CHAPTER IV

Study of Phytochemicals and Antioxidant Properties of Wild Edible Plants

Phytochemicals are the bioactive organic compounds which are naturally present in the plants. These bioactive compounds differ extensively in their structure and mechanisms of action, and possess many biological properties such as antioxidant, antimicrobial, anticancer and many other activities [1, 2]. In cellular metabolism, free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are abundantly produced due to aging and external influences like stresses, ionizing radicals, pollutions, and synthetic pesticides [3]. These radicals initiate the lipid and protein oxidations which result in cell structural damage, tissue injury or gene mutation leading to the development of various health disorders such as cancer, Alzheimer's disease, atherosclerosis, diabetes mellitus, hypertension, and ageing processes in the human body [4, 5]. The antioxidants compounds from plants slow down or delay or stop the oxidation processes in the cell by scavenging free radicals or chelating metal ions and thereby protect the human body from oxidative stress related diseases [6]. Antioxidant compounds obtained from plant sources have advantages as they are less toxic, more effective and low cost [7]. Polyphenol compounds are the large group of secondary metabolites which can be categorized into simple phenols, phenolic acids, flavonoids, lignin and tannins, and these are responsible for colour, bitterness, acerbic taste, odour, flavour, and several biological properties including antioxidants [8].

In this chapter, the methanol extracts of wild edible plants were screened for the presence of phytochemical constituents and evaluated for total phenolic content and total flavonoid content. Antioxidant activities of methanol extracts were investigated by DPPH, ABTS, H_2O_2 and FRAP methods, and the results are reported herein.

IV.1 Materials and Methods

IV.1.1 Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2´-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt [ABTS] and quercetin were purchased from Himedia

Laboratories Pvt. Ltd., Mumbai (India), Folin-Ciocalteu's reagents, hydrogen peroxide, ascorbic acid were obtained from Merck, Mumbai (India), gallic acid from Central Drug House Pvt. Ltd., New Delhi (India), trolox and 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) were obtained from Sigma Aldrich, Bangalore (India) and other chemicals were of analytical grade.

IV.1.2 Sample preparation

The powdered sample prepared as per the procedure mentioned in the **Section II.2.3** (**Page no. 73**) was extracted with methanol using 1:10 ratio (w/v) by stirring vigorously at normal temperature for 72 h. The mixture was filtered using Whatman No. 1 filter paper and evaporated using Buchi Rotavapor (R-215, Switzerland) at 40^{o}C. The dried extract was kept in the plastic container at 4^{o}C for analysis.

IV.1.3 Phytochemical screening

The dried methanol extracts of plant species were re-dissolved in methanol (1 mg/mL) for qualitative analysis of phytochemical constituents following the reported methods [9, 10].

IV.1.3.1 Test for alkaloids

To detect the alkaloids, two experiments were performed by adding two different reagents *viz*. Wagner's reagent and Dragondroff's reagent. First, a few drops of 1% HCl was added into 2 mL extract, warmed, and filtered followed by addition of 0.1 mL of Wagner's reagent. A brownish red precipitate indicates the presence of alkaloids. Secondly, 0.1 mL of Dragondroff's reagent was added in the same solution taken separately in another test tube. An orange precipitate indicates the presence of alkaloids.

IV.1.3.2 Test for saponins

Saponin also known as frothing agent was detected by mixing 1 mL of extract with 20 mL distilled water followed by shaking vigorously in a graduated cylinder for 15 min. Frothing persistence for a few seconds indicates the presence of saponins.

IV.1.3.3 Test for cardiac glycosides

Keller-Killiani's test is performed to detect the presence of cardiac glycosides in the plant extract. For this test, 2 mL of extract was added with 1 mL glacial acetic acid

followed by addition of a few drops $FeCl_3$ solution. After that 2 mL conc. H_2SO_4 was added very carefully. The formation of reddish brown with a bluish green upper layer indicates the presence of deoxy-sugar which indicated the presence of cardiac glycosides.

IV.1.3.4 Test for steroids

To detect the presence of steroids, Liebermann-Burchard reaction was performed by adding 2 mL extract, 1 mL acetic anhydride and 2 mL conc. H_2SO_4. The formation of reddish violet colour at the junction indicates the presence of steroids. Another method for the detection of steroids was also performed by mixing 1 mL of the extract with 10 mL of chloroform and equal volume of conc. H_2SO_4 was added slowly from the side of the test tube. The upper layer turns red and lower layer shows yellow with green fluorescence confirming the presence of steroids. This test is known as Salkowski test.

IV.1.3.5 Test for anthraquinones

To detect anthraquinones, Borntrager's test was performed by adding 3 mL petroleum ether to 5 mL of extract and it was vigorously shaken followed by the addition of 2 mL of ammonia solution (25%). Formation of red colour indicates the presence of anthraquinone.

IV.1.3.6 Test for coumarins

It was detected by adding 3 mL of NaOH (10%) in 2 mL of extract which formed yellow colour indicating the presence of coumarins.

IV.1.3.7 Test for phenols

To detect the presence of phenols, ellagic acid test was performed. The test solution was added with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The formation of muddy brown precipitate indicates the presence of phenols.

IV.1.3.8 Test for tannin

It was detected by mixing 5 mL of extract with 1 mL of $FeCl_3$ (5%) solution. Colouration of greenish black indicates the presence of tannins.

IV.1.3.9 Test for flavonoids

To detect the presence of flavonoids, Shinoda's test was performed by mixing 2 mL of extract with conc. HCl and magnesium ribbon. Pink-tomato red colour indicates the presence of flavonoids.

IV.1.3.10 Test for anthocyanins

It was detected by adding 2 mL of extract with 2 mL of HCl (2 N) and conc. NH_3. The colour change from pink-red to blue-violet indicates the presence of anthocyanins.

IV.1.3.11 Test for phlobatannins

It was detected by boiling 5 mL of extract with 2 mL of aqueous HCl (2%) and the formation of red precipitate indicates the presence of phlobatannins.

IV.1.3.12 Test for lignin

It was detected by adding 2 mL of 2 % (w/v) furfuraldehyde to the 5 mL test solution. Formation of red colour indicates the presence of lignin.

IV.1.3.13 Test for protein

The presence of proteins was detected by adding few drops of Ninhydrin reagent to 2 mL extract and the appearance of blue colour indicated the presence of amino acid. It was also confirmed by adding 2 mL Millon's reagent to the 2 mL crude extract. The formation of white precipitate which turned red upon gentle heating indicates the presence of proteins.

IV.1.3.14 Test for starch

It was detected by adding 2 mL of iodine solution to 3 mL of crude extract. A dark blue or purple coloration indicates the presence of the carbohydrate.

IV.1.4 Determination of antioxidant properties

IV.1.4.1 DPPH free radical scavenging activity

The free radical scavenging capacities of methanol extracts of the wild edible plants were studied following the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay [11]. The dried methanol extract was re-dissolved in methanol (1 mg/mL) and different concentrations (2, 5, 10, 50, 100, 200, 500 µg/mL) were prepared in separate test tubes to examine the scavenging capacities. After that, 3 mL of methanolic DPPH (0.1 mM) was added in

every test tube and the solution was mixed properly and allowed to incubate for 30 min in the dark at room temperature. After incubation, each solution was measured for absorbance at 517 nm using UV-VIS spectrophotometer (Perkin Elmer, Lambda 35). Similarly, the standard ascorbic acid (2, 5, 10, 50, 100, 200, 500 µg/mL) was also measured following same procedure. Methanol was taken as the blank solution and 1 mL methanol with 3 mL of methanolic DPPH solution was taken as the control. The inhibition (%) was calculated by the following equation.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The concentration (µg/mL) of the plant extract was plotted against the percentage inhibition and the IC_{50} was calculated from the linear regression equation obtained from the graph.

IV.1.4.2 ABTS radical scavenging activity

The free radical scavenging activity of methanol extracts was determined by using ABTS assay [12]. ABTS radical cation ($ABTS^{\cdot+}$) was created by adding 5 mL of both ABTS (7 mM) and potassium persulfate (2.45 mM) solutions and it was incubated in the dark at room temperature for 12–16 h. The ABTS radical solution was then diluted in 1:60 (v/v) with methanol to bring the initial absorbance to 0.70 ± 0.02 at 734 nm. After that, different concentrations (20 – 300 µg/mL) of sample and standard was prepared and 2 mL of working ABTS solution was added in each set and mixed thoroughly. After 6 min, the absorbance of solution was measured in 734 nm using UV-Vis Spectrophotometer. A graph of percentage inhibition was plotted against concentration of the extract. Similarly, experiments were also done for standard trolox using the same concentrations. The methanol (1 mL) and working ABTS solution (2 mL) were taken as a control and the methanol was taken as a blank solution. The IC_{50} value was calculated from the linear regression equation obtained from the graph of percentage inhibition and the results were expressed in µg/mL of dry extract. The inhibition percentage was calculated by the following equation.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Here, control is the solution of the ABTS radical cation without the test sample.

IV.1.4.3 H_2O_2 scavenging activity

The non-radical H_2O_2 scavenging capacities of the methanol extracts were examined at 230 nm spectrophotometrically [13]. For the experiment, 20 mM H_2O_2 was prepared by diluting 227 µL of 30% H_2O_2 in 99.773 mL phosphate buffer saline (PBS) of pH 7.4. After that, different concentrations of sample and standard were prepared (5 - 25 µg/mL) and then 2 mL of working solution of H_2O_2 was added in each test solution and the absorbance was measured at 230 nm after 10 min of reaction time using UV-Vis Spectrophotometer. Phosphate buffer saline was used as blank solution and ascorbic acid as the standard. The IC_{50} was calculated from the linear regression equation obtained from the graph. The % inhibition was calculated by the following equation.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Here control is the working solution of H_2O_2 without the test sample.

IV.1.4.4 Ferric reducing antioxidant power (FRAP) assay

The FRAP value was determined by following the method of Benzie *et al.* [14]. The FRAP reagent contains three different solutions *viz.* 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in HCl (40 mM) and $FeCl_3.6H_2O$ (20 mM). FRAP reagent was prepared by addition of 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3.6H_2O$ solution. To obtain the calibration curve, different concentration of standard trolox (25 – 1000 µM) was prepared. After that, 4 mL of FRAP reagent was added in all the solutions and incubated for 30 min in the dark and the absorbance was measured at 593 nm using UV-Vis spectrophotometer. For the sample, 40 µL of extract was allowed to react with 4 mL of FRAP solution and after 30 min incubation in the dark the absorbance was measured. The FRAP value was determined from the linear regression equation obtained from the graph. The data were expressed in µM trolox equivalent (TE)/g of dry extract.

IV.1.5 Determination vitamin C content

The vitamin C content was determined by titrating against 2, 6-dichlorophenol indophenol (0.02 M) [15]. Briefly, 2 g of the fresh plant sample was grounded in 10 mL oxalic acid (4%) and the volume was adjusted to 100 mL with oxalic acid (4%)

followed by filtrating with Whatman No. 1 filter paper. The filtrate was used for determination of vitamin C content. Pure ascorbic acid was taken as a standard by dissolving (1 mg/mL) in 4% oxalic acid and it was diluted to 100 µg/mL. Then 5 mL of standard ascorbic acid or sample solution was taken in 100 mL conical flask followed by addition of 10 mL of oxalic acid (4%) and it was then titrated against the standard dye solution which gives a pink colour at the end point that persists only a few seconds.

$$\text{Vitamin C (mg/100 g)} = \frac{0.5\ \text{mg} \times V_2 \times 100\ \text{mL}}{V_1 \times 5\ \text{mL} \times \text{Weight of test sample}} \times 100$$

Where, V_1 = mL of dye required to neutralize the standard ascorbic acid.

