was kept in the dark for 12–16 h at room temperature. The radical cation solution was again diluted to 1 : 60 (v/v) with methanol until the initial absorbance becomes 0.706 ± 0.02 at 734 nm. 1 mg/mL extract or standard was diluted in different concentration from 20–300 μg/mL and to this, 2 mL diluted $\text{ABTS}^{\cdot+}$ working solution was added and the absorbance was measured at 734 nm after 6 min using Perkin Elmer UV-Vis Spectrophotometer (Lambda 35, USA). A graph was plotted using inhibition (%) against concentration of standard trolox. The methanol was taken as blank and IC_{50} was obtained from the linear regression equation from the graph of percentage inhibition and the results were expressed in μg/mL of dry extract. The % inhibition was calculated as:

$$\%\,\text{Inhibition} = \left[(\text{A}_{control} - \text{A}_{sample})/\text{A}_{control} \times 100\right]$$

where, $\text{A}_{control}$ is concentration of ABTS radical cation without the test sample, A_{sample} is the absorbance of sample or standard.

2.4.3. H_2O_2 scavenging assay

Hydrogen peroxide scavenging activities of samples were determined spectrophotometrically at 230 nm [23]. A solution of 20 mM H_2O_2 was made from 30% H_2O_2 by diluting 226 μL in 99.8 mL phosphate buffer saline (pH 7.4). Various concentration of sample ranging from 5–25 μg/mL was prepared and 2 mL of H_2O_2 was added, incubated for 10 min and the absorbance was taken at 230 nm using Perkin Elmer UV-Vis spectrophotometer (Lambda 35, USA). Phosphate buffer saline was taken as blank for zeroing and ascorbic acid as positive control.

The IC_{50} value was determined from the graph obtained from the percentage of inhibition and the results were presented in μg/mL of dry extract.

$$\%\ \text{inhibition} = \left[(A_{control} - A_{sample})/A_{control} \times 100\right]$$

where, $A_{control}$ is concentration of H_2O_2 without the test sample, A_{sample} is the absorbance of sample or standard.

2.4.4. Ferric reducing antioxidant power (FRAP) assay

FRAP value was evaluated using the method of Benzie et al. [24]. The stock solution contains 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$. The working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3.6H_2O$. 40 μL of the sample was allowed to react with 3960 μL of FRAP solution and incubated in the dark for 30 min. The absorbance was taken at 593 nm using Perkin Elmer UV-Vis spectrophotometer (Lambda 35, USA) and standard trolox was taken in different concentration starting from 25-1000 μM for obtaining calibration curve. The data were expressed in μM trolox equivalent (TE)/g of extract.

2.5. Evaluation of total phenolic content (TPC)

Total phenolic content was evaluated using Folin-Ciocalteu's reagent spectrophotometrically [21]. Different concentrations (10, 20, 40, 60, 80, 100 μg/mL) of standard gallic acid were prepared and to each 2.5 mL of 10% Folin-Ciocalteu's reagent was added and incubated for 5 min. After 5 min, 2 mL of 7.5% Na_2CO_3 solution was added to the mixture, incubated in the dark for 30 min, and the absorbance was taken at 765 nm using Perkin Elmer UV-VIS spectrophotometer (Lambda 35, USA). For analysis of samples, 40 μL was taken and all the reagents were added as in standard. The reagent blank was prepared by adding 1 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2 mL of 7.5% Na_2CO_3 solution. The values were obtained using the calibration curve of gallic acid and the total phenolic content was presented as milligrams of gallic acid equivalents per gram dry extract (mg GAE/g dry extract).

2.6. Evaluation of total flavonoid content (TFC)

Total flavonoid content of plant extract was also evaluated spectrophotometrically at 510 nm [25]. A methanol solution (1 mL) of extract (1 mg/mL) or solutions of standard quercetin (10, 20, 40, 60, 80, 100 μg/mL) was taken in 0.5 mL of 5% $NaNO_2$ solution and 0.5 mL of 10% $AlCl_3$ solution. After 5 min, 2 mL of NaOH solution (4%) was added and incubated for 15 min at room temperature and the absorbance was read against the blank at 510 nm using Perkin Elmer UV-VIS spectrophotometer (Lambda 35, USA). Blank solution was made by adding the entire reagent except sample or standard. A calibration curve was being made using standard quercetin and the value of total flavonoid was presented as milligrams of quercetin equivalents per gram extract (mg QE/g dry extract).

