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RESEARCH ARTICLE

Evaluation of in vitro antioxidant and brine shrimp lethality bioassay of different extracts of Polygonum plebeium R. Br.

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Abstract

Antioxidants are substances which has the ability to scavenge free radicals and help to decrease the incidence of oxidative stress induced damage. As synthetic antioxidants have some side effect, now a day's great deal of effort being expanded to find effective antioxidant from natural sources for the treatment and prevention of oxidative stress induced damage. *Polygonum plebeium* R. Br. (Family: Polygonaceae) commonly known as "Chemti sag", "Dubia Sag", "Anjaban" is widely distributed in Bangladesh and used as a vegetable. The present study was carried out to investigate antioxidant and cytotoxic potential of aerial parts of *Polygonum plebeium* R. Br. in vitro. The aerial parts of the plant were extracted with different solvents (petroleum ether > ethyl acetate > methanol > water) i.e. from non-polar to polar. Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, glycosides, steroids, saponins and carbohydrates. All the extracts showed promising antioxidant and cytotoxic potential. Methanolic extract was found to contain highest amount of phenolic compounds ((7.773 ± 0.547 mg/g GAE) and flavonoids contents was highest in petroleum ether extracts (11.88 ± 0.204 mg/g QE). Both the methanolic and ethyl acetate extracts showed to have possessed similar total antioxidant capacity and expressed as ascorbic acid equivalents. In DPPH free radical scavenging assay, methanolic extracts showed highest scavenging potential with IC_{50} value of 43.63 µg/ml followed by ethyl acetate extracts with IC_{50} value of 72.62 µg/ml compared to standard ascorbic acid having IC_{50} value of 18.34 µg/ml. Both methanolic and water extracts had similar nitric oxide scavenging potential and were highest among the extracts. Reducing power was found to increase with increasing the concentration of the plant extracts and the extracts were found to contain low amount of alkaloids. In brine shrimp lethality bioassay, water extracts of the plant showed most potent cytotoxic action with LC_{50} value of 23.72 µg/ml followed by the petroleum ether extracts with LC_{50} value of 46.04µg/ml where standard cytotoxic agent vincristine used as positive control. This study provides scientific basis for the use of *Polygonum plebeium*R. Br. as a natural antioxidant.

INTRODUCTION

Free radicals are produced continuously in human body and human body has endogenous enzyme system to protect the body from free radical mediated damage. It is well accepted that reactive oxygen species such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), peroxyl ($OOH^{\cdot}$, $ROO^{\cdot}$) radicals are produced under oxidative stress and are responsible for many degenerative diseases like aging, cancer, cardiovascular diseases, neurodegenerative disorders, etc (Huang et al., 2012). To counter the effect of such reacting species, body has its own mechanism which are produced either endogenously or received from external sources commonly known as antioxidants. Antioxidants protect vital cellular compartments from these species from the formation or removal of these agents from the body (Hasan et al., 2014).

Many plant species have been studied to find effective antioxidant for the treatment and prevention of free radical mediated harmful effects and many plants have been found promising antioxidant potential. Such plants are known as medicinal plants simply explained as plant or its parts having medicinal properties and in fact their chemical constituents are mainly responsible for pharmacological activity (Alhadi et al., 2015). Plants are commonly enriched in phenols, flavonoids, tanins, lignins, alkaloids, essential oils, terpenes, sterols, etc. which are mainly responsible for various pharmacological effects (Huang et al., 2012; Patwardhan et al., 2004). People of all around the world have the tradition of using various plants in the treatment of many ailments and almost 80% of population of the world still depends on traditional use of plant base pharmaceuticals for primary health care need specially observed in rural areas (Ahmad et al., 2013).Plant resources have been emphasized world wide as precious sources of raw material and hold a great promise for the discovery and development of new drugs.

Bangladesh is a store house of various kinds of plants having medicinal value. *Polygonum plebeium* is one of the important plant of ploygonaceae family locally known as “Chemti Sag”, “Dubia Sag”, “Anjaban”, Small knotweed in English is a prostrate, diffusely branched herb (Ghani A., 1998). *Polygonum plebeium* is widely distributed throughout the old tropics and native to India, Pakistan, Madagaskar, Srilanka and Bangladesh (IUCN Red List of Threatened Species). This plant forms dense prostrate mass in rivers, canals and drying out pools; diffusely branched, very variable, sub erect or prostrate under shrubs with a woody rootstock, ochreas lacerate, flowers in axillary clusters, perianth rose (Swapna et al., 2011). This is used as vegetable in Bangladesh. Powdered herb of *Polygonum plebeium* is used in the treatment of pneumonia and rootstock is used against bowel complaints (Swapna et al., 2011; Ghanthikumar et al., 2008; Hameed et al., 2010). Katewa and Galav (2005) also reported the use of its ash with oil on eczema. Various phytochemical reports have been published regarding this family from which the plant belong include flavonoids, essential oils, tannins, triterpenoids, unsaturated sterols, steroidal sapogenins (saponins) and alkaloids (Ahmad et al., 2013). The present study was designed to investigate different phytochemical groups, antioxidant activity and brine shrimp lethality (cytotoxicity) of the different extracts of aerial parts of *Polygonum plebeium* R Br.