V_2 = mL of dye required to neutralize the sample.

0.5 mg = Concentration of standard ascorbic acid present in 5 mL.

IV.1.6 Evaluation of total phenolic content (TPC)

The TPC was determined spectrophotometrically by using Folin-Ciocalteu's reagent and standard gallic acid [11]. Standard gallic acid of different concentration (10, 20, 40, 60, 80 and 100 µg/mL) was prepared in methanol and 2.5 mL of Folin-Ciocalteu's reagent (10 %) was added in each solution. After that it was allowed to react for 5 min followed by the addition of 2 mL of Na_2CO_3 (7.5 %) solution and incubated in the dark for 30 min. The absorbance was taken at 765 nm using UV-VIS spectrophotometer and from the standard calibration graph a linear regression equation was obtained. Then 40 µg/mL of methanol extract was added with all the reagents used in standard and the absorbance was read at 765 nm. The reagent blank was prepared by adding 2.5 mL of Folin-Ciocalteu's reagent (10 %), 1 mL methanol and 2 mL of Na_2CO_3 (7.5 %) solution. The TPC content was calculated from the linear regression equation obtained from the standard graph and the results were presented in milligrams of gallic acid equivalents (GAE) per gram dry extract (mg GAE/g dry extract).

IV.1.7 Evaluation of total flavonoid content (TFC)

The TFC content was determined by using quercetin as the standard following the reported method [16]. Different concentrations (10, 20, 40, 60, 80, 100 µg/mL) of standard quercetin were prepared. To these, 0.5 mL of 5 % (w/v) $NaNO_2$ solution and 0.5 mL of 10 % (w/v) $AlCl_3$ solution were added. After 5 min reaction time, 2 mL of NaOH (4%) solution was added to stop the reaction and incubated at room temperature

for 15 min. The absorbance was read at 510 nm using UV-VIS spectrophotometer and from the standard calibration graph a linear regression equation was obtained. Then, 40 µL of methanol extract was taken and all the reagents used in standard were added and absorbance was read at 510 nm. Blank solution was prepared by adding all the reagents except sample or standard. From the calibration curve of standard, TFC was calculated and expressed in milligram of quercetin equivalents (QE) per gram of dry extract (mg QE/g dry extract).

IV.1.8 Statistical analysis

All the experiments were carried out for three independent replicates and the data were represented in terms of mean ± standard deviation. OriginPro 8.5 software (MA 01060, OriginLab Corporation, USA) was used for statistical analysis and executed by the one-way ANOVA *t*-test at $p < 0.05$.

IV.2 Results and Discussion

IV.2.1 Phytochemical Screening

The results of qualitative phytochemical analysis in methanolic extracts of plants were summarized in **Table IV.1**. The study indicated the presence of numerous biologically active compounds which are considered to have several medicinal properties such as anthelmintic, antioxidant, antimicrobial, and other biological properties. Phytochemicals are the bioactive organic compounds which are naturally present in the plants and they differ widely in their structure, mode of action and biological activities [1, 2]. It has been reported that most of the secondary metabolites can be extracted in the polar solvents which can then be isolated and identified [17]. The preliminary phytochemical analysis in the methanolic extracts of plants indicated the presence of biologically important compounds and these were phenolics, flavonoids, tannins, anthocyanins, alkaloid, saponin, cardiac glycoside, steroids, anthraquinone, coumarin, phlobatannins, lignin, proteins and starch (**Table IV.1**). In this study, two different reagents *viz*. Wagner's and Dragondroff's reagents were used for the detection of alkaloids and it was observed that both the reagents indicted the presence of alkaloids in the methanolic extracts of all plants. Alkaloids are the secondary metabolites and they are generally found in almost all the plants with varying composition which have different physiological functions. It has been reported that alkaloids possess painkiller, anti-malarial and antimicrobial properties [18, 19].

Table IV.1: Phytochemical screening of methanol extracts of wild edible plants

Phytochemical Constituents	***S. zeylanica***	***C. hirsuta***	***N. herpeticum***	***B. lanceolaria***	***S. peguensis***	***T. angustifolium***	***O. javanica***	***M. perpusilla***	***D. cordata***	***C. sinensis***	***S. media***	***P. chinensis***	***A. acidum***	***E. foetidum***	***L. javanica***	***P. perfoliatum***	***E. fluctuans***
Alkaloids (Wagner reagent)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids (Dragondroff's reagent)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Steroids (Liebermann test)	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Steroids (Salkowski test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	+	-	-	+	-	-	+	-	-	+	+	+	-	+	+	+
Coumarin	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanin	-	-	+	+	-	+	-	+	-	+	+	+	+	-	+	+	-
Phlobatannins	-	-	+	-	-	+	+	-	-	+	+	+	+	-	-	+	-
Lignin	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-
Protein (Ninhydrin test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Protein (Millon's test)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

Positive (+) indicates present, Negative (–) indicates absent.

In this study, saponins, steroids (Salkowski test), phenols, and flavonoids were also detected in the methanolic extracts of all the plant species **(Table IV.1)**. Saponins have bitterness taste and have properties like haemolytic, precipitating and coagulating of RBC, foaming and cholesterol binding properties [18, 20]. Saponins are poisonous at high doses and are known to cause cattle poisoning [21]. However, they also have beneficial pharmacological properties including anti-parasitic, anti-inflammatory, and anti-viral properties [22]. The phytosterols or steroids in the plants have important pharmacological properties due to their relationship with sex hormones and cholesterol since these compounds act as the precursor for synthesizing hormones [23]. It is known that the phenolic compounds have numerous biological roles for human health such as protection from apoptosis, fast aging process, cardiovascular diseases, inflammation, atherosclerosis, and angiogenesis [24, 26, 27]. Cardiac glycosides were detected in all the plant species except in the methanol extract of *O. javanica* (**Table IV.1)**. Glycosides are naturally cardio-active drugs which are used for the treatment of congestive heart problem [28]. Glycosides are reported for antifungal activity for example treatment of skin diseases [29, 30], cardiac arrhythmia, [26], and anti-diabetic properties and also they regulate Na^+/K^+ pump in the cell [31]. However, the anthraquinone was detected only in nine plant species (**Table IV.1)**. The coumarin was not detected in four species *viz. B. Lanceolaria, T. angustifolium, C. sinensis* and in *S. media* (**Table IV.1)**. Tannins were not detected in three species *viz. S. zeylanica, S. media* and *L. javanica* (**Table IV.1)**. Tannins have antioxidant, antimicrobial and cytotoxic activities [32, 33] and they have the ability to inhibit HIV replication selectively [34]. In our study, phlobatannins were detected in *N. herpeticum, T. angustifolium, O. javanica, C. sinensis, S. media, P, chinensis, A. acidum* and *P. perfoliatum.* Phlobatannins have been reported to have analgesic, anti-inflammatory and wound healing properties [35]. In present study, protein was detected in all the plant samples by using both Ninhydrin and Millon's reagents. It has been reported that the plant proteins (polypeptides and lectins) show antimicrobial properties [36]. Proteins in the plants are the huge group of macromolecules which act as antimicrobial agents and plants can protect themselves against some microbial pathogens by producing several antimicrobial proteins [37]. Lignin was not detected in *S. zeylanica, D. cordata, A. acidum, E. foetidum* and *E. fluctuans*. The starch was not detected in all the plant species extract except *P. chinensis* (**Table IV.1**). From the results (**Table IV.1**), it has been observed that almost all the phytochemicals studied are extractable with methanol.

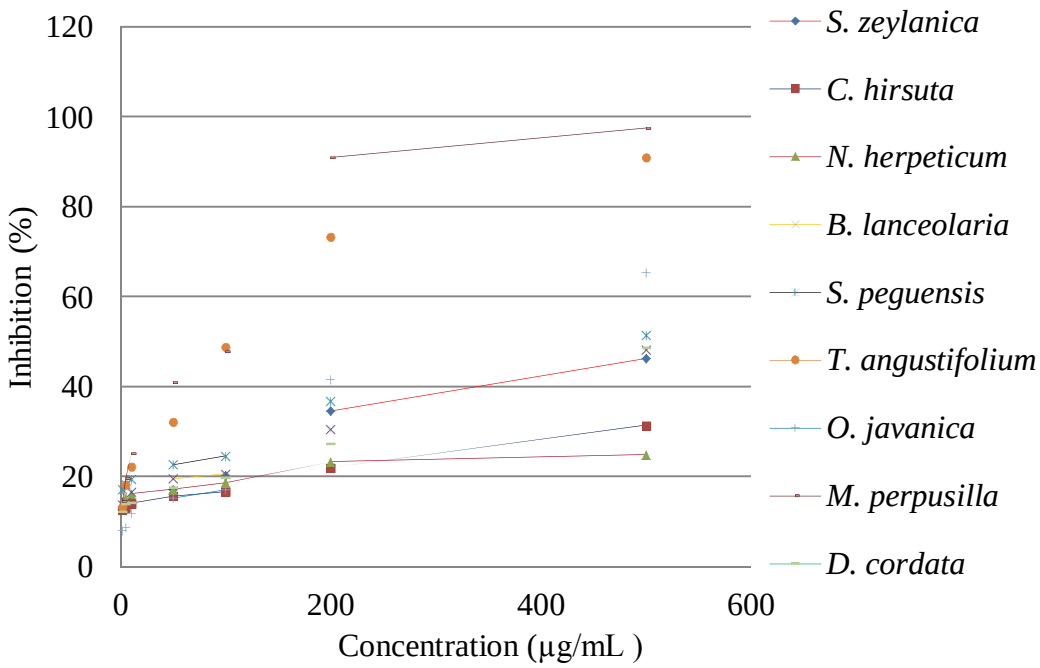

Fig.IV.1a: Plot of inhibition (%) of DPPH assay against concentration of sample extract.

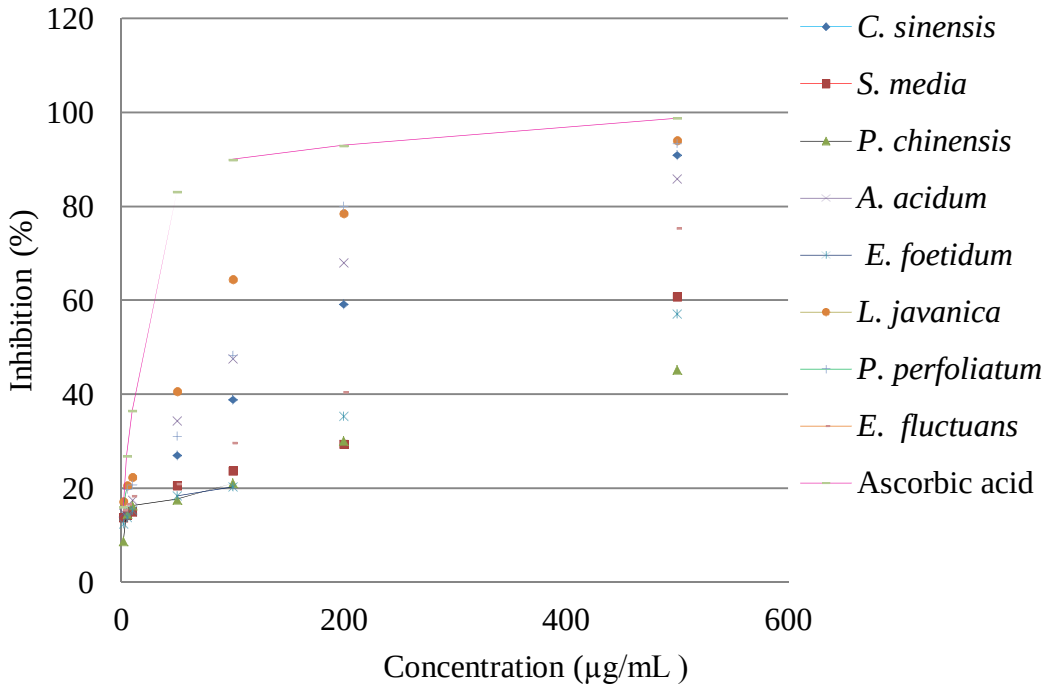

Fig.IV.1b: Plot of inhibition (%) of DPPH assay against concentration of sample extract.