2.7. Statistical analysis

The results of all the experiments were expressed as mean of triplicate readings ± standard deviation. Standard deviations were calculated at Microsoft Excel. Relative significant differences among the means were determined by one-way ANOVA *t*-test at $p < 0.05$ using OriginPro 8.5 software (OriginLab Corporation, MA 01060 USA). Pearson's correlation study was done using SPSS 13.0 software.

Table 1
Phytochemical screening of methanolic extracts of eleven wild edible plants

Phytochemical constituents	Test	*B. lanceolaria*	*T. angustifolium*	*O. javanica*	*D. cordata*	*C. sinensis*	*S. media*	*A. acidum*	*E. foetidum*	*L. javanica*	*P. perfoliatum*	*E. fluctuans*
Alkaloids	Wagner's reagent	+	+	+	+	+	+	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+	+	+	+	+	+	+
Saponins	Frothing test	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	–	+	+	+	+	+	+	+	+
Steroids	Liebermann-Burchard test	–	–	+	+	+	+	+	+	+	+	+
	Salkowski's test	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	Modified Borntrager's test	–	–	–	–	–	+	+	–	+	+	+
Coumarins		–	–	+	+	–	–	+	+	+	+	+
Phenols		+	+	+	+	+	+	+	+	+	+	+
Tannins	Gelatine	+	+	+	+	+	–	+	+	–	+	+
Flavonoids	Shinoda's test	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins		+	+	–	–	+	+	+	–	+	+	–
Phlobatannins		–	+	+	–	+	+	+	–	–	+	–
Lignin	Lignin test	+	+	+	–	+	+	–	–	+	+	–
Proteins	Ninhydrin test	+	–	+	+	–	–	–	+	+	+	–
	Millon's test	+	+	–	–	+	–	+	–	–	+	–
Starch	Iodine test	–	–	–	–	–	–	–	–	–	–	–

Negative (–) indicates absent and positive (+) indicates present.

3. Results and discussion

3.1. Phytochemical screening

Phytochemicals are bioactive organic compounds which are found naturally in the plants. Plants are very good sources of biomolecules that differ extensively in their structure, mechanisms of action, and biological properties [26, 27]. The screening of phytochemical constituents present in the eleven wild edible plants was performed using the methanol extract. The phytochemical constituents investigated were alkaloid, saponin, cardiac glycoside, steroid, anthraquinone, coumarin, phenolic compounds, tannin, flavonoid, anthocyanins, phlobatannins, lignin, proteins and starch. The results are presented in Table 1 which showed the presence of many biologically active compounds and considered to have medicinal properties like antimicrobial, antioxidant, anthelmintic and also exhibit other biological activities. These plants could be considered as value-added products for various pharmacological uses and could serve as potent starting materials in formulation of various dietary supplements.

3.2. Antioxidant properties

In the present study, DPPH, ABTS, H_2O_2 and FRAP assays were used to assess the *in vitro* antioxidant capacities in the methanol extracts of eleven wild edible plants. DPPH method is an easy, rapid, sensitive and routinely used method for the determination of antioxidant activity. The radical scavenging activity in plant extract is determined based on its ability to quench the DPPH free radical. Antioxidants in the plant extracts react with DPPH, a stable free radical and convert 1, 1-diphenyl-2-picrylhydrazyl to a stable

Table 2
DPPH free radical scavenging activity of methanolic extract of wild edible plants