Materials and Method:

Study Design:

The present protocol was designed to investigate the presence of various phytochemical groups, antioxidant and cytotoxic potentiality of extracts of aerial parts of *Polygonum plebeium* R. Br. extracted with different solvent i.e., non polar to polar i.e., petroleum ether > ethyl acetate > methanol > water.

Collection and Identification of plant material:

The whole plant was collected from Jahangirnagar University, Savar, Dhaka, Bangladesh and identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka (Accession number: 38349).

Chemicals and Reagents:

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, sodium nitroprusside, sodium phosphate, sulphanilamide, phosphoric acid and napthylethylenediamine were obtained from SD Fine Chem. Ltd, India. Quercetin, Gallic acid and Folin- Ciocalteu reagent(FCR), Vincristine, Di-methyl sulphoxide (DMSO), ammonium molybdate was obtained from Merck, Germany. Ferric chloride and neocaprine were obtained from Sigma Chemical Co.

Extraction process:

Roots of the plant were discarded and aerial parts were sliced into small pieces. It was sun-dried and then, dried in an oven at reduced temperature (not more than 50^0C) to make suitable for grinding purpose. The plant part was then grounded to make powder using a grinder and then stored in air tight container with necessary marking for identification and kept in cool, dark and dry place for the investigation.

The powdered plant material was used for extraction by Soxhlet apparatus at elevated temperature (65^{o}C) using petroleum ether, ethyl acetate& methanol consecutively with 500 ml of each solvent. After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. After methanol extraction was completed, the plant material were dried and soaked into distilled water (1L). The plant material was kept in water for 7 days in sealed container accompanying occasional shaking and string. All the extracts were filtered individually through fresh cotton bed and the filtrates obtained were dried at 40^{o}C to get final concentrated extracts. Each extracts was kept in suitable container with proper labeling and stored in cool and dry place (Cannel, 1998).

Evaluation of phytochemical groups:
The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Determination of total phenol content:
The content of total phenolic compounds in plant extracts were determined by Folin–Ciocalteu Reagent (FCR) (Folin and Ciocalteu, 1927). The FCR actually measures a sample's reducing capacity. 1.0 mL of each plant extracts or standard of different concentration solution were taken in test tubes and 5 mL of Folin – ciocalteu (Diluted 10 fold) reagent solution was added to the test tubes. 4 mL of Sodium carbonate solution was added into the test tubes. The test tubes were incubated for 30 minutes at 20°C to complete the reaction (Only for standard). The test tube was incubated for 1 hour at 20^{0} C to complete the reaction (Only for extract).The absorbances of the solutions were measured at 765 nm using a spectrophotometer against blank. The Total content of phenolic compounds in plant ethanol extracts in Gallic acid equivalents (GAE) were calculated by the following formula equation

$C = (c \times V)/m$
Where:
C = total content of phenolic compounds, mg/g plant extract, in GAE;
c = the concentration of Gallic acid established from the calibration curve, mg/mL;
V = the volume of extract, mL;
m = the weight of pure plant ethanol extract, g.

Determination of Flavonoid content:
Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002).1 mL of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2mL of 1 M potassium acetate and 5.6 mL of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/mL of sample.

Determination of Total Antioxidant Capacity:
The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α- tocopherol, and carotenoids. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.(Prieto et al., 1999). Sample extracts (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against blank. Total antioxidant capacity of the extract was measured from the regression equation prepared from the concentration versus optical density of ascorbic acid.

DPPH free radical scavenging assay:
DPPH scavenging activity of the plant was measured by the method developed by Manzocco et al., (1998). The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated from the measured absorbance data. Ascorbic acid was used as a reference or standard antioxidant in this assay method. The percentage (%) inhibition activity was calculated from the following equation

$(A_0 – A_1)/A_0\} \times 100$
Where,
A_0 is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated.

Nitric Oxide Scavenging Capacity Assay:
Nitric oxide radical scavenging capacity was estimated on the basis of Griess-Illosvoy reaction (Govindarajan et al., 2006). In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). 4.0 ml of each fraction and standard (ascorbic acid) was added into 1.0 ml of Sodium nitroprusside (5 mM) solution and incubated for 2 hours at 30^{o}C to complete reaction. Then 2.0 ml solution was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent and absorbance of the solution was measured at 550 nm using a spectrophotometer (Shimadzu UV PC-1600) against blank. Percentage scavenging activity was calculated from

$[(A_0–A_1)/A_0]$ x100

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were calculated.