Table IV.2: Linear equations of different samples of DPPH assay for IC_{50} calculation

Plants	**Linear equations** **Y = mx + C**	**R^2 values**
S. zeylanica	Y = 0.0686x + 14.311	R^2 = 0.9445
C. hirsuta	Y = 0.037x + 13.368	R^2 = 0.9896
N. herpeticum	Y = 0.0205x + 15.997	R^2 = 0.8381
B. lanceolaria	Y = 0.0679x + 14.918	R^2 = 0.9876
S. peguensis	Y = 0.0687x + 18.682	R^2 = 0.9722
T. angustifolium	Y = 0.1532x + 23.758	R^2 = 0.8663
O. javanica	Y = 0.1171x + 9.4916	R^2 = 0.9618
M. perpusilla	Y = 0.1662x + 27.558	R^2 = 0.7946
D. cordata	Y = 0.0709x + 13.265	R^2 = 0.9975
C. sinensis	Y = 0.1562x + 17.865	R^2 = 0.9591
S. media	Y = 0.092x + 14.032	R^2 = 0.9916
P. chinensis	Y = 0.0657x + 13.834	R^2 = 0.9510
A. acidum	Y = 0.1447x + 22.548	R^2 = 0.8603
E. foetidum	Y = 0.0893x + 13.588	R^2 = 0.9850
L. javanica	Y = 0.1524x + 29.423	R^2 = 0.7890
P. perfoliatum	Y = 0.1593x + 24.484	R^2 = 0.8497
E. fluctuans	Y = 0.119x + 16.279	R^2 = 0.9978
Ascorbic acid	Y = 0.1366x + 46.577	R^2 = 0.4835

Table IV.3: DPPH free radical scavenging activities in methanol extracts of plants

Plants	Concentration (µg/mL) and its inhibition (%)							IC_{50}
	2	5	10	50	100	200	500	(µg/mL)
Sz	12.60 ±0.28^{a}	13.82 ±0.05^{a}	14.91 ±0.14^{a}	17.19 ±0.35^{a}	20.31 ±0.09^{a}	34.61 ±0.42^{a}	46.25 ±0.33^{a}	519.90 ±2.88^{a}
Ch	12.73 ±0.09^{a}	13.20 ±0.09^{b}	14.04 ±0.09^{b}	15.63 ±0.09^{b}	16.63 ±0.10^{b}	22.00 ±0.18^{b}	31.42 ±0.19^{b}	989.98 ±6.07^{b}
Nh	14.48 ±0.07^{b}	15.14 ±0.07^{c}	16.15 ±0.04^{c}	17.18 ±0.11^{a}	18.67 ±0.51^{c}	23.28 ±0.22^{c}	24.85 ±0.07^{c}	1658.47 ±2.72^{c}
Bl	13.70 ±0.04^{c}	14.24 ±0.11^{d}	16.56 ±0.11^{c}	19.50 ±0.18^{c}	20.58 ±0.14^{a}	30.56 ±0.22^{d}	48.13 ±0.15^{d}	516.34 ±2.52^{d}
Sp	17.18 ±0.07^{d}	18.28 ±0.34^{e}	19.47 ±0.41^{d}	22.61 ±0.12^{d}	24.54 ±0.15^{d}	36.81 ±0.15^{e}	51.43 ±0.16^{e}	455.76 ±0.87^{e}
Ta	13.32 ±0.16^{c}	18.23 ±0.24^{e}	22.24 ±0.15^{e}	32.25 ±0.19^{e}	48.87 ±0.19^{e}	73.31 ±0.23^{f}	90.94 ±0.12^{f}	171.21 ±0.57^{f}
Oj	7.99 ±0.15^{e}	8.78 ±0.15^{f}	11.85 ±0.16^{f}	15.25 ±0.19^{b}	17.07 ±0.16^{f}	41.64 ±0.15^{g}	65.39 ±0.15^{g}	345.80 ±1.07^{g}
Mp	14.59 ±0.12^{b}	19.71 ±0.23^{g}	25.17 ±0.15^{g}	41.09 ±0.28^{f}	47.92 ±0.19^{g}	91.00 ±0.27^{h}	97.54 ±0.15^{h}	134.96 ±0.35^{h}
Dc	12.29 ±0.25^{a}	13.93 ±0.07^{a}	14.27 ±0.12^{b}	17.76 ±0.12^{g}	19.97 ±0.16^{a}	27.39 ±0.15^{i}	48.71 ±0.19^{i}	516.04 ±2.50^{i}
Cs	13.88 ±0.19^{c}	15.20 ±0.15^{c}	15.54 ±0.19^{a}	26.97 ±0.24^{h}	38.87 ±0.15^{h}	59.17 ±0.19^{j}	90.89 ±0.15^{j}	205.62 ±0.99^{j}
Sm	13.77 ±0.18^{c}	14.29 ±0.13^{d}	15.07 ±0.18^{a}	20.66 ±0.18^{i}	23.79 ±0.13^{i}	29.51 ±0.18^{k}	60.86 ±0.22^{k}	391.04 ±1.11^{k}
Pc	8.75 ±0.09^{f}	14.65 ±0.13^{d}	16.33 ±0.09^{c}	17.62 ±0.27^{g}	21.14 ±0.22^{j}	30.08 ±0.31^{d}	45.21 ±0.27^{l}	550.68 ±2.86^{l}
Aa	14.71 ±0.18^{b}	15.19 ±0.13^{c}	17.41 ±0.18^{h}	34.35 ±0.18^{j}	47.62 ±0.22^{g}	68.08 ±0.22^{l}	85.92 ±0.18^{m}	189.67 ±0.22^{m}
Efo	12.36 ±0.18^{a}	13.73 ±0.09^{a}	15.34 ±0.18^{a}	18.41 ±0.27^{k}	20.33 ±0.22^{a}	35.28 ±0.18^{m}	57.12 ±0.18^{n}	407.54 ±0.65^{n}
Lj	17.21 ±0.17^{d}	20.63 ±0.21^{h}	22.34 ±0.26^{e}	40.63 ±0.21^{l}	64.55 ±0.30^{k}	78.60 ±0.17^{n}	94.11 ±0.21^{p}	135.00 ±1.49^{p}
Pp	16.08 ±0.17^{g}	19.82 ±0.15^{g}	20.78 ±0.17^{i}	31.07 ±0.13^{m}	48.26 ±0.17^{g}	80.08 ±0.17^{p}	93.39 ±0.17^{q}	160.14 ±0.39^{q}
Ef	15.44 ±0.27^{h}	16.69 ±0.17^{i}	18.37 ±0.13^{j}	21.01 ±0.21^{n}	29.71 ±0.13^{l}	40.49 ±0.10^{q}	75.42 ±0.21^{r}	283.40 ±1.15^{r}
AA	15.94 ±0.14^{i}	26.93 ±0.19^{j}	36.57 ±0.28^{k}	83.11 ±0.23^{p}	90.04 ±0.23^{m}	93.03 ±0.47^{r}	98.84 ±0.10^{s}	25.01 ±0.52^{s}

Sz = S. zeylanica, Ch = C. hirsuta, Nh = N. herpeticum, Bl = B. lanceolaria, Sp = S. peguensis, Ta = T. angustifolium, Oj = O. javanica, Mp = M. perpusilla, Dc = D. cordata, Cs = C. sinensis, Sm = S. media, Pc = P. chinensis, Aa = A. acidum, Efo = E. foetidum, Lj = L. javanica, Pp = P. perfoliatum and Ef = E. fluctuans, AA = Ascorbic acid, Values were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.2 Antioxidant properties

In this study, DPPH, ABTS, H_2O_2 and FRAP methods were adopted to determine the *in vitro* antioxidant properties of methanol extracts of wild edible plants.

IV.2.2.1 DPPH free radical scavenging activity

DPPH assay is one of the most sensitive and very simple methods for evaluation of *in-vitro* antioxidant activities in which 1, 1-diphenyl-2-picrylhydrazyl radical is converted to a stable molecule (1, 1-diphenyl-2-picrylhydrazine) by accepting a hydrogen radical or an electron from the test sample and this is measured at 517 nm [38; 39]. The antioxidant capacity can be measured on the basis of the IC_{50} value of sample which is the inhibitory concentration of test sample that could inhibit or scavenge the oxidation of free radicals by 50%. It is inversely proportional to the antioxidant activity *i.e.* lower the value of IC_{50} of a sample, higher is the antioxidant potential [40].

In this study, the DPPH assay percentage inhibition of plant extracts against concentration was plotted as shown in the **Fig.IV.1a** and **Fig.IV.1b** and the linear regression equations shown in the **Table IV.2** were obtained from the graphs for IC_{50} value calculation. The DPPH free radical scavenging activities in methanol extracts of plants along with IC_{50} values are shown in the **Table IV.3**. In this investigation, ascorbic acid was taken as a reference antioxidant sample. The methanol extracts of all the plants samples exhibited increasing DPPH free radical scavenging activities with increasing the concentration of extracts in each plant species (**Table IV. 3**). Similarly, Ng *et al.* [41] also reported that the plant extracts showed increasing capability of trapping DPPH free radicals with increasing concentration of plant extracts. In this study (**Table IV.3**), the standard ascorbic acid displayed 98.84 ± 0.10% inhibition at the concentration 500 µg/mL and showed an IC_{50} value of 25.01 ± 0.52 µg/mL. In comparison to the reference ascorbic acid, *M. perpusilla* and *L. javanica* extracts showed the highest DPPH radical scavenging activities with IC_{50} value of 134.96 ± 0.35 µg/mL and 135.0 ± 1.49 µg/mL, respectively followed by *P. perfoliatum* (IC_{50} 160.14 ± 0.39 µg/mL), *T. angustifolium* (IC_{50} 171.21 ± 0.57 µg/mL), *A. acidum* (IC_{50} 189.67 ± 0.22 µg/mL), *C. sinensis* (IC_{50} 205.62 ± 0.99 µg/mL), and *E. fluctuans* (IC_{50} 283.40 ± 1.15 µg/mL). The lowest antioxidant capacity was exhibited by *N. herpeticum* with IC_{50} values (1658.47 ± 2.72 µg/mL) followed by *C. hirsuta* (IC_{50} 989.98 ± 2.88 µg/mL.