Plant extract/ Standard	Concentration (μg/mL) and its inhibition (%)							IC_{50} value (μg/mL)
	2	5	10	50	100	200	500	
B. lanceolaria	13.70 ± 0.04^{a}	14.24 ± 0.11^{a}	16.56 ± 0.11^{a}	19.50 ± 0.18^{a}	20.58 ± 0.14^{a}	30.56 ± 0.22^{a}	48.13 ± 0.15^{a}	516.34 ± 2.52^{a}
T. angustifolium	13.32 ± 0.16^{a}	18.23 ± 0.24^{b}	22.24 ± 0.15^{b}	32.25 ± 0.19^{b}	48.87 ± 0.19^{b}	73.31 ± 0.23^{b}	90.94 ± 0.12^{b}	171.21 ± 0.57^{b}
O. javanica	7.99 ± 0.15^{b}	8.78 ± 0.15^{c}	11.85 ± 0.16^{c}	15.25 ± 0.19^{c}	17.07 ± 0.16^{c}	41.64 ± 0.15^{c}	65.39 ± 0.15^{c}	345.80 ± 1.07^{c}
D. cordata	12.29 ± 0.25^{c}	13.93 ± 0.07^{a}	14.27 ± 0.12^{d}	17.76 ± 0.12^{d}	19.97 ± 0.16^{d}	27.39 ± 0.15^{d}	48.71 ± 0.19^{d}	516.04 ± 2.50^{a}
C. sinensis	13.88 ± 0.19^{a}	15.20 ± 0.15^{d}	15.54 ± 0.19^{e}	26.97 ± 0.24^{e}	38.87 ± 0.15^{e}	59.17 ± 0.19^{e}	90.89 ± 0.15^{b}	205.62 ± 0.99^{d}
S. media	13.77 ± 0.18^{a}	14.29 ± 0.13^{a}	15.07 ± 0.18^{e}	20.66 ± 0.18^{f}	23.79 ± 0.13^{f}	29.51 ± 0.18^{f}	60.86 ± 0.22^{e}	391.04 ± 1.11^{e}
A. acidum	14.71 ± 0.18^{d}	15.19 ± 0.13^{d}	17.41 ± 0.18^{f}	34.35 ± 0.18^{g}	47.62 ± 0.22^{g}	68.08 ± 0.22^{g}	85.92 ± 0.18^{f}	189.67 ± 0.22^{f}
E. foetidum	12.36 ± 0.18^{c}	13.73 ± 0.09^{a}	15.34 ± 0.18^{e}	18.41 ± 0.27^{h}	$20.33 \pm 0.22^{a,d}$	35.28 ± 0.18^{h}	57.12 ± 0.18^{g}	407.54 ± 0.65^{g}
L. javanica	17.21 ± 0.17^{e}	20.63 ± 0.21^{e}	22.34 ± 0.26^{b}	40.63 ± 0.21^{i}	64.55 ± 0.30^{h}	78.60 ± 0.17^{i}	94.11 ± 0.21^{h}	135.00 ± 1.49^{h}
P. perfoliatum	16.08 ± 0.17^{f}	19.82 ± 0.15^{f}	20.78 ± 0.17^{g}	31.07 ± 0.13^{j}	48.26 ± 0.17^{i}	80.08 ± 0.17^{j}	93.39 ± 0.17^{i}	160.14 ± 0.39^{i}
E. fluctuans	15.44 ± 0.21^{f}	16.69 ± 0.17^{g}	18.37 ± 0.13^{h}	21.01 ± 0.21^{k}	29.71 ± 0.13^{j}	40.49 ± 0.10^{k}	75.42 ± 0.21^{j}	283.40 ± 1.15^{j}
Ascorbic acid	15.94 ± 0.14^{f}	26.93 ± 0.19^{h}	36.57 ± 0.28^{i}	83.11 ± 0.23^{l}	90.04 ± 0.23^{k}	93.03 ± 0.47^{l}	98.84 ± 0.10^{k}	25.01 ± 0.52^{k}

Results are expressed as mean of 3 replicates ± standard deviation. The values with different letters in a column are significantly different from each other at $p < 0.05$.

