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# *In-vitro* investigation of antioxidant activity and phytochemical screening of *Persicaria glabra*

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**Abstract**
This study was conducted to evaluate the antioxidant property and perform the phytochemical screening of *Persicaria glabra.* The plant belongs to the Polygonaceae family and locally used analgesic agent. This study provides a scientific basis for the use of *Persicaria glabra* in traditional medicine. The whole plant was extracted using methanol. The extract was subjected to antioxidant activity determination and phytochemical screening. The phytochemical screening of *Persicaria glabra* showed the presence of alkaloids, flavonoids, glycosides, phenol, phlobatannin, resins, sterol and tannins but showed absence of tannin, resin and quinone. The total phenolic content of the methanolic extract of the leaves of *Persicaria glabra* was found to be 106.877 (mg of GAE / gm of extractives). Methanolic extract solution presented notable free radical scavenging activity with an $IC_{50}$ value of 5.524µg/ml which is comparable with the value of standard Ascorbic Acid, which provided an $IC_{50}$ value of 3.01µg/ml.

**Keywords:** Antioxidant, total phenolic content, DPPH, methanol, *Persicaria glabra*

## Introduction
Medicinal plants are an essential part of traditional medicines. The traditional drug is the collection of the knowledge, practices, and skills which are actually constructed on the theories, experiences and beliefs of indigenous to various cultures, whether explainable or not, used to keep good health as well as in the diagnosis, prevention, improvement or treatment of mental and physical illness. Traditional medicines use parts like leafs or roots to treat different diseases. Nature is the basic source of 87% of drugs used to treat all type of human diseases. About 25% of recommended drugs made from the plant. In developing countries, around 80% people depend on traditional based medications for their wellbeing [1]. In this project, I have focused on traditional plants used in Bangladesh to discover some unknown pharmacological effect of those plants like antioxidant, antimicrobial and cytotoxicity to discover a new source of drugs to treat diseases efficiently and cost effectively. Phytochemical investigations have been run on 15% medicinal plants [2]. *Persicaria glabra* is one of the traditional plants that is used as an analgesic agent in Sylhet and anti-cancer agent and the juice of this herbs is used as painkiller and leaf paste is used as an anti-cancer drug. The other species of *Persicaria* genus possesses several activities. Seeds of *Persicaria barbata* (L). are to possess antiemetic, purgative, stimulant properties and can be used to relieve colic pain [3]. Infusion of *Persicaria orientalis* (l.) is a good tonic and can be used in ulcerative colitis and remedy of Fever [4]. *Persicaria hydropiper* contains flavonoids and chalcones which show powerful antioxidant effects and can be used to reverse oxidative stress-causing pathologic conditions, for example arteriosclerosis and cancer [5]. Based on the several activities provided by the other species of *Persicaria* genus, *Persicaria glabra* as been chosen to perform the phytochemical screening and analysis of antioxidant property.

## Materials and Methods
### Plant collection and identification
The whole plant of *Persicaria glabra* was collected in the month of October 2016 from Khagrachari, Bangladesh. After that, its verification (Verification code number: 45023) was done by the National Herbarium of Bangladesh (NHB), Mirpur, Dhaka by submitting plant sample.

**Chemicals**
1, 1-diphenyl-2-picrylhydrazyl (DPPH), Potassium mercuric