These results are similar to the free radical scavenging activity of some leafy vegetables like *Centella asiatica, Chenopodium album, Paederia foetida* etc. with IC_{50} ranging from 61.5 µg/mL to 1946 µg/mL reported by Dasgupta *et al.* Moreover, they also have reported the lower IC_{50} value of 95.5 µg/mL in *E. fluctuans* in comparison to the present study [42]. Similarly, Awah *et al.* [43] showed more scavenging power in comparison to the current study in some Nigerian medicinal plants with IC_{50} value of DPPH assay ranging from 8.8 ± 0.2 to 24.1 ± 2.0 µg/mL. Similar work has been done in edible plants of Oman by Ruchi *et al.* [44] and they reported the lowest DPPH IC_{50} value (4.45 µg/mL) in the ethanol extract of *Anogeissus dhofarica* and highest in *Acalypha indica* (37.9 ± 0.1 µg/mL) which indicated greater antioxidant power compared to this study. The IC_{50} values of the present study was also comparable with the results of some wild edible fruits of Manipur, where they reported IC_{50} values from 181.21 ± 2.0 µg/mL in *Phyllanthus emblica* to 2717.46 ± 363.6 µg/mL in *Hodgsonia macrocarpa* [45]. Moreover, the antioxidant capacity of water and ethanol extract of some spices also showed more antioxidant capacity than the present study with IC_{50} value ranging from 5.7 ± 0.4 to 48.0 ± 0.1 µg/mL in water extract and 12.0 ± 0.1 to 65.2 ± 0.1 µg/mL in ethanol extract [46]. Similarly, in methanol extract of wild edible plants *Allium ampeloprasum* showed IC_{50} value 15120 ± 12.1 µg/mL which indicates very less antioxidant in comparison to our highest IC_{50} (1658.47 ± 2.72 µg/mL) in *N. herpeticum* [47]. Satpathy *et al.* [48] also reported close IC_{50} value (590 ± 0.13 to 730 ± 0.05 µg/mL) in methanol extract of edible fruits of raw *Spondias pinnata*. Similarly in traditional wild edible plants such as *Asparagus acutifolius, Bryonia dioca* and *Tamus communis* of Peninsula showed IC_{50} values 423 ± 24 µg/mL 640 ± 49 µg/mL and 203 ± 30 µg/mL respectively, which are also comparable with *S. peguensis* (IC_{50} 455.76 ± 0.87 µg/mL), *P. chinensis* (550.68 ± 2.86 µg/mL) and *C. sinensis* (205.62 ± 0.99 µg/mL) of this study [49]. Islary *et al.* [50] also assessed the antioxidant potential of two wild fruits of Assam in which the IC_{50} value of DPPH assay was reported as 168.001 ± 2.645 µg/mL and 364.33 ± 5.507 µg/mL, respectively in methanol extracts of *Aporosa dioica* and *Ottelia alismoides* which are also similar to the findings of the present study.

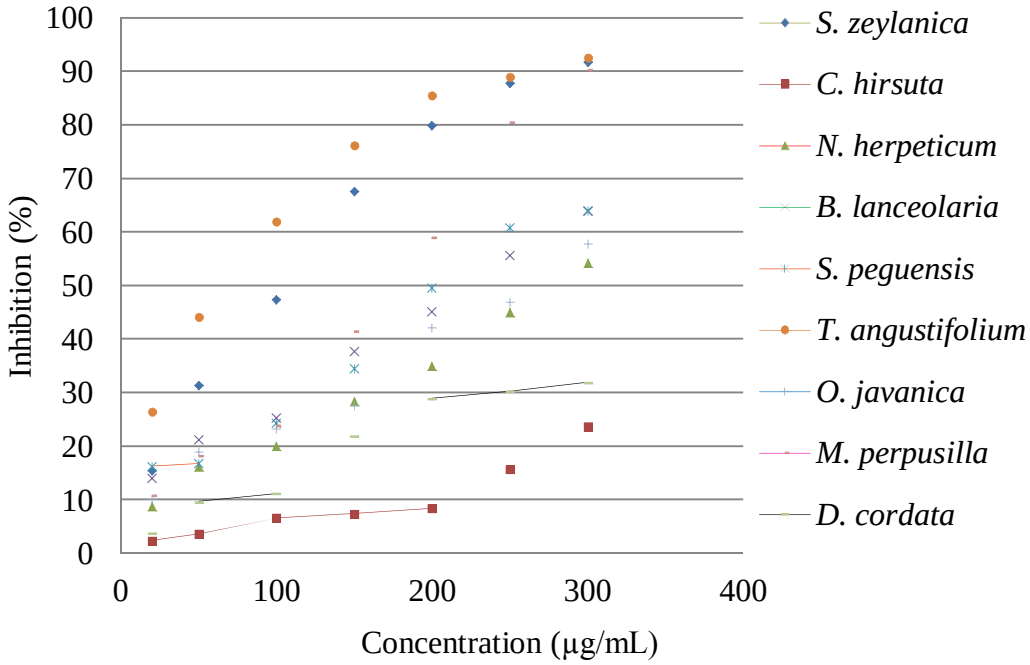

Fig.IV.2a: Plot of inhibition (%) of ABTS assay against concentration of sample extract.

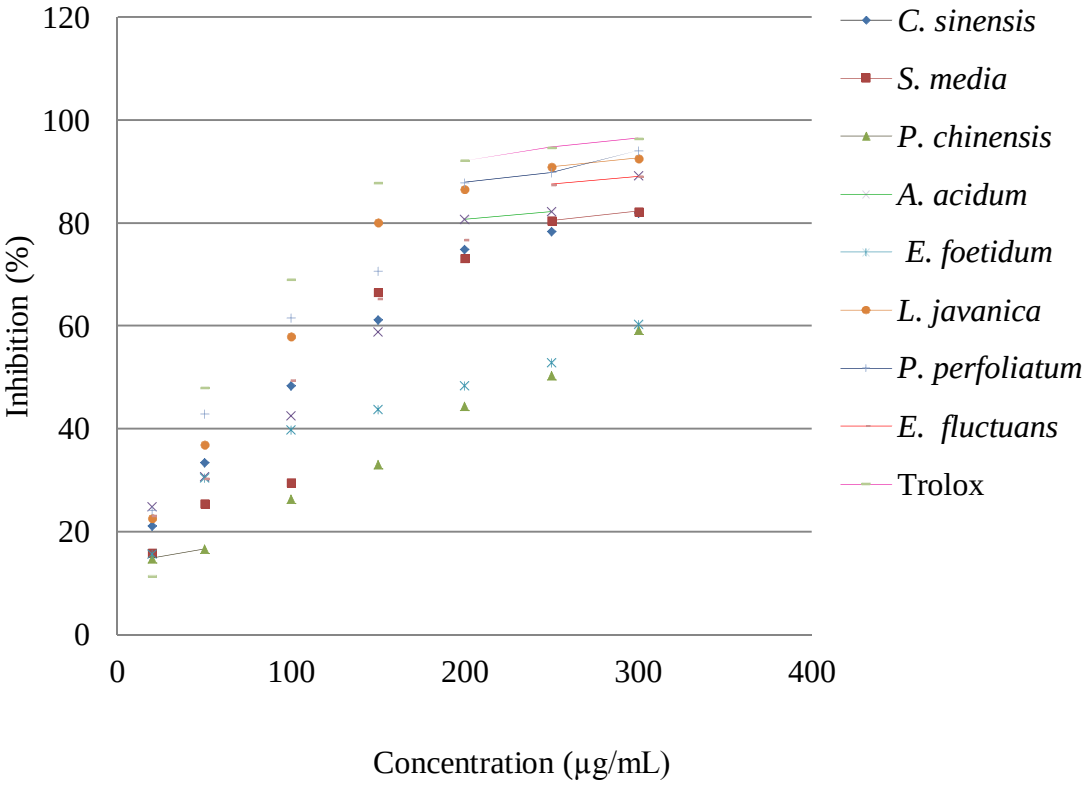

Fig.IV.2b. Plot of inhibition (%) of ABTS assay against concentration of sample extract.

Table IV.4: Linear equations of different samples of ABTS assay for IC_{50} calculation

Plants	Linear equations Y= mx + C	R^2 values
S. zeylanica	Y = 0.2768x + 17.886	R^2 = 0.9491
C. hirsuta	Y = 0.0679x - 0.68970	R^2 = 0.8678
N. herpeticum	Y = 0.1561x + 5.7934	R^2 = 0.9898
B. lanceolaria	Y = 0.1778x + 10.392	R^2 = 0.9932
S. peguensis	Y = 0.1927x + 8.5829	R^2 = 0.973
T. angustifolium	Y = 0.2292x + 32.963	R^2 = 0.9019
O. javanica	Y = 0.1625x + 7.5508	R^2 = 0.9778
M. perpusilla	Y = 0.2984x + 0.7098	R^2 = 0.9805
D. cordata	Y = 0.1065x + 3.3794	R^2 = 0.9377
C. sinensis	Y = 0.2211x + 23.293	R^2 = 0.9432
S. media	Y = 0.2623x + 13.286	R^2 = 0.9077
P. chinensis	Y = 0.1639x + 9.9320	R^2 = 0.9942
A. acidum	Y = 0.2493x + 20.343	R^2 = 0.9616
E. foetidum	Y = 0.1377x + 20.568	R^2 = 0.9162
L. javanica	Y = 0.2555x + 27.765	R^2 = 0.8871
P. perfoliatum	Y = 0.243x + 30.1410	R^2 = 0.9155
E. fluctuans	Y = 0.2521x + 21.697	R^2 = 0.9607
Trolox	Y = 0.2703x + 30.085	R^2 = 0.7794

IV.2.2.2 ABTS free radical scavenging activity

In this study, the percentage inhibition of plant extracts against the concentration was plotted which are shown in the **Fig.IV.2a** and **Fig.IV.2b** and the linear regression equations shown in the **Table IV.4** were obtained from the graphs for IC_{50} value calculation. The ABTS radical scavenging activities in methanol extracts of plants along with IC_{50} values are shown in the **Table IV.5**. In this study, a concentration dependent scavenging activity was observed in each sample extract. The highest antioxidant activity was found in the extract of *T. angustifolium* with IC_{50} value of 74.3 ± 0.29 µg/mL followed by *P. perfoliatum* with IC_{50} value of 81.67 ± 0.28 µg/mL and *L. javanica* with IC_{50} value 86.99 ± 0.27 µg/mL. The ABTS IC_{50} values found in *E.*