molecule 1, 1-diphenyl-2-picrylhydrazine by accepting hydrogen radical or an electron leading to a decrease absorbance at 517 nm [14]. IC_{50} value is the inhibitory concentration of the crude extract that could scavenge 50% ROS or inhibit oxidation by 50%. IC_{50} value is inversely related to the activity and lower IC_{50} value means higher antioxidant activity. The DPPH free radical scavenging activity of the plant species is shown in Table 2. In this investigation, all the plant extracts were compared with standard ascorbic acid and the methanol extract of the plants exhibited DPPH free radical scavenging activity. All the methanol extracts of the plants showed noticeable free radical scavenging activities in concentration-dependent manner and scavenging activity increased with increasing the concentration of each individual plant extract (Table 2). Similar to this study, Ng et al. [10] also reported that the plant extract is capable of trapping the DPPH free radical in a dose-dependent manner. The results of present study (Table 2) showed that *L. javanica* ($94.11 \pm 0.21\%$) had the highest DPPH radical scavenging activity with an IC_{50} value of 135.0 ± 1.49 μg/mL followed by *P. perfoliatum* ($IC_{50} = 160.14 \pm 0.39$ μg/mL), *T. angustifolium* ($IC_{50} = 171.21 \pm 0.57$ μg/mL), *C. sinensis* ($IC_{50} = 205.62 \pm 0.99$ μg/mL) and *A. acidum* ($IC_{50} = 189.67 \pm 0.22$ μg/mL), and *B. lanceolaria* ($48.13 \pm 0.15\%$) exhibited the lowest antioxidant activity with IC_{50} value of 516.34 ± 2.52 μg/mL. While the standard ascorbic acid displayed $98.84 \pm 0.10\%$ inhibition at the concentration of 500 μg/mL and showed an IC_{50} value of 25.01 ± 0.52 μg/mL.

$ABTS^{+}$ radical is a stable free radical species which accepts an electron or hydrogen radical from antioxidant compounds to become a stable molecule and thus prevents initiation or propagation of free-radical chain reaction or oxidation of other molecules. The ABTS assay is routinely used for evaluation of antioxidant capacity of plant extracts to scavenge free radicals [28]. ABTS radical scavenging activity of the plant species is shown in Table 3 and the highest activity was found in the extract of *T. angustifolium* ($94.62 \pm 0.14\%$) followed by *P. perfoliatum* ($94.08 \pm 0.31\%$) and *L. javanica* ($92.62 \pm 0.18\%$) with an IC_{50} value of 74.3 ± 0.29 μg/mL, 81.67 ± 0.28 μg/mL and 86.99 ± 0.27 μg/mL respectively, whereas *D. cordata* ($31.90 \pm 0.30\%$) displayed the lowest scavenging activity among the selected plant species with an IC_{50} value of 437.77 ± 3.93 μg/mL. Trolox was used as standard in ABTS assay and showed an IC_{50} value 73.67 ± 0.74 μg/mL (Table 3). This study revealed concentration-dependent scavenging activity and it was observed that the methanol extract of *T. angustifolium* (74.3 ± 0.29 μg/mL) and the standard trolox (73.67 ± 0.74 μg/mL) showed almost similar activity. Hence, *T. angustifolium* can be considered as a powerful antioxidant. It was reported that the high molecular weight phenolic

Table 3
ABTS radical scavenging activity of methanolic extract of wild edible plants