Assessment of reducing power capacity:
Reducing power capacity assessment of the plant extracts was determined using the method described by Oyaizu et al, (1986). 2.0 ml of each plant extracts or standard of different concentration solutions were taken and 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$, 1% solution was added into each test tubes. The test tubes were incubated for 10 min at 50^{o} C and 2.5 ml of trichloroacetic acid, 10% solution was added. The resultant mixtures were centrifuged at 3000 rpm for 10 min and 2.5 ml supernatant solution were withdrawn from each of the mixtures and mixed with 2.5 ml of distilled water. Then 0.5 ml of ferric chloride ($FeCl_3$), 0.1% solution was added. The absorbances of the solutions were measured at 700 nm using a spectrometer against a typical blank solution.

Cupric Reducing Antioxidant Capacity:
Cupric reducing antioxidant capacity of the plant extracts was determined following the method described by Resat et al.,(2004). 500 µl of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes. 1.0 ml of 0.01 M $CuCl_2.2H_2O$ solution and 1.0 ml of ammonium acetate buffer (pH 7.0) was added into the test tubes. Then 1.0 ml of 0.0075 ml of Neocaproin solution and 600 µl of distilled water was added into the test tubes. The total mixture was incubated for 1 hour at room temperature then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank.

Total alkaloid Content Determination:
Total alkaloid content was determined by slightly modified Fazel et al., 2008 method. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.D.

Brine Shrimp lethality bioassay for cytotoxic activity:
Cytotoxicity of the plant extracts were determined by Brine Shrimp lethality bioassay described by Meyer et al., (1982). Brine Shrimp eggs are hatched in simulated seawater to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of DMSO (Di-methyl sulphoxide). Ten nauplii are taken in vials containing 5 ml of simulated seawater. The samples of different concentrations are added. Survivors are counted after 24 hours. The median lethal concentration, LC_{50} values of the test samples after 24 hours are obtained by a plot of percentage of dead Shrimps against the logarithm of the sample concentration using Microsoft Excel. Vincristine sulphate is usually used as the reference cytotoxic drug.

Statistical analysis:
Values are presented as mean ± SD (standard deviation). IC_{50} and LC_{50} values were obtained with the help of Microsoft excel 2007. One way ANOVA followed by Tukey multiple comparisons were performed to analyze the data using Graph pad prism version 5.00 (Graph pad Software Inc., San Diego, CA, USA).

Result & Discussion:

Preliminary phytochemical group evaluation:
In the present study, preliminary phytochemical screening study of extracts under consideration was done to identify the presence of various bioactive components. Preliminary phytochemical screening of the crude extracts of aerial parts of *Polygonum plebeium* showed the presence of different phytochemical constituents that can be summarized in the table 1.

There is not enough study to report the presence of specific phytochemical constituents in *Polygonum plebeium* R. Br.. Hasan et al., (2009) reported the presence of flavonoids, saponins, steroids and alkaloids in the leaves of *Polygonum plebeium* R. Br.. In our study, we have extracted the aerial parts of the plant with different solvents and preliminary phytochemical screening confirmed the presence of alkaloid in all extracts. Methanolic, petroleum ether and ethyl acetate extracts of the aerial parts of *Polygonum plebeium* R. Br. were rich in flavonoids, glycosides and steroids. Methanolic and water extracts confirmed the presence of saponin and the presence of carbohydrate was only detectable in methanol extracts. It can be assumed that *Polygonum plebeium* R. Br. are rich in various type phytochemical constituents that may account for its various pharmacological properties.

Evaluation of antioxidant activity

Total phenol content:
Total phenolic contents of the extracts under consideration were calculated using the calibration curve of Gallic acid ($y= 0.009x + 0.098$, $R^2 = 0.983$) and were expressed as Gallic acid equivalents (GAE) per gram of plant extracts. In this study, we found that methanolic extracts contain highest amount of phenols (7.773 ± 0.547 mg/g). Phenol contents of the extracts were found to decrease in the following order: Methanolic extracts > Petroleum ether extract > Ethyl acetate extract > Water extract (Table: 2).
Plant kingdom is a great source of phenolic compounds and they have ideal structural chemistry for antioxidant potential. Antioxidant activities of phenolic compounds arise from their high reactivity as hydrogen or electron donor which can stabilize unpaired electrons and from their potential to chelate metal ions (Hasan et al., 2014). So, phenolics are important component derived from plant source and the result strongly suggest that extracts of *Polygonum plebeium* R. Br showed mild to moderate amount of phenolic content which is expressed in Gallic acid equivalents (table 2). Huang et al.,(2012) also expressed the polyphenolic contents of methanolic and water extracts of *Polygonum plebeium* R. Br. and expressed as µg/ml of catechin equivalent of dry weight. So, the present study demonstrates that, the plant under investigation is a great source of phenolic compounds which may have some strong physiological effect.