fluctuans, S. zeylanica, C. chinensis and *M. perpusilla* were 112.23 ± 0.14, 115.99 ± 0.12, 120.8 ± 0.55 and 165.18 ± 0.25 µg/mL, respectively. However, higher IC_{50} values were observed in *C. hirsuta* and *D. cordata* (**Table IV.5**) showing the lowest antioxidant activity. Trolox was taken as a standard in this study and showed an IC_{50} value of 73.67 ± 0.74 µg/mL. This study revealed that the methanol extract of *T. angustifolium* (IC_{50} 74.3 ± 0.29 µg/mL) showed almost similar antioxidant activity to the standard trolox (73.67 ± 0.74 µg/mL) and therefore, *T. angustifolium* is considered to have powerful antioxidant property. The IC_{50} values of the present study showed higher in comparison to the methanol and dichloromethane extract of medicinal plant *Abies pindrow* with IC_{50} value 8.00 ± 0.001 µg/mL and 16.00 ± 0.001 µg/mL respectively, which showed stronger antioxidant activity. However, the acetone extract of this plant showed IC_{50} value 99.00 ± 0.004 µg/mL, which is comparable with our results [51]. Similarly in methanol extract of medicinal plant *Curcuma alismatifolia* leaves showed IC_{50} value ranging from 225.2 ± 1.25 to 288.1 ± 1.35 µg/mL in ABTS assay and these results were also in accordance with the present study [52]. On the other hand, our results are higher free radical scavenging activity in comparison to the results reported in methanol extract of some medicinal plants like *Aizoon canariense, Asphodelus tenuifolius* and *Emex spinosus,* where IC_{50} value ranged from 5.79 ± 0.54 to 19.78 ± 0.01 mg/mL collected from different sites [53]. Kim *et al.* [54] reported the ABTS radical scavenging activity in some green leafy vegetables which ranged 4.34 ± 0.04 to 63.53 ± 2.70 mg TE/g DW. The antioxidant capacity of methanol and *n*-hexane extract of phytococktail of different plants was evaluated by Dhar *et al.* [55] and reported IC_{50} values as 181.98 µg/mL and 183.373 µg/mL, respectively which are comparable to current study. Wojdylo *et al.* [56] also reported the ABTS IC_{50} value in 32 selected edible herbs which ranged from 0.45 ± 0.01 to 346 ± 5.34 µM trolox/100 g DW. Similarly, Wong *et al.* [57] also evaluated the antioxidant capacity of methanol extract of Malaysian wild edible plants with ABTS assay and reported the antioxidant activity with IC_{50} values ranging from 5.81 ± 1.85 to 100.79 ± 11.75 µmol TE/g DW in methanol extract which also support our study. However, in hot water extract, it ranged from 34.81 ± 5.85 to 197.26 ± 14.69 µmol TE/g DW. It was reported that the polyphenols with high molecular mass exhibit more ABTS radical scavenging capacities and their effectiveness depends on the nature of -OH groups, number of aromatic rings, and molecular masses [58, 59].

Table IV.5: ABTS free radical scavenging activities in methanol extracts of plants

Plants	Concentration (µg/mL) and its inhibition (%)							IC_{50}
	20	50	100	150	200	250	300	µg/mL
Sz	15.46 ±0.14^{a}	31.40 ±0.08^{a}	47.44 ±0.08^{a}	67.63 ±0.14^{a}	79.91 ±0.14^{a}	87.86 ±0.14^{a}	91.71 ±0.08^{a}	115.99 ±0.12^{a}
Ch	2.33 ±0.09^{b}	3.60 ±0.18^{b}	6.62 ±0.23^{b}	7.36 ± 0.24^{b}	8.42 ±0.15^{b}	15.73 ±0.15^{b}	23.74 ±0.18^{b}	746.46 ±1.90^{b}
Nh	8.74 ±0.14^{c}	16.19 ±0.22^{c}	20.00 ±0.14^{c}	28.34 ±0.08^{c}	35.01 ±0.22^{c}	44.98 ±0.23^{c}	54.27 ±0.22^{c}	283.23 ±0.49^{c}
Bl	14.07 ±0.57^{d}	21.25 ±0.28^{d}	25.29 ±0.35^{d}	37.70 ±0.21^{d}	45.12 ±0.21^{d}	55.63 ±0.28^{d}	63.95 ±1.00^{d}	222.69 ±0.96^{d}
Sp	16.24 ±0.24^{e}	16.74 ±0.23^{c}	24.37 ±0.24^{e}	34.48 ±0.23^{e}	49.58 ±0.23^{e}	60.84 ±0.15^{e}	63.99 ±0.16^{d}	214.86 ±0.65^{e}
Ta	26.49 ±0.22^{f}	44.19 ±0.30^{e}	61.94 ±0.29^{f}	76.25 ±0.29^{f}	85.49 ±0.22^{f}	88.98 ±0.22^{f}	94.60 ±0.14^{e}	74.30 ±0.29^{f}
Oj	10.05 ±0.32^{g}	18.97 ±0.31^{f}	23.22 ±0.39^{g}	27.63 ±0.17^{g}	42.19 ±0.32^{g}	46.91 ±0.23^{g}	57.80 ±0.39f	261.14 ±1.44^{g}
Mp	10.81 ±2.01^{h}	18.24 ±0.22^{g}	23.82 ±0.16^{g}	41.58 ±0.22^{h}	58.95 ±0.14^{h}	80.54 ±0.22^{h}	90.29 ±0.23^{g}	165.18 ±0.25^{h}
Dc	3.78 ±0.51^{i}	9.67 ± 0.30^{h}	11.14 ±0.30^{h}	21.89 ±0.22^{i}	28.91 ±0.22^{i}	30.28 ±0.30^{i}	31.90 ±0.30^{h}	437.77 ±3.93^{i}
Cs	21.17 ±0.29^{j}	33.43 ±0.22^{i}	48.43 ±0.30^{i}	61.27 ±0.16^{j}	74.90 ±0.37^{j}	78.43 ±0.22^{j}	81.96 ±0.22^{i}	120.8 ±0.55^{j}
Sm	15.96 ±0.31^{a,e}	25.52 ±0.30^{j}	29.65 ±0.38^{j}	66.51 ±0.38^{k}	73.21 ±0.31^{k}	80.51 ±0.30^{h}	82.27 ±0.23^{i}	139.96 ±0.65^{k}
Pc	14.81 ±0.23^{k}	16.64 ±0.23^{c}	26.38 ±0.08^{k}	33.07 ±0.24^{l}	44.44 ±0.15^{l}	50.43 ±0.23^{k}	59.25 ±0.23^{j}	244.36 ±0.50^{l}
Aa	24.85 ±0.43^{l}	30.71 ±0.22^{k}	42.53 ±0.22^{l}	58.91 ±0.36^{m}	80.71 ±0.22^{m}	82.21 ±0.30^{l}	89.19 ±0.22^{g}	118.93 ±0.63^{m}
Efo	15.63 ±0.30^{a}	30.47 ±0.45^{k}	39.79 ±0.37^{m}	43.80 ±0.37^{n}	48.37 ±0.31^{n}	52.88 ±0.31^{m}	60.35 ±0.22^{j}	213.77 ±1.57^{n}
Lj	22.61 ±0.15^{m}	36.99 ±0.24^{l}	57.96 ±0.15^{n}	80.09 ±0.15^{p}	86.57 ±0.09^{p}	90.92 ±0.15^{n}	92.62 ±0.18^{k}	86.99 ±0.27^{p}
Pp	24.14 ±0.31^{n}	42.88 ±0.23^{m}	61.57 ±0.23^{f}	70.66 ±0.32^{q}	87.90 ±0.39^{q}	89.82 ±0.23^{p}	94.08 ±0.31^{e}	81.67 ±0.28^{q}
Ef	23.13 ±0.22^{p}	30.33 ±0.22^{k}	49.57 ±0.22^{p}	65.31 ±0.22^{r}	76.76 ±0.14^{r}	87.50 ±0.31^{q}	89.05 ±0.29^{g}	112.23 ±0.14^{r}
Trolox	11.41 ±0.22^{q}	47.98 ±0.14^{n}	69.10 ±0.22^{q}	87.89 ±0.29^{s}	92.17 ±0.17^{s}	94.76 ±0.14^{r}	96.46 ±0.22^{l}	73.67 ±0.74^{s}

Sz = S. zeylanica, Ch = C. hirsuta, Nh = N. herpeticum, Bl = B. lanceolaria, Sp = S. peguensis, Ta = T. angustifolium, Oj = O. javanica, Mp = M. perpusilla, Dc = D. cordata, Cs = C. sinensis, Sm = S. media, Pc = P. chinensis, Aa = A. acidum, Efo = E. foetidum, Lj = L. javanica, Pp = P. perfoliatum and Ef = E. fluctuans; values were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

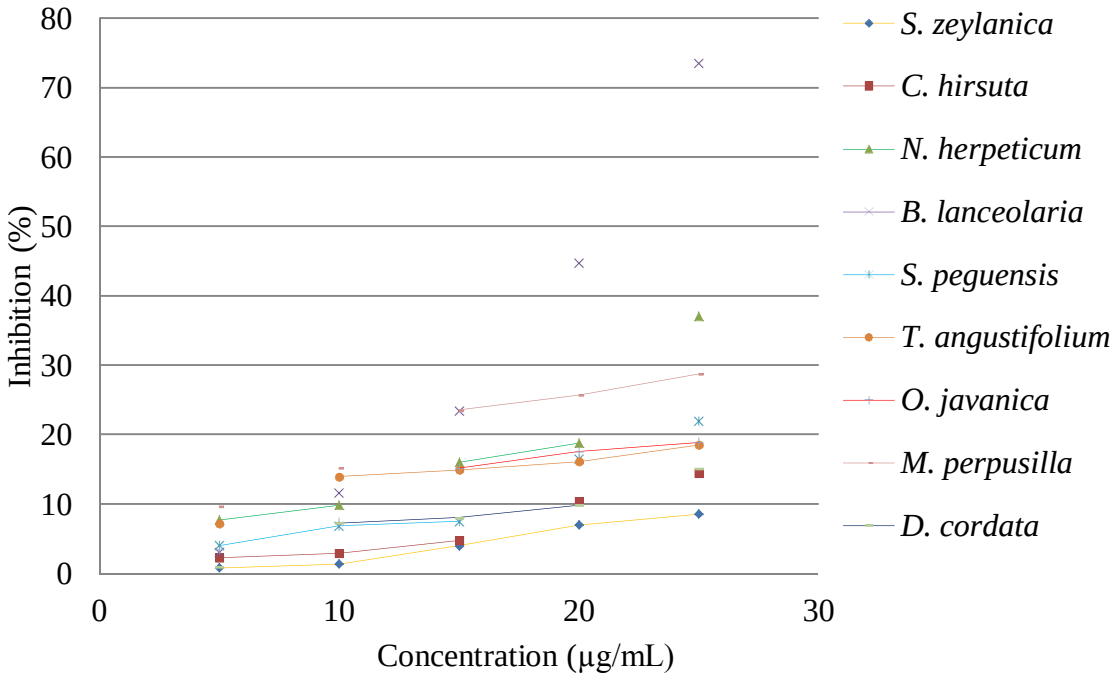

Fig.IV.3a: Plot of inhibition (%) of H_2O_2 assay against concentration of sample extract.