Plant extract/ Standard	Concentration (μg/mL) and its inhibition (%)							IC_{50} value (μg/mL)
	20	50	100	150	200	250	300	
B. lanceolaria	14.07 ± 0.57^{a}	21.25 ± 0.28^{a}	25.29 ± 0.35^{a}	37.70 ± 0.21^{a}	45.12 ± 0.21^{a}	55.63 ± 0.28^{a}	63.95 ± 21.0^{a}	222.69 ± 0.96^{a}
T. angustifolium	26.49 ± 0.22^{b}	44.19 ± 0.30^{b}	61.94 ± 0.29^{b}	76.25 ± 0.29^{b}	85.49 ± 0.22^{b}	88.98 ± 0.22^{b}	94.62 ± 0.14^{b}	74.3 ± 0.29^{b}
O. javanica	10.05 ± 0.32^{c}	18.97 ± 0.31^{c}	23.22 ± 0.39^{c}	27.63 ± 0.17^{c}	42.19 ± 0.32^{c}	46.91 ± 0.23^{c}	57.80 ± 0.39^{c}	261.14 ± 1.44^{c}
D. cordata	3.78 ± 0.51^{d}	9.67 ± 0.30^{d}	11.14 ± 0.30^{d}	21.89 ± 0.22^{d}	28.91 ± 0.22^{d}	30.28 ± 0.30^{d}	31.90 ± 0.30^{d}	437.77 ± 3.93^{d}
C. sinensis	21.17 ± 0.29^{e}	33.43 ± 0.22^{e}	48.43 ± 0.30^{e}	61.27 ± 0.16^{e}	74.90 ± 0.37^{e}	78.43 ± 0.22^{e}	81.96 ± 0.22^{e}	120.8 ± 0.55^{e}
S. media	15.96 ± 0.31^{f}	25.52 ± 0.30^{f}	29.65 ± 0.38^{f}	66.51 ± 0.38^{f}	73.21 ± 0.31^{f}	80.51 ± 0.30^{f}	82.27 ± 0.23^{f}	139.96 ± 0.61^{f}
A. acidum	24.85 ± 0.43^{g}	30.71 ± 0.22^{g}	42.53 ± 0.22^{g}	58.91 ± 0.36^{g}	80.71 ± 0.22^{g}	82.21 ± 0.30^{g}	89.19 ± 0.22^{g}	118.93 ± 0.63^{g}
E. foetidum	15.63 ± 0.30^{f}	30.47 ± 0.45^{g}	39.79 ± 0.37^{h}	43.80 ± 0.37^{h}	48.37 ± 0.31^{h}	52.88 ± 0.31^{h}	60.35 ± 0.22^{h}	213.77 ± 1.57^{h}
L. javanica	22.61 ± 0.15^{h}	36.99 ± 0.24^{h}	57.96 ± 0.15^{i}	80.09 ± 0.15^{i}	86.57 ± 0.09^{i}	90.92 ± 0.15^{i}	92.62 ± 0.18^{i}	86.99 ± 0.27^{i}
P. perfoliatum	24.14 ± 0.31^{g}	42.88 ± 0.23^{i}	61.57 ± 0.23^{b}	70.66 ± 0.32^{j}	87.90 ± 0.39^{j}	89.82 ± 0.23^{j}	94.08 ± 0.31^{b}	81.67 ± 0.28^{j}
E. fluctuans	$23.13 \pm 0.22^{h,g}$	30.33 ± 0.22^{g}	49.57 ± 0.22^{j}	65.31 ± 0.22^{k}	76.76 ± 0.14^{k}	87.50 ± 0.31^{k}	89.05 ± 0.29^{g}	112.23 ± 0.14^{k}
Trolox	11.41 ± 0.22^{i}	47.98 ± 0.14^{j}	69.10 ± 0.22^{k}	87.89 ± 0.29^{l}	92.17 ± 0.17^{l}	94.76 ± 0.14^{l}	96.46 ± 0.22^{j}	73.67 ± 0.74^{l}

Results are expressed as mean of 3 replicates ± standard deviation. The values with different letters in a column are significantly different from each other at $p < 0.05$.

compounds have more ability to quench ABTS free radicals and their effectiveness depends on the molecular weight, number of aromatic rings, and nature of hydroxyl group's substitution than the specific functional groups [29, 30].

H_2O_2 scavenging activity of methanolic extract of the plants is shown in Table 4 and this activity was compared with the standard ascorbic acid. The study showed the highest percentage of scavenging activity in *B. lanceolaria* extract with an IC_{50} value of 20.37 ± 0.01 μg/mL, while standard ascorbic acid revealed an IC_{50} value of 19.02 ± 0.01 μg/mL. *P. perfoliatum* extract showed the lowest H_2O_2 scavenging activity exhibiting an IC_{50} value of 376.75 ± 14.12 μg/mL. Hydrogen peroxide is a non-radical reactive oxygen species and is not a very reactive, but sometimes it is toxic to the cells in living organisms as it has the ability to penetrate cell membranes which may give rise to hydroxyl radicals and singlet oxygen, and thus initiation of oxidation takes place in the cells [31]. Therefore, neutralizing H_2O_2 by natural antioxidant sources is very essential for protection of biological or food systems. Food polyphenols have been shown to protect mammalian and bacterial cells from cytotoxicity induced by H_2O_2 particularly the compounds with the orthodihydroxy phenolic structure, catechin, quercetin, caffeic acid ester, and gallic acid ester [32].