Total flavonoid content determination:
Aluminum chloride colorimetric method was used to determine total flavonoid contents of different extracts of aerial parts of *Polygonum plebeium* R. Br. Total flavonoid content was estimated using the standard curve of quercetin ($y= 0.009x – 0.036$, $R^2 = 0.972$) and expressed as Quercetin Equivalents (QE). In our present study we found that, petroleum ether extract contain highest amount of flavonoid (11.88 ± 0.065 mg/g QE), followed by ethyl acetate extract (9.16 ± 0.067 mg/g QE) of aerial parts of *Polygonum plebeium* R. Br. (Table 2).

The antioxidant properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions and inhibition of enzymes responsible for free radical generation. Depending on their structure flavonoids are able to scavenge practically all known ROS (Gracia et al., 1997). The plant extracts under investigation have been shown to possess mild to moderate amount of flavonoids.

Total antioxidant capacity assay:
Total antioxidant capacity of different extracts were evaluated by phosphomolybdenum method and expressed as Ascorbic Acid Equivalents (AAE). Total antioxidant capacity of the extracts were estimated using the standard curve of ascorbic acid ($y= 0.004x – 0.008$, $R^2 = 0.997$) and methanol extract and ethyl acetate extract of the plant possess almost equal antioxidant potential. (Table: 2).

The total antioxidant activity was evaluated from their ability to reduce phosphate/Mo(VI) complex to phosphate/Mo (V). Total phenol or flavonoid content assay only determines the amount of total amount of phenolic or flavonoid compounds, but this does not represent the total antioxidant activity of the constituents present in the extract. Therefore, the result of total antioxidant capacity assay is more important for the determination of antioxidant property of an extract (Alam et al., 2012). More over this is a quantitative method as antioxidant activity is expressed as AAE. According to recent study, in many plant extracts there is highly positive relationship between total phenol and antioxidant activity.

DPPH free radical scavenging activity:
DPPH (1,1-diphenyl 1-2-picrylhydrazyl) radical scavenging is a popular and reliable method for evaluation of free radical scavenging activity of compounds or antioxidant capacity of plant extracts. The percent (%) of inhibition were plotted against log concentration and from the graph IC_{50} value was calculated. In our present study, we found that methanolic extracts of aerial parts of *Polygonum plebeium* R. Br. possess most DPPH free radical scavenging potential with IC_{50} value of 43.65 µg/ml, followed by ethyl acetate extract with IC_{50} value of 77.62 µg/ml. The IC_{50} value of standard drug ascorbic acid was 18.34 µg/ml (table: 3).

In DPPH radical scavenging assays, the crude extracts of *polygonum plebeium* R. Br.showed dose dependent scavenging of DPPH radicals in a way similar to that of the reference antioxidant ascorbic acid and all the extracts possess good to moderate DPPH scavenging potential. The relatively stable organic free radical DPPH is used to investigate the scavenging properties of crude extracts. The DPPH radical is scavenged in presence of antioxidants through the donation of electrons and thus forming the reduced free radicals. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Huang et al., 2012). Huang et al., (2012) also investigated the DPPH scavenging potential of *Polygonum plebeium* R. Br. and expressed it as µg/ml glutathione equivalents (GSH) and confirmed that the plant under investigation has good free radical scavenging potential.

Nitric oxide (NO) scavenging capacity assay:
Nitric oxide (NO) scavenging activity was determined by decrease in its absorbance at 550nm, induced by antioxidants. All the extracts under investigation showed a dose dependent scavenging of NO similar to the standard antioxidant compound ascorbic acid. However, maximum scavenging of NO was found with methanol extract of aerial part of *P. plebejum* with an IC_{50} value of 5.13µg/ml ;(table 3), the result is comparable to ascorbic acid which was taken as the standard (4.79µg/ml).
Nitric oxide (NO) is a physiologically important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various biochemical processes. Excess generation and accumulation of nitric oxide are implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, Alzheimer's, and arthritis (Sainani et al., 1997). Different extracts of *P. plebeium* was able to scavenge NO produced from nitroprusside at a considerable level. Several reports have pointed out the role of flavonoids and phenolic compounds (the presence of which has been ascertained in the present study) in NO scavenging.

Cupric Reducing Antioxidant Capacity (CUPRAC):
Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing concentrations of the different extracts of *Polygonum plebeium* R. Br.. The standard ascorbic acid showed the highest reducing capacity in CUPRAC method. Comparison of graph of CUPRAC between ascorbic acid & different extracts are given in figure 3.