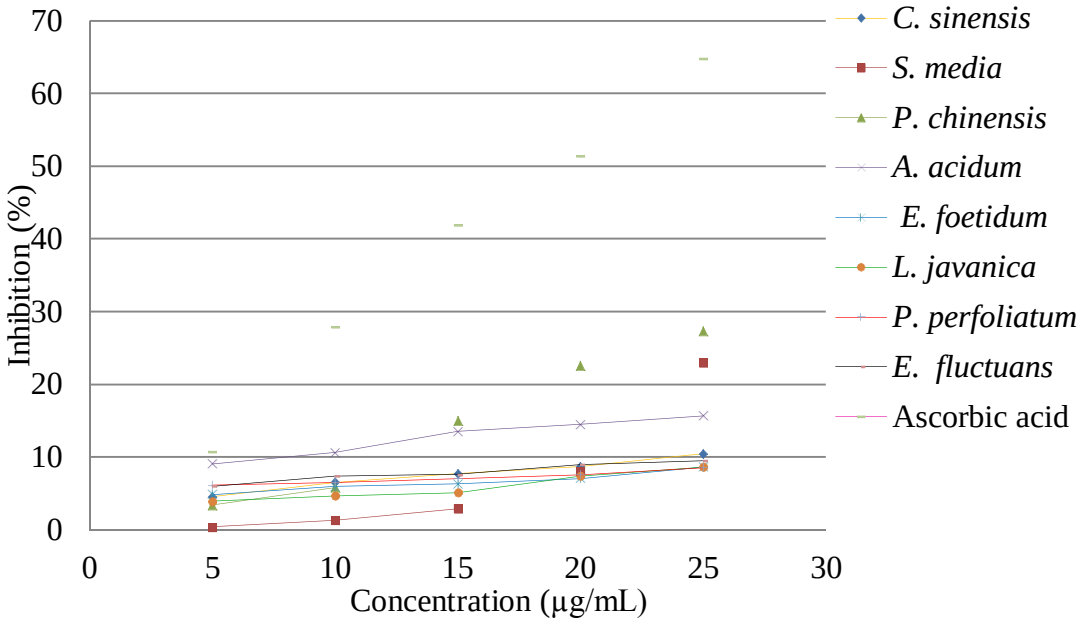

Fig.IV.3b: Plot of inhibition (%) of H_2O_2 assay against concentration of sample extract.

IV.2.2.3 H_2O_2 scavenging activity

In this study, the **H_2O_2** assay percentage inhibition of plant extracts against the concentration was plotted which are shown in the **Fig.IV.3a** and **Fig.IV.3b** and the linear regression equations presented in the **Table IV.6** were obtained from the graphs for IC_{50} value calculation. The H_2O_2 scavenging activities in methanol extracts of plants along with IC_{50} values are summarized in the **Table IV.7**. In this study also, a concentration dependent scavenging activity was observed in each plant extract. The highest percentage inhibition of H_2O_2 scavenging activity was found in *B. lanceolaria* (73.52 ± 0.04%) in 25 µg/mL concentration with an IC_{50} value of 20.37 ± 0.01 µg/mL, while the standard ascorbic acid showed an IC_{50} value of 19.02 ± 0.01 µg/mL. On the other hand, *P. perfoliatum* extract showed the lowest H_2O_2 scavenging activity exhibiting an IC_{50} value of 376.75 ± 14.12 µg/mL followed by *E. fluctuans* with an IC_{50} value of 260.35 ± 7.62 µg/mL. The higher scavenging activities were also observed in *N. herpeticum* (IC_{50} 38.54 ± 0.19 µg/mL**)**, *P. chinensis* (IC_{50} 42.26 ± 0.04 µg/mL), *M. perpusilla* (IC_{50} 45.20 ± 0.09 µg/mL), *S. peguensis* (IC_{50} 57.49 ± 0.26 µg/mL) and *S. media* (IC_{50} 56.32 ± 0.17 µg/mL). In this study, *B. lanceolaria, N. herpeticum, P. chinensis, M. perpusilla, S. peguensis* and *S. media* are showing good antioxidant capacity in terms of H_2O_2 scavenging activities. These findings are in agreement with the IC_{50} value of edible herbs *viz. Tridax procumbens* (56.96 µg/mL) reported by Mir *et al*. [60]. Similarly, Gul *et al.* [25] also reported with IC_{50} value ranged from 22.6 ± 5.0 to 138 ± 12.0 µg/mL in aqueous seed extract of medicinal plant *Abelmoschus moschatus* in different temperature which connects the result of this study. Nabavi *et al.* [61] reported an IC_{50} value of 1138 ± 77.1 µg/mL in *Mespilus germanica* indicating lower antioxidant activity compared to the current study. Subramanian *et al*. [62] reported the IC_{50} values 63.67 and 87.18 µg/mL in *Shorea roxburghii* stem bark for methanol and acetone extracts, respectively. Pawar *et al.* [63] also reported the IC_{50} value of ethanol extract of *Asteracantha longifolia* as 60.77 ± 1.34 µg/mL. H_2O_2, a non-radical reactive oxygen species, can oxidise biological macromolecules such as proteins or enzymes, nucleic acids, lipids, and carbohydrates by generating powerful reactive oxygen species like hydroxyl radical (˙OH) [64, 65]. Therefore, neutralization of such reactive species through natural antioxidants from plants is very essential for protection of biological molecules by inhibiting oxidation reactions. It has been reported that the polyphenols such as catechin, quercetin, caffeic acid ester and gallic acid ester found in fruits and vegetables defend bacterial and mammalian cells from cytotoxicities induced by H_2O_2 [58].

Table IV.6: Linear equations of different samples of H_2O_2 assay for IC_{50} calculation

Plants	**Linear equations**	**R^2 values**
S. zeylanica	Y = 0.4224x - 2.0354	R^2 = 0.9651
C. hirsuta	Y = 0.6388x - 2.626	R^2 = 0.9112
N. herpeticum	Y = 1.3558x - 2.453	R^2 = 0.8461
B. lanceolaria	Y = 3.4832x - 20.978	R^2 = 0.9441
S. peguensis	Y = 0.9097x - 2.3185	R^2 = 0.9094
T. angustifolium	Y = 0.4954x + 6.693	R^2 = 0.8546
O. javanica	Y = 0.8224x + 0.1245	R^2 = 0.9289
M. perpusilla	Y = 0.9742x + 5.951	R^2 = 0.9488
D. cordata	Y = 0.6204x - 1.088	R^2 = 0.9191
C. sinensis	Y = 0.2781x + 3.4017	R^2 = 0.9858
S. media	Y = 1.0374x - 8.443	R^2 = 0.7718
P. chinensis	Y = 1.2904x - 4.55	R^2 = 0.9753
A. acidum	Y = 0.3428x + 7.524	R^2 = 0.9663
E. foetidum	Y = 0.1738x + 3.929	R^2 = 0.9527
L. javanica	Y = 0.2422x + 2.313	R^2 = 0.9358
P. perfoliatum	Y = 0.1186x + 5.3678	R^2 = 0.9708
E. fluctuans	Y = 0.1716x + 5.322	R^2 = 0.9582
Ascorbic acid	Y = 2.6354x - 0.155	R^2 = 0.9904

Table IV.7: H_2O_2 scavenging activities in methanol extracts of plants

Plants	Concentration (µg/mL) and its inhibition (%)					IC_{50}
	5	10	15	20	25	(µg/mL)
Sz	0.76±0.03^{a}	1.33±0.04^{a}	3.95±0.03^{a}	6.95±0.03^{a}	8.51±0.04^{a}	123.19±0.54^{a}
Ch	2.27±0.06^{b}	2.89±0.03^{b}	4.74±0.06^{b}	10.39±0.03^{b}	14.49±0.06^{b}	82.34±0.53^{b}
Nh	7.71±0.07^{c}	9.80±0.91^{c}	16.02±0.07^{c}	18.77±0.07^{c}	37.12±0.02^{c}	38.54±0.19^{c}
Bl	2.99±0.04^{d}	11.64±0.04^{d}	23.46±0.07^{d}	44.74±0.07^{d}	73.52±0.04^{d}	20.37±0.01^{d}
Sp	3.99±0.09^{e}	6.81±0.07^{e}	7.46±0.07^{e}	16.46±0.08^{e}	21.91±0.07^{e}	57.49±0.26^{e}
Ta	7.18±0.06^{f}	13.95±0.08^{f}	14.89±0.11^{f}	16.12±0.06^{e}	18.48±0.11^{f}	87.39±0.67^{f}
Oj	3.42±0.11^{e}	7.34 ±0.08^{g}	15.12±0.08^{f}	17.54±0.09^{f}	18.88±0.13^{f}	60.63±0.52^{g}
Mp	9.63±0.13^{g}	15.23±0.11^{h}	23.51±0.06^{d}	25.7±0.14^{g}	28.75±0.13^{g}	45.20±0.09^{h}
Dc	0.88±0.11^{h}	7.24±0.13^{g}	8.04±0.13^{g}	9.84±0.06^{h}	15.09±0.11^{h}	82.37±0.23^{b}
Cs	4.54±0.16^{i}	6.52±0.27^{e}	7.71±0.07^{e}	8.68±0.09^{i}	10.41±0.09^{i}	167.65±1.93^{i}
Sm	0.38±0.07^{j}	1.32±0.11^{a}	2.90±0.11^{h}	8.03±0.11^{j}	22.96±0.11^{j}	56.32±0.17^{j}
Pc	3.40±0.07^{d,e}	5.82±0.07^{i}	14.96±0.09^{f}	22.56±0.05^{k}	27.29±0.07^{k}	42.26±0.04^{k}
Aa	9.06±0.12^{k}	10.60±0.15^{j}	13.50±0.12^{i}	14.48±0.06^{l}	15.69±0.13^{l}	123.83±1.14^{l}
Efo	4.83±0.11^{i}	5.92±0.04^{i}	6.27±0.05^{j}	7.05±0.02^{a}	8.61±0.07^{a}	265.37±9.85^{m}
Lj	3.93±0.11^{e}	4.68±0.07^{k}	5.10±0.11^{k}	7.39±0.11^{a,m}	8.63±0.09^{a}	196.91±0.45^{n}
Pp	6.11±0.04^{l}	6.49 ±0.07^{e}	7.02±0.07^{l}	7.58±0.07^{m}	8.53±0.07^{a}	376.75±4.12^{p}
Ef	6.00±0.09^{l}	7.41±0.06^{g}	7.59±0.11^{e,g}	8.97±0.04^{i}	9.51±0.06^{m}	260.35±7.62^{q}
AA	10.73±0.02^{m}	27.91±0.04^{l}	41.96±0.07^{m}	51.42±0.07^{n}	64.86±0.07^{n}	19.02±0.01^{d}

Sz = S. zeylanica, Ch = C. hirsuta, Nh = N. herpeticum, Bl = B. lanceolaria, Sp = S. peguensis, Ta = T. angustifolium, Oj = O. javanica, Mp = M. perpusilla, Dc = D. cordata, Cs = C. sinensis, Sm = S. media, Pc = P. chinensis, Aa = A. acidum, Efo = E. foetidum, Lj = L. javanica, Pp = P. perfoliatum and Ef = E. fluctuans, AA = Ascorbic acid, values were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.2.4 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is also another effective and simple method which is used to study the *in-vitro* antioxidant property of the plant extracts and it is based on the power of antioxidant compounds to reduce ferric (III) ions to ferrous (II) ions [66]. Stronger antioxidant capacity of the sample is exhibited by the higher FRAP values. FRAP values were calculated from the linear regression equation ($Y = 0.0007x + 0.1272$; $R^2 = 0.9903$) of standard trolox (**Fig.IV.4**) and the results are presented in **Table IV.8**. The FRAP values in methanol extracts of the plant species ranged from 38.57 ± 7.14 μM TE/g dry extract (DE) showing the lowest antioxidant activity in *S. zeylanica* to 855.23 ± 10.91 μM TE/g DE showing the highest antioxidant capacity in *M. perpusilla*. In this study, higher antioxidant capacities were also exhibited by *L. javanica* (799.28 ± 7.14 μM TE/g DE), *P. perfoliatum* (621.90 ± 7.43 μM TE/g DE), *T. angustifolium* (581.42 ± 10.7 μM TE/g DE), and *C. sinensis* (457.61 ± 7.43 μM TE/g DE). The FRAP values (12.84 ± 2.95 to 119.97 ± 16.73 μmol TE/g DW sample) reported in methanol extract of some wild edible plants such as *Gonostegia hirta, Heckeria umbellatum, Lasia spinosa* etc. of Malaysia are comparable to the present findings. However, some of the FRAP values of hot water extract of these edible plants are similar and some are lower (20.32 ± 4.75 to 200.56 ± 38.44 μM TE/g DW sample) in comparison to our findings [57]. Similarly, Katalinic *et al.* [67] also reported the FRAP value ranging from 59 to 25234 μM/L in 70 different types of medicinal plants such as *Melissae folium, Malvae herba, Althaeae radix,* etc., some of which are more than our highest level (855.23 ± 10.91 μM TE/g DW) and some are similar to our findings. Moreover, the phytochemical study of three medicinal plants of Bahrain reveals the FRAP value ranging from 570 to 2210 μM/g DW which corresponds to our results [53]. It has also reported the antioxidant capacity of 32 edible herbs with FRAP value ranging from (13.8 ± 1.10 to 2133 ± 6.87 μM trolox/100 g DW) which are also comparable to the present findings [56]. The FRAP result reported by Li *et al.* [68] ranged from 0.17 ± 0.00 to 178.43 ± 14.31 μM Fe(II)/g FW in 51 numbers of wild edible flowers, which are also comparable with some results of present study. Islary *et al.* [50] also reported the antioxidant activity of edible fruits such as *Aporosa dioica* and *Ottelia alismoides* with FRAP value 106.583 ± 5.204 μM TE/g DE and 44.083 ± 7.637 μM TE/g DE which resembles with the findings of present study.