FRAP assay is another method which is used to determine the antioxidant property of the plant extracts. It is also a simple, inexpensive and widely employed method for the evaluation of antioxidant activity and is based on the power of antioxidants to reduce ferric (III) ions to ferrous (II) ions [33]. Higher FRAP value indicates the stronger antioxidant capacity. The results of FRAP assay of the plants studied are presented in Table 5 and the values were calculated from the linear regression equation of standard trolox ($y = 0.0007x + 0.1272$; $r^2 = 0.9903$). The FRAP values of methanol extracts of the plants (Table 5) varied from 64.76 ± 7.43 to 799.28 ± 7.14 μM TE/g dry extract showing the strongest antioxidant activity in *L. javanica* extract, while *D. cordata* revealed the lowest activity. The high activity of the extract may be due to the presence of antioxidant compounds in the plants which could react with free radicals to stabilize and terminate radical chain reactions by donating an electron. However, lower levels of FRAP value was reported by Wong et al. [34] in some selected Malaysian wild edible plants. Generally, the values obtained in FRAP method indicate all the electron-donating reductants in the sample extracts [35].

Table 4
Hydrogen peroxide scavenging activity of methanolic extract of wild edible plants

Plant extract/ Standard	Concentration (μg/mL) and its inhibition (%)					IC_{50} value (μg/mL)
	5	10	15	20	25	
B. lanceolaria	2.99 ± 0.04^{a}	11.64 ± 0.04^{a}	23.46 ± 0.07^{a}	44.74 ± 0.07^{a}	73.52 ± 0.04^{a}	20.37 ± 0.01^{a}
T. angustifolium	7.18 ± 0.06^{b}	13.95 ± 0.08^{b}	14.89 ± 0.11^{b}	16.12 ± 0.06^{b}	18.48 ± 0.11^{b}	87.39 ± 0.67^{b}
O. javanica	3.42 ± 0.11^{c}	7.34 ± 0.08^{c}	15.12 ± 0.08^{b}	17.54 ± 0.09^{c}	18.88 ± 0.13^{b}	60.63 ± 0.52^{c}
D. cordata	0.88 ± 0.11^{d}	7.24 ± 0.13^{c}	8.04 ± 0.13^{c}	9.84 ± 0.06^{d}	15.09 ± 0.11^{c}	82.37 ± 0.23^{d}
C. sinensis	4.54 ± 0.16^{e}	6.52 ± 0.27^{d}	7.71 ± 0.07^{c}	8.68 ± 0.09^{e}	10.41 ± 0.09^{d}	167.65 ± 1.93^{e}
S. media	0.38 ± 0.07^{f}	1.32 ± 0.11^{e}	2.90 ± 0.11^{d}	8.03 ± 0.11^{f}	22.96 ± 0.11^{e}	56.32 ± 0.17^{f}
A. acidum	9.06 ± 0.12^{g}	10.60 ± 0.15^{f}	13.50 ± 0.12^{e}	14.48 ± 0.06^{g}	15.69 ± 0.13^{f}	123.83 ± 1.14^{g}
E. foetidum	4.83 ± 0.11^{e}	5.92 ± 0.04^{g}	6.27 ± 0.05^{f}	7.05 ± 0.02^{h}	8.61 ± 0.07^{g}	265.37 ± 9.85^{h}
L. javanica	3.93 ± 0.11^{c}	4.68 ± 0.07^{h}	5.10 ± 0.11^{g}	$7.39 \pm 0.11^{h,i}$	8.63 ± 0.09^{g}	196.91 ± 0.45^{i}
P. perfoliatum	6.11 ± 0.04^{h}	6.49 ± 0.07^{d}	7.02 ± 0.07^{h}	7.58 ± 0.07^{i}	8.53 ± 0.07^{g}	376.75 ± 14.12^{j}
E. fluctuans	6.00 ± 0.09^{h}	7.41 ± 0.06^{c}	7.59 ± 0.11^{c}	8.97 ± 0.04^{e}	9.51 ± 0.06^{h}	260.35 ± 7.62^{k}
Ascorbic acid	10.73 ± 0.02^{i}	27.91 ± 0.04^{i}	41.96 ± 0.07^{i}	51.42 ± 0.07^{j}	64.86 ± 0.07^{i}	19.02 ± 0.01^{l}