Reducing power capacity assessment:
Reducing power of the tested extracts were assessed using ferric to ferrous reduction activity as determined spectrophotometrically from the formation of Perls's Prussian blue color complex (Yildirim et al., 2000). The extracts were found to display excellent reducing power. Reducing power was found to increase with increasing concentration of the extracts in all cases and was comparable to the standard (Ascorbic Acid). Among the extract, methanol extract of aerial part exhibited the most reducing power (Fig. 4). This result indicates that the extracts may consist of polyphenolic compounds that usually show greater reducing power.
The reducing capacity of the plant extracts is an important indicator of its potential antioxidant activity (Huang et al., 2012).The extracts were found to display excellent reducing power and reducing power was found to increase with increasing concentration of the extracts in all cases and was comparable to the standard (Ascorbic Acid)

Total alkaloid content determination:
Total alkaloid content was calculated using the calibration curve of atropine($y=0.085x-0.048$, $R^2= 0.812$) and results are summarized into the table 2.

Brine shrimp lethality bioassay for cytotoxic activity:
All the extracts were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, water extract of aerial part was found to be the most toxic to Brine Shrimp nauplii, with LC_{50} of 23.72 µg/ml whereas anticancer drug Vincristine sulphate showed LC_{50} value 2.47 µg/ml. The order at which cytotoxic potential of the test samples decreased was as follows: Water extract > Petroleum ether extract >Methanolic extract> Ethyl acetate extract (table 3).

Brine shrimp lethality bioassay has been considered as practical, safe and economic method for the evaluation of bioactivities of crude extracts. In brine shrimp lethality bioassay, all the extracts showed considerable lethality. The observed cytotoxic action may be due to the presence of different phytochemical constituents confirmed during phytochemical group evaluation test.

Conclusion:

The results from this study indicate that the extracts of *Polygonum plebeium* R. Br. possess good to moderate antioxidant and cytotoxic potential. Polyphenolic compounds, falvonoids and various other phytochemicals present in the plant may be responsible for its antioxidant and cytotoxic potential and can be a great source of natural antioxidant and new cytotoxic compound. It is also obvious that the difference of activity in different extracts. Further study is suggested to understand the mechanism of observed activities and to isolate and characterize active phytochemical compounds that are responsible for it's activity and to carry out advance research on cancer as in its preliminary study the plant showed promising result.

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Table 1:

Phytochemical Test		**Crude extracts of aerial parts of *Polygonum plebeium***			
		Methanol extract	**Ethyl acetate extract**	**Petroleum ether extract**	**Water extract**
Alkaloid	Mayer's test	_	+	+	_
	Hager's test	+	+	++	+
	Wagner's test	+	+	++	+
	Dragandroff's test	+	+	++	+
Carbohydrate		+	_	_	_
Flavonoid		+	+	+	_
Glucoside		_	_	_	_
Glycoside		+	+	+	_
Saponin		+	_	_	+
Steroid		+	+	+	_
Tanin		_	_	_	_

Table 1: Result of phytochemical screening of crude extracts of aerial parts of *Polygonum plebeium* R. Br., "+" indicates presence, "++" indicates strong presence, "-" indicates absence.

Table 2:

Extracts	Total Phenol	Total Flavonoid	Total Antioxidant	Total Alkaloid
Methanol	7.773±0.547[a]	6.437±0.220[c]	4.164±0.003[b]	2.398±0.146[a]
Pet ether	0.835±0.236[c]	11.88±0.204[a]	1.692±0.033[c]	1.418±0.003[c]
Ethyl acetate	6.151±0.173[b]	9.159±0.211[b]	4.478±0.007[a]	1.697±0.036[b]
Water	1.423±0.376[c]	1.644±0.177[d]	0.666±0.04[d]	1.577±0.010[bc]

Table 2: Result of antioxidant activity of different extracts of aerial parts of *Ploygonum plebeium*R. Br. Values are presented as Mean±SD (Standard Deviation). Values in the same column with different superscripts are significantly different from one another (P<0.05). One way ANOVA followed by Tukey Multiple comparison was performed to analyze this data set.

Table 3:

Extracts	DPPH free radical scavenging assay, IC_{50} (µg/ml)	Nitric oxide scavenging assay, IC_{50} (µg/ml)	Brine shrimp lethality bioassay, LC_{50} (µg/ml)
Methanolic	43.65	5.13	114.34
Petroleum ether	155.08	14.79	46.04
Ethyl acetate	72.62	14.45	309.49
Water	114.81	5.37	23.72
Ascorbic acid	18.34	4.79	-
Vincristine	-	-	2.47

Table 3: IC_{50} and LC_{50} values of different extracts in DPPH, Nitric oxide, brine shrimp lethality bioassay.