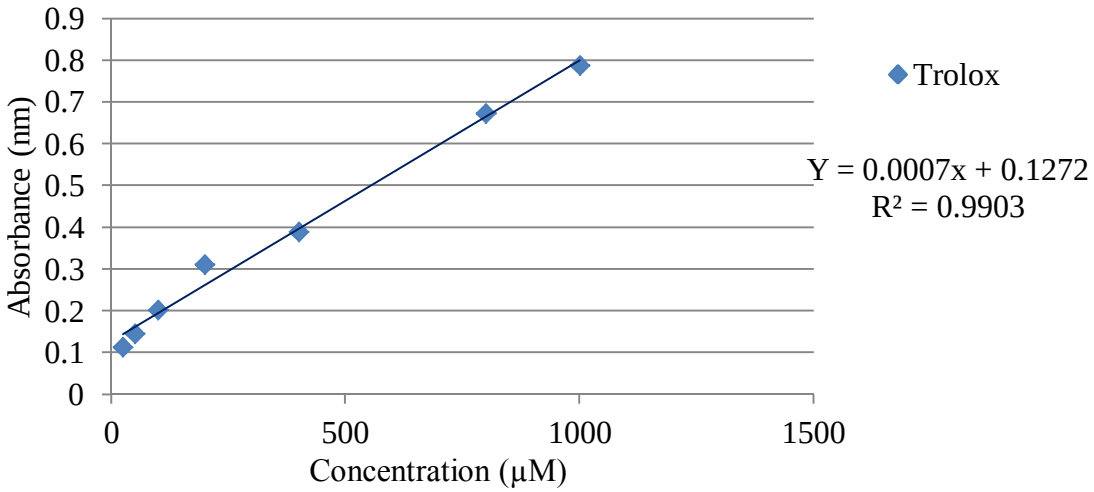

Fig.IV.4: Standard graph of FRAP assay

Table IV.8: FRAP values in methanol extracts of wild edible plants

Plants	Absorbance at 593 nm	FRAP (µM TE/g dried extract)
S. zeylanica	0.13±0.02	38.57±7.14^{a}
C. hirsuta	0.14±0.01	50.47±5.45^{b}
N. herpeticum	0.14±0.01	60.01±3.57^{c}
B. lanceolaria	0.21±0.02	308.80±8.98^{d}
S. peguensis	0.15±0.01	115.9±5.45e
T. angustifolium	0.29±0.03	581.42±10.71^{f}
O. javanica	0.15±0.01	98.09±5.45g
M. perpusilla	0.36±0.03	855.23±10.91^{h}
D. cordata	0.14±0.02	64.76±7.43^{i}
C. sinensis	0.25±0.02	457.61±7.43^{j}
S. media	0.24±0.02	406.42±7.14^{k}
P. chinensis	0.15±0.02	88.57±7.14^{l}
A. acidum	0.24±0.02	423.09±8.98^{m}
E. foetidum	0.16±0.02	127.85±7.14^{n}
L. javanica	0.35±0.02	799.28±7.14^{p}
P. perfoliatum	0.30±0.02	621.90±7.43^{q}
E. fluctuans	0.17±0.02	156.42±7.14^{r}

Results were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.3 Determination of vitamin C content

Vitamin C (ascorbic acid) is one of the water soluble vitamins which play important roles in maintaining a healthy life style and it has potential antioxidant property [11, 69]. The variation of vitamin C contents in the wild edible vegetables of present study are given in the **Table IV.9**. In this study, the vitamin C contents were found ranging from 11.39 ± 0.00 mg/100 g FW in *A. acidum* to 85.71 ± 5.71 mg/100 g FW in *N. herpeticum*. Higher amounts of vitamin C were also found in *T. angustifolium* (79.06 ± 0.02 mg/100 g FW), *P. perfoliatum* (57.74 ± 4.3 mg/100 g FW), *M. perpusilla* (57.07 ± 1.59 mg/100 g FW), and *S. zeylanica* (40.37 ± 1.62 mg/100 g FW). The results obtained in this study are similar to the vitamin C contents of some underutilized leafy vegetables (11 to 85 mg/100 g FW) except *Coleus aromaticus* (3 mg/100 g FW) and *Delonix elata* (295 mg/100 g FW) [70] and some edible plants of Thailand were also comparable ranging from 0.98 ± 0.03 mg/100 g to 48.5 ± 0.13 mg/100 g DW [69]. Our findings are also comparable with the findings of some endemic fruits from Manipur reported by Khomdram *et al.* [45] which ranged from 8.60 ± 2.15 to 86.16 ± 11.04 mg/100 g FW except *Phyllanthus emblica* (375.68 ± 110.6 mg/100 g FW) in comparison to the present study. Similarly, Lim *et al.* [71] also reported vitamin C content in several tropical fruits of Malaysia, ranged from 3.9 ± 1.0 to 144 ± 60 mg/100 g FW which supports our findings. Moreover, the evaluation of vitamin C content of some unexplored edible plants of Eastern Himalayan hot spot showed very closed results (20.7 ± 11.3 to 53.4 ± 6.09 mg/100g FW) to the present study [72]. Similarly, Sarma *et al.* [73] reported higher vitamin C content in some underutilised aroids growing in hilly regions of Assam, which ranged from 74.10 ± 1.00 to 114.2 ± 1.10 mg/100 g FW which were very close to the findings of present study. However, higher values of ascorbic acid (444.9 ± 64.3 to 627.6 ± 126.2 mg/100 g FW) in *Amaranthus hybridus* collected from various part of Nairobi City, Kenya was reported by Onyango *et al.* [74] in comparison to our findings. Similarly, Martins *et al.* [49] also reported higher value of ascorbic acid 142 ± 12 mg/100 g DW in edible plant *Asparagus acutifolius* in comparison to our findings. It has been reported that an adult person requires about 50 mg/day of vitamin C content for maintaining a healthy life [75].

Table IV.9: Vitamin C contents in mg/100 g fresh weight of plants

Plants	Weight of sample for analysis (g)	Titrant value (mL)	Vitamin C (mg/100 g FW)
S. zeylanica	2.04	1.43±0.05	$40.37±1.62^{a}$
C. hirsuta	2.04	1.26±0.11	$35.62±3.24^{b}$
N. herpeticum	2.01	3.00±0.20	$85.71±5.71^{c}$
B. lanceolaria	2.00	0.20±0.00	$11.45±0.00^{d}$
S. peguensis	2.01	0.53±0.05	$15.24±1.65^{e}$
T. angustifolium	2.03	1.40±0.00	$79.06±0.02^{f}$
O. javanica	3.05	0.36±0.05	$13.77±2.16^{g}$
M. perpusilla	2.08	2.06±0.05	$57.07±1.59^{h}$
D. cordata	2.03	0.70±0.10	$39.51±5.64^{i}$
C. sinensis	2.03	0.53±0.11	$30.16±6.53^{j}$
S. media	3.06	1.00±0.00	$37.40±0.00^{k}$
P. chinensis	2.06	1.00±0.00	$27.78±0.00^{l}$
A. acidum	3.02	0.30±0.00	$11.39±0.00^{d}$
E. foetidum	2.05	0.30±0.10	$16.72±5.57^{m}$
L. javanica	2.04	0.40±0.00	$22.42±0.00^{n}$
P. perfoliatum	3.04	1.53±0.11	$57.74±4.34^{p}$
E. fluctuans	2.05	0.26±0.05	$14.94±3.23^{q}$

FW = Fresh weight, Results were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

IV.2.4 Determination of total phenolic contents (TPC) and total flavonoid contents (TFC)

The **Fig.IV.5** and **Fig.IV.6** are the standard graphs of gallic acid and quercetin for the determination TPC and TFC, respectively. The TPF and TFC in methanol extracts of the plants determined from the linear curve of standard gallic acid ($Y = 0.0212x + 0.3098$; $R^2 = 0.9971$) and standard quercetin ($Y = 0.0014x + 0.0799$; $R^2 = 0.9859$) are presented in **Table IV.10**.

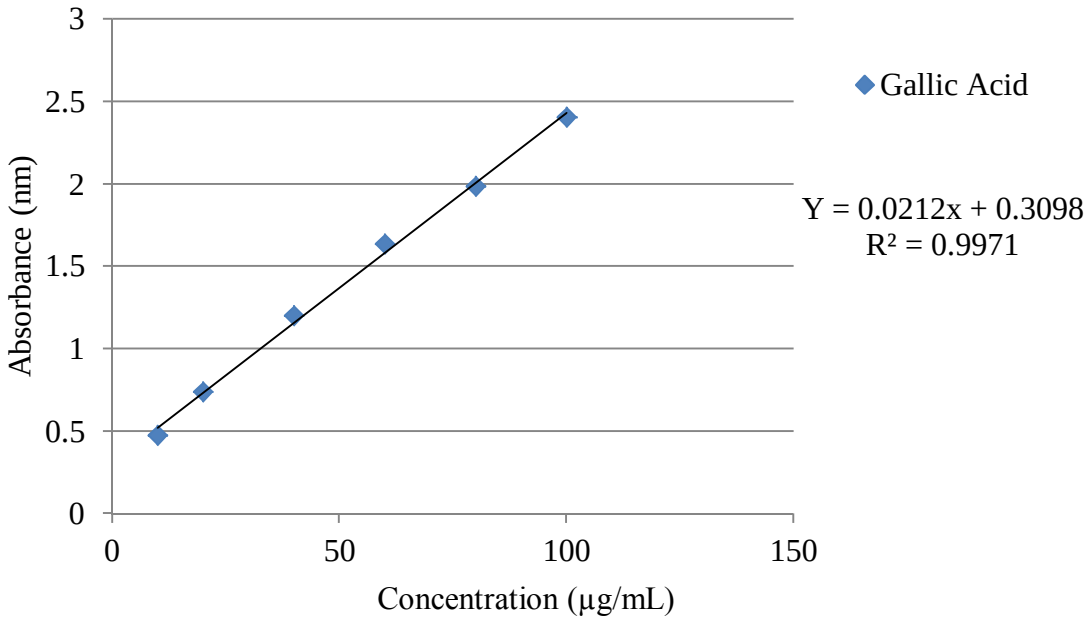

Fig.IV.5: Standard graph for determination of total phenolic content.