Results are expressed as mean of 3 replicates ± standard deviation. The values with different letters in a column are significantly different from each other at $p < 0.05$.

Table 5
Ferric reducing antioxidant power (FRAP) and phytochemical contents of wild edible plants

Plant extract	FRAP value (μM TE/g extract)	Total phenolic content (mg GAE/g dry extract)	Total flavonoid content (mg QE/g dry extract)
B. lanceolaria	308.80 ± 8.98^{a}	36.39 ± 2.96^{a}	1.01 ± 0.10^{a}
T. angustifolium	581.42 ± 10.71^{b}	54.08 ± 7.20^{b}	1.36 ± 1.03^{a}
O. javanica	98.09 ± 5.45^{c}	171.22 ± 8.90^{c}	0.47 ± 0.10^{b}
D. cordata	64.76 ± 7.43^{d}	29.71 ± 5.40^{d}	0.77 ± 1.03^{b}
C. sinensis	457.61 ± 7.43^{e}	26.96 ± 9.81^{e}	0.77 ± 0.10^{b}
S. media	406.42 ± 7.14^{f}	67.45 ± 7.07^{f}	$0.23 \pm 0.10^{b,c}$
A. acidum	423.09 ± 8.98^{g}	30.11 ± 2.96^{g}	1.19 ± 0.10^{a}
E. foetidum	127.85 ± 7.14^{h}	105.18 ± 3.11^{h}	1.30 ± 0.10^{a}
L. javanica	799.28 ± 7.14^{i}	91.43 ± 4.14^{i}	2.55 ± 0.10^{d}
P. perfoliatum	621.90 ± 7.43^{j}	265.95 ± 4.76^{j}	4.34 ± 1.03^{e}
E. fluctuans	156.42 ± 7.14^{k}	269.49 ± 2.96^{k}	$0.83 \pm 0.10^{a,b}$

Results are expressed as mean of 3 replicates ± standard deviation. The values with different letters in a column are significantly different from each other at $p < 0.05$.

3.3. Total phenolic and flavonoid contents

Total phenolic and flavonoid contents of the plants are presented in Table 5. The phenolic contents in the methanol extracts were determined through a linear curve of standard gallic acid ($y = 0.0212x + 0.3098$; $r^2 = 0.9971$) and flavonoid contents through a linear curve of standard quercetin ($y = 0.0014x + 0.0799$; $r^2 = 0.9859$). The TPC in the methanol extract of plants varied from 26.96 ± 9.81 to 269.49 ± 2.96 mg GAE/g dry extract. *E. fluctuans* extract showed the highest phenolic content (269.49 ± 2.96 mg GAE/g) followed by

Table 6
Pearson's correlation coefficients of antioxidant activity (DPPH, ABTS, H_2O_2, FRAP), TPC and TFC in eleven wild edible plants

	DPPH	ABTS	H_2O_2	FRAP	TPC	TFC
DPPH	1					
ABTS	0.82[a]	1				
H_2O_2	–0.48	–0.41	1			
FRAP	–0.76[a]	–0.74[a]	0.23	1		
TPC	–0.25	–0.27	0.69[b]	–0.07	1	
TFC	–0.54	–0.41	0.73[b]	0.62[b]	0.46	1

a, Correlation is significant at $p < 0.01$; b, Correlation is significant at $p < 0.05$.