Figure 1:

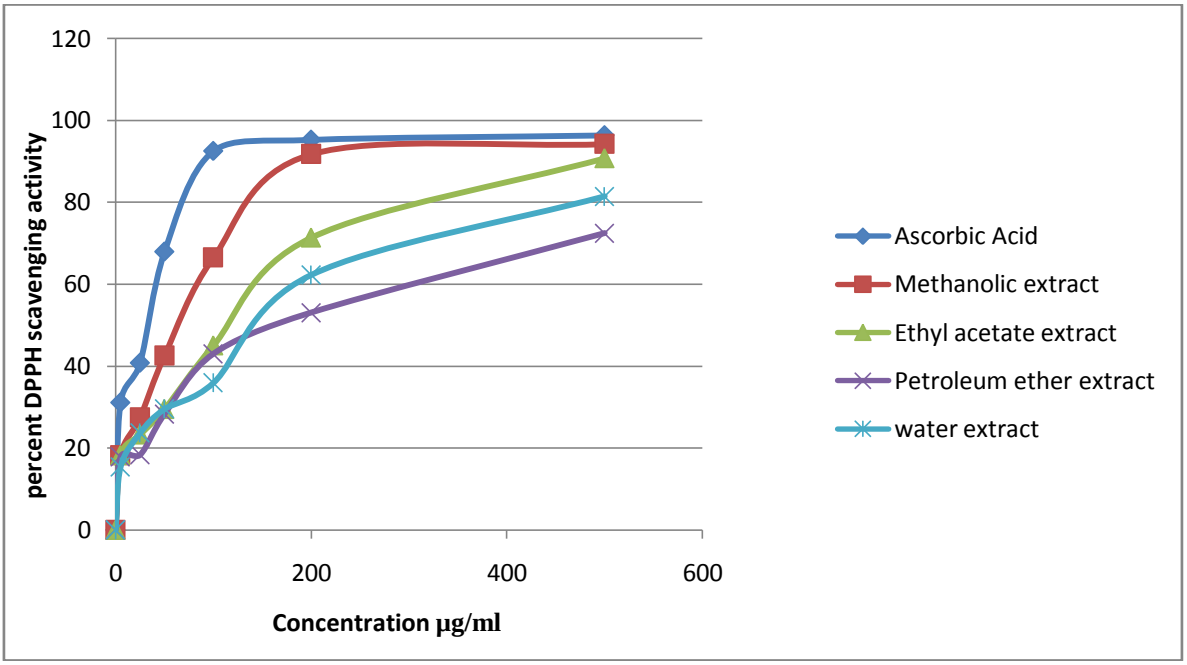

Figure 1: DPPH scavenging activity of different extracts of *Polygonum plebeium*R. Br.

Figure 2:

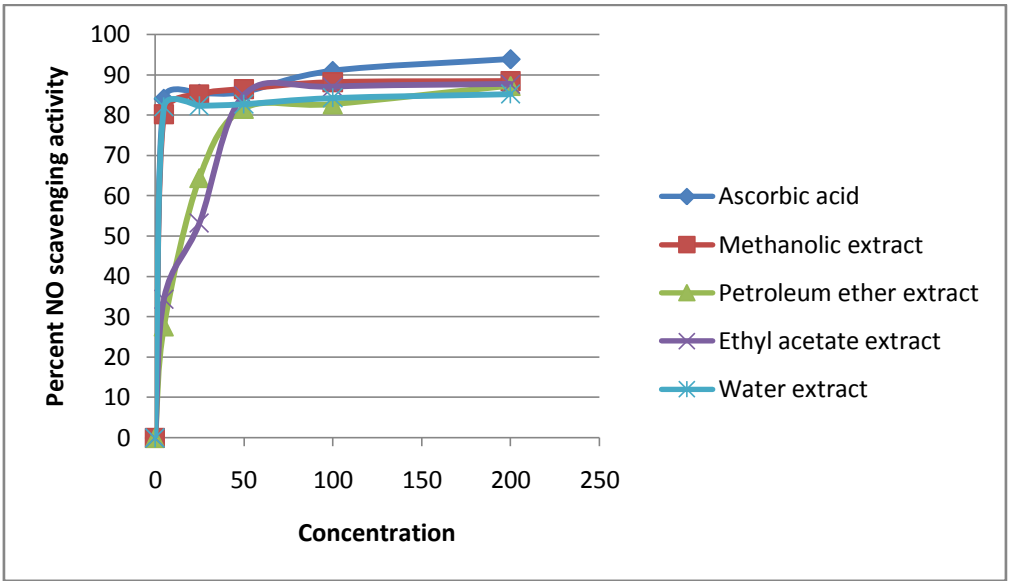

Figure 2: Nitric oxide scavenging activity of different extracts of *Polygonum plebeium R. Br.*