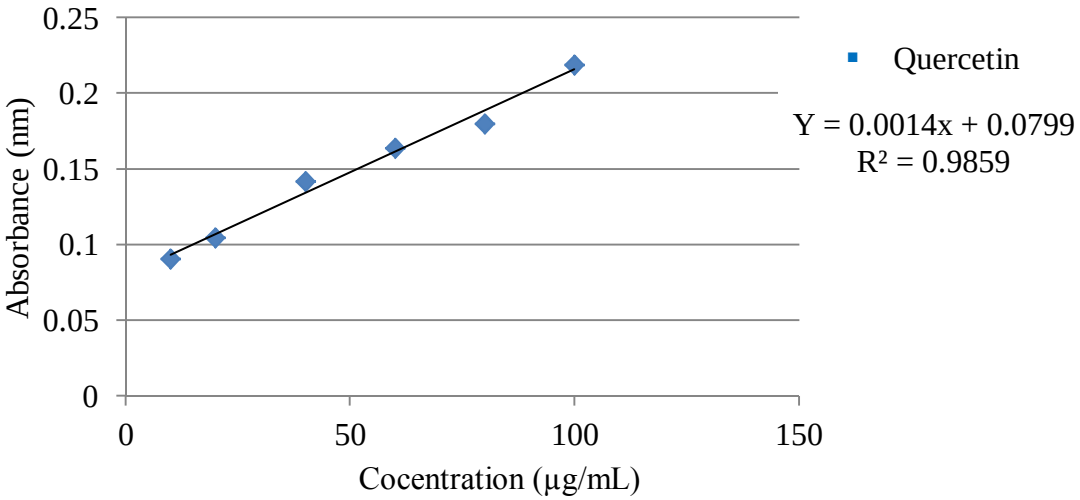

Fig.IV.6: Standard graph for determination of total flavanoid content.

Table IV.10: Total phenolic and total flavonoid contents of wild edible plants

Plants	Absorbance of sample at 765 nm	TPC (mg GAE/g dry extract)	Absorbance of sample at 510 nm	TFC (mg QE/g dry extract)
S. zeylanica	0.34±0.01	36.39±5.31^{a}	0.01± 0.00	0.23 ± 0.10^{a}
C. hirsuta	0.37±0.01	71.77±5.81^{b}	0.01± 0.00	0.47 ± 0.23^{a}
N. herpeticum	0.31±0.00	10.45±0.68^{c}	0.01±0.00	0.29 ± 0.11^{a}
B. lanceolaria	0.34±0.00	36.39±2.96^{a}	0.04±0.00	1.01 ± 0.10^{b}
S. peguensis	0.35±0.01	54.08±7.21^{d}	0.03±0.00	0.77 ± 0.19^{a,b}
T. angustifolium	0.45±0.01	54.08±7.20^{d}	0.06±0.00	1.36 ± 1.03^{b}
O. javanica	0.33±0.01	171.22±8.90^{e}	0.01±0.00	0.47 ± 0.10^{a}
M. perpusilla	0.51±0.01	239.62±5.4^{f}	0.08±0.00	1.66 ± 0.10^{b}
D. cordata	0.33±0.01	29.71±5.40^{g}	0.03±0.00	0.77 ± 1.03^{a}
C. sinensis	0.36±0.01	26.96±9.81^{h}	0.03±0.00	0.77 ± 0.10^{a}
S. media	0.33±0.00	67.45±7.07^{i}	0.01±0.00	0.23 ± 0.10^{a}
P. chinensis	0.35±0.00	52.12±2.35^{j}	0.02±0.00	0.65 ± 0.17^{a}
A. acidum	0.40±0.00	30.11±2.96^{k}	0.05±0.00	1.19 ± 0.10^{b}
E. foetidum	0.38±0.00	105.18±3.11^{l}	0.06±0.00	1.30 ± 0.10^{b}
L. javanica	0.53±0.00	91.43±4.14^{m}	0.13±0.00	2.55 ± 0.10^{c}
P. perfoliatum	0.54±0.00	265.95±4.75^{n}	0.23±0.00	4.34 ± 1.03^{d}
E. fluctuans	0.38±0.01	269.49±2.96^{p}	0.03±0.00	0.83 ± 0.10^{a,b}

Results were expressed as mean of three replicates ± standard deviation and the data with different letters in a column are significantly different from each other at $p < 0.05$.

In this study, the TPC varied from 10.45 ± 0.68 mg GAE/g dry extract (DE) in *N. herpeticum* to 269.49 ± 2.96 mg GAE/g DE in *E. fluctuans.* Higher amounts of TPC were also found in *P. perfoliatum* (265.95 ± 4.76 mg GAE/g DE), *M. perpusilla* (239.62 ± 5.4 mg GAE/g DE), *O. javanica* (171.22 ± 8.90 mg GAE/g DE) and *E. foetidum* (105.18 ± 3.11 mg GAE/g DE). However, the flavonoid content was found ranging from 0.23 ± 0.10 mg QE/g DE both in *S. media* and *S. zeylanica* to 4.34 ± 1.03 mg QE/g DE which was the highest in *P. perfoliatum* followed by *L. javanica* (2.55 ± 0.10 mg QE/g DE). Marwah *et al*. [44] also reported the phenolic content ranging from 14.9 ±

1.6 to 454 ± 16.3 mg GAE/g in ethanol extract of some edible plants which resembles with the results of the present study. Similarly, Mata *et al.* [46] reported penolic content of ethanol extract (63.1 ± 3.0 to 113.0 ± 6.2 mg/g of extract) and in aqueous extract (57.9 ± 1.6 to 74.9 ± 3.3 mg/g of extract) of five Portuguese food spices, which resembles with the results of current study. Moreover, Maisuthisakul *et al*. [40] also reported similar result of TPC ranging from 6.4 ± 0.1 to 63.4 ± 0.5 mg GAE/g DW in some Thai indigenous edible herbs but higher value of TFC has reported which ranged from 3.7 ± 0.2 to 25.5 ± 0.1 mg RE/g DW in comparison to the present result. In a study of selected wild plants reported by Arunachalam *et al*. [58], the TPC were found in the range of 0.69 to 19.65 mg GAE/g DW and TFC were found in the range of 0.19 ± 0.02 to 8.37 ± 2.62 mg catechin equivalent per gram of dry weight. Similarly, Ng *et al.* [41] reported lower amounts of TPC in tropical wild vegetables that ranged from 1.8 mg to 4.1 mg GAE/g FW and TFC that ranged from 0.4 mg to 1.4 mg rutin equivalents/g FW. Saikia *et al*. [76] also studied some non-conventional vegetables from North-East India and reported TPC in the range of 4.62–14.74 mg GAE/g dry weight and TFC in the range of 0.65–7.72 mg QE/g DW. The TPC (1.29 ± 0.47 to 19.39 ± 2.46 mg GAE/g dry weight sample) and TFC (0.30 ± 0.02 to 6.13 ± 2.13 mg CAE/g dry weight sample) methanol extract of some wild edible plants like *Gonostegia hirta, Heckeria umbellatum, Lasia spinosa* etc. showed lower phenolic and flavonoid content than the current study [57]. Similarly, the lower amounts of TPC (0.08 to 0.31 mg Ferulic acid equivalent (FAE)/g dry weight) and TFC (0.03 to 0.15 mg rutin equivalent (RE)/g dry weight) in some leafy vegetable has been reported in comparison to our results [77]. Moreover, the TPC of 51 wild edible flower also showed lower phenolic content ranging from 0.50 ± 0.01 to 24.36 ± 1.11 mg GAE/g wet weight in aqueous extract, whereas in fat soluble fraction, it ranged from 0.13 ± 0.02 to 11.48 ± 0.56 mg GAE/g wet weight which were much lower in comparison to the present study [68]. However, higher values of TPC were reported by Xia *et al.* [78] in six wild plants that ranged from 278.7 ± 24.4 to 417.3 ± 38.3 mg GAE/g dry weight. Al-Tohamy *et al.* [79] also reported the similar value of TPC which ranged from 19.48 to 65.48 mg GAE/g DW and also reported the higher amount of flavonoid which ranged from 2.90 to 11.09 mg QE/g DW in comparison to the present study. It has been reported that the phenolics compounds in plants are responsible for odour, flavour, colour, and bitterness taste [8] and also for biological properties such as anticarcinogenic, antioxidant, antimicrobial, antimutagenic, antiallergic and anti-inflammatory properties [38, 57, 80, 81]. The higher amounts of TPC and TFC indicates stronger antioxidant activities and they have

countless roles for reducing risk of numerous diseases occurred in human health due to oxidative damages [82].

IV.3 Conclusion

In conclusion, the study of methanol extracts of wild edible plants indicated the presence of numerous biologically active compounds which are considered to have several medicinal properties such as anthelmintic, antioxidant, antimicrobial, and other biological properties. The study of *in vitro* antioxidant capacities in methanol extracts showed increasing scavenging activities with increasing concentration. In DPPH assay, *M. perpusilla* showed the best antioxidant activity with an IC_{50} value 134.96 ± 0.35 µg/mL followed by *L. javanica* (IC_{50} 135.0 ± 1.49 µg/mL) whereas in ABTS method, *T. angustifolium* exhibited the highest antioxidant activity with IC_{50} value 74.3 ± 0.29 µg/mL followed by *P. perfoliatum* (IC_{50} 81.67 ± 0.28 µg/mL) and *L. javanica* (IC_{50} 86.99 ± 0.27 µg/mL). However, the highest antioxidant capacity indicated by the H_2O_2 assay was the methanol extract of *B. lanceolaria* with IC_{50} value of 20.37 ± 0.01 µg/mL. The FRAP value was found the highest in *M. perpusilla* (855.23 ± 10.91 µM TE/g DE) followed by *L. javanica* (799.28 ± 7.14 µM TE/g DE), *P. perfoliatum* (621.90 ± 7.43 µM TE/g DE) and *T. angustifolium* (581.42 ± 10.7 µM TE/g DE). Higher amounts of TPC were found in *E. fluctuans* (269.49 ± 2.96 mg GAE/g DE), *P. perfoliatum* (265.95 ± 4.76 mg GAE/g DE) and *M. perpusilla* (239.62 ± 5.4 mg GAE/g DE). *P. perfoliatum* showed the highest TFC of 4.34 ± 1.03 mg QE/g DE. In this study, the vitamin C content was found the highest in *N. herpeticum* (85.71 ± 5.71 mg/100 g FW) followed by *T. angustifolium* (79.06 ± 0.02 mg/100 g FW), *P. perfoliatum* (57.74 ± 4.3 mg/100 g FW), and *M. perpusilla* (57.07 ± 1.59 mg/100 g FW). Thus, it can be concluded that these wild plants *viz. M. perpusilla*, *L. javanica*, *P. perfoliatum*, *T. angustifolium*, *B. lanceolaria*, *N. herpeticum*, and *E. fluctuans* have strong antioxidant capacity and can be considered as the good sources of natural antioxidants.

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