P. perfoliatum (265.95 ± 4.76 mg GAE/g) and the lowest being in *C. sinensis* (26.96 ± 9.81 mg GAE/g). Higher amounts of phenolic contents were also found in *O. javanica* and *E. foetidum* which were 171.22 ± 8.90 and 105.18 ± 3.11 mg GAE/g dry extract, respectively. While the flavonoid content was found the lowest in *S. media* (0.23 ± 0.10 mg QE/g dry extract) and the highest being in *P. perfoliatum* (4.34 ± 1.03 mg QE/g dry extract). However, Xia et al. [36] reported higher phenolic content in six edible wild plants which was found ranging from 278.7 ± 24.4 to 417.3 ± 38.3 mg GAE/g dry weight. The phenolic contents of selected wild edible plants reported by Wong et al. [32] was found varying from 0.69 to 19.65 mg GAE/g dry weight and the flavonoid content from 0.19 ± 0.02 to 8.37 ± 2.62 mg catechin equivalent per gram of dry weight. Similarly, Ng et al. [10] also reported phenolic content of selected tropical wild vegetables that ranged from 1.8 to 4.1 mg GAE/g fresh weight and flavonoid content varied from 0.4 to 1.4 mg rutin equivalents/g fresh weight. Phenolic compounds are widely distributed in plants. Phenolic compounds such as phenolic acids, flavonoids, tocopherols *etc*. are natural antioxidants obtained from plants and they possess antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory properties [14, 34, 37, 38]. It was reported that the antioxidant property of phenolic compounds is due to their redox properties, hydrogen donating abilities, and singlet oxygen quenchers [14, 21, 29]. Higher amount of phenolic and flavonoid compounds corresponds to their stronger antioxidant capacity. Therefore, phenolics and flavonoids have many essential roles in decreasing the risk of various human diseases [7].

3.4. Correlation

Pearson's correlation study of antioxidant property of the plant extracts showed that there was a strong positive correlation significantly at $p < 0.01$ between DPPH and ABTS radical scavenging assays (Table 6) and this can be attributed to the fact that both methods are based on the similar reaction mechanism. This is in agreement with other study reported by Bunea et al. [39]. The present study also showed a positive correlation between FRAP assay and H_2O_2 assay. FRAP assay was positively correlated with TFC significantly at $p < 0.05$. Similar to this study, Ku et al. [40] also reported a positive correlation between FRAP assay and flavonoids. Positive correlations were also observed between TPC and TFC with antioxidant activity assayed by H_2O_2 radical scavenging assay. Several studies showed that the antioxidant capacity of plant material is very well-correlated with total phenolic compounds and the contribution of phenolic compounds to the overall antioxidant activity is mainly due to their redox properties involved in the plant materials [39, 41, 42]. In this investigation, a positive correlation was also seen between TPC and TFC which was in agreement with the study reported by Ku et al. [40]. It is well-known that phenolic and flavonoid compounds with certain structures particularly with the hydroxyl group in the molecule can act as proton donating and exhibit antioxidant property [42].

4. Conclusion

The study of eleven wild edible plants showed the presence of several important phytochemical constituents in the methanol extracts which are associated with various biological activities. The results of DPPH, ABTS, H_2O_2 and FRAP assays exhibited potent antioxidant properties. Both DPPH and FRAP methods showed the strongest antioxidant activity in the extract of *L. javanica*. ABTS and H_2O_2 assays indicated the highest antioxidant activities in *T. angustifolium* and *B. lanceolaria,* respectively. The TPC was found maximum in the extract of *E. fluctuans* and *P. perfoliatum* displayed the highest TFC. The evaluation of TPC and TFC established the food values of plants which are linked to free radical scavenging activities. A positive correlation of DPPH with ABTS, FRAP with TFC, H_2O_2 with FRAP, TPC and TFC, and TPC with TFC were also seen in this study. The antioxidant properties of the plants that revealed in this study indicate their role towards various oxidative stress related diseases and could be supportive in stopping or slowing the growth of various types of human diseases. These plants are good sources of natural antioxidants and would act as a food supplement.

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