Figure 3:

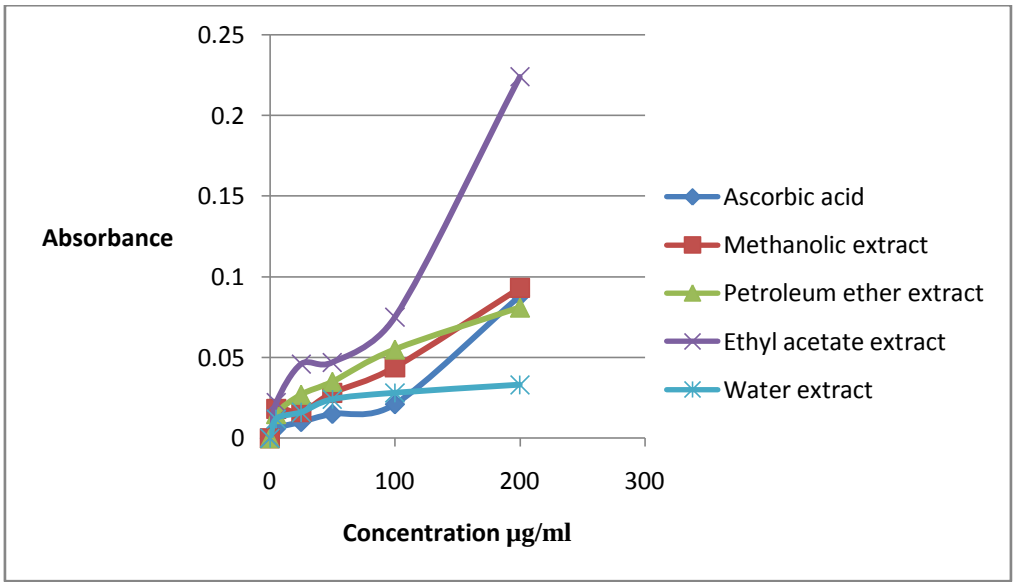

Figure 3:CUPRAC assessment of different extracts of *Polygonum plebeium* R. Br..

Figure 4:

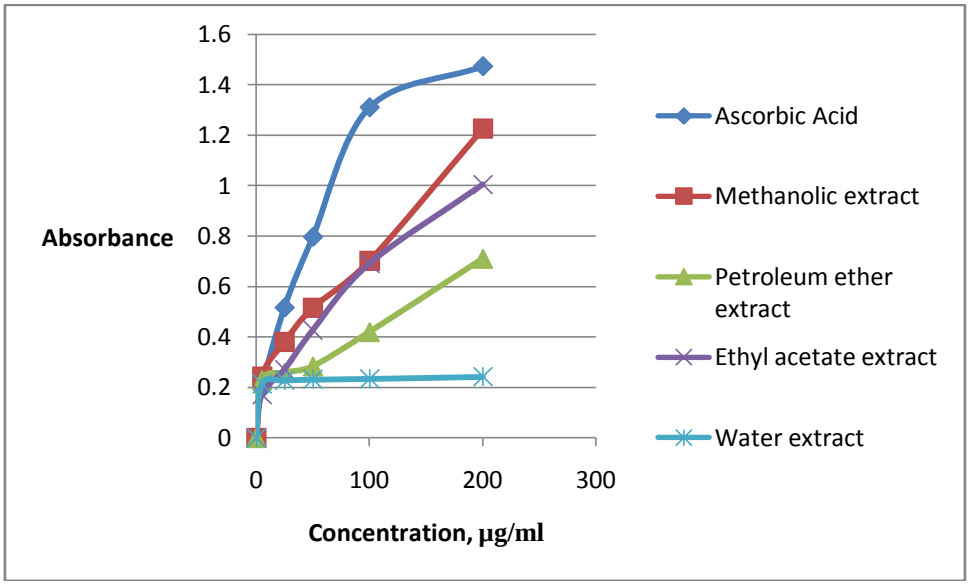

Figure 4: Reducing power capacity assessment of different extracts of *Polygonum plebeium* R. Br.

References:

Ahamd, MS., Naqvi, SS., Mushtaq, S., Ramzan, F., Sami, A., Batool, S., Haq, I., Mirza, B. 2013. Phytochemical and biological evaluation of *Polygonum amplexicaule* rhizome extracts. Canadian Journal of Pure and Applied Sciences, Vol. 7, No. 3, pp. 2491-2498.

Alam, MN., Roy, S., Anisuzzaman, SM., Rafiquzzaman, M., 2012. Antioxidant activity of the ethanolicextracts of leaves stems and fruits of *Solanum nigrum*. Pharmacognosy Communications, 2(3): 67-71.

Alhadi, EA., Khalid, HS., Alhassan, MS., Ali, AA., Babiker, SG., Alabdeen, EMZ., Kabbashi, AS. 2015. Antioxidant and cytotoxicity activity of *Cordia africana*in Sudan. Adv Med Plant Res, 3(2): 29-32.

Cannel, RJP. 1998. How to approach the isolation of a natural product, In: Natural products isolation, 1st edition (Humana press, New Jersy), pp. 1-55.

Chang, C., Yang, M., Wen, H., Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anala, 10: 178-182.

Fazel, S., Hamidreza, M., Rouhollah, G., Mohammadreza, V. 2008. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J. Pharm. Sci., 32: 17-20.

Folin, O. and Ciocalteu, V. 1927. On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry, 27: 627-650.

Ghani A, 1998. Medicinal Plants of Bangladesh, 1st Ed. pp. 63. The Asiatic Society of Bangladesh, Dhaka.

Ghani A, 2003. Medicinal Plants of Bangladesh. 2nd Edition (The Asiatic Society of Bangladesh, Dhaka, Bangladesh), pp. 31, 39-40, 418, 500-504, 589-590.

Govindarajan, R., Vijayakumar, M., Shirwikar, A., Rawat, AKS., Mehrotra, S., Pushpangadan, P. 2006. Antioxidant activity of *Desmodium gangeticum* and its phenolics in arthritic rats. Acta Pharm, 56: 489–496.

Gracia, OB., Castilo, J., Marin, FR., Ortuno, A., Rio, JAD. 1997. Uses and properties of citrus flavonoids. J. Agric. Food Chem, 45: 4505-4515.

Hasan, AN., Wahed, TB., Alam, MN., Bristy, NJ., Hossain, MS., Wahed, SB. 2014. Antioxidant activity and brine shrimp lethality bioassay of the ethanolic and water extracts of fruits and leaves of *Olea europaea*. Aust. J. Basic & Appl. Sci.,8(17): 412-420, 2014.

Hasan, MK., Kabir, AKL., Mistry, S. 2009. Chemical and Biological Investigation of Leaves of *Polygonum plebejum*. S. J. Pharm. Sci. 2(2): 66-71.

Hussain, F., Ahmad, B., Hameed, I., Dastagir, G., Sanaullah, PS. 2010. Antibacterial, antifungal and insecticidal activities of some selected medicinal plants of polygonaceae. African Journal of Biotechnology Vol. 9(31), pp. 5032-5036.

Katewa, SS. and Galav, PK., 2005. Traditional herbal medicines from Shekhawati region of Rajasthan. Indian Journal of Traditional Knowledge, Vol. 4(3), pp 237-245.

Manzocco, L., Anese, M. and Nicoli, MC., 1998. Antioxidant properties of tea extracts as affected by processing. Lebens-mittel-Wissenschaft Und-Technologie, 31: 694-698.

Meyer, BN., Ferrigni, NR., Putnam, JE., Jacobsen, LB. 1982. Brine shrimp: A convenient general bioassayfor active plant constituents. *Planta Med.,* 45: 31-34.

Oyaizu, M.1986. Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307-315.

Patwardhan, B., Vaidha, ADB., Chorghade, M., 2004. Ayurveda and natural products drug discovery. CurrSci 86: 789-799.

Prieto, P., Pineda, M., and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, 269: 337-341.

Resat, A., Kubilay, G., Mustafa, O., Saliha, EK. 2004. Novel total antioxidant capacity index of dietary polyphenols and vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. *J. Agric. Food Chem.*, 52: 7970-7981.

Sainani, GS., Manika, JS., Sainani, RG. 1997. Oxidative stress: a key factor in pathogenesis of chronic diseases. Med Update, 1: 1.

Swapna, MM., Prakashkumar, R., Anoop, KP., Manju, CN., Rajith, NP. 2011. A review on the medicinal and edible aspects of aquatic and wetland plants of India. Journal of Medicinal Plants Research Vol. 5(33), pp. 7163-7176.

The IUCN Red List of Threatened Species, http://www.iucnredlist.org/details/177128/0 (Accessed on July 22, 2015).

Vanila, D., Ghanthikumar, S., Manickam, VS. 2008. Ethnomedicinal Uses of Plants in the Plains Area of the Tirunelveli-District, Tamilnanu, India. Ethnobotanical Leaflets 12: 1198-1205.

Yildirim, A., Mavi, A., Oktay, M., Kara, AA. 2000. Comparison of antioxidant and antimicrobial activities of *Tilia.* J. Agric. Food Chem. 48, 5030-5034.

Yu-Ling, HO., Huang, SS., Deng, JS., Lin, YH., Chang, YS., Huang, GJ. 2012. In vitro antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. Botanical Studies (2012) 53: 55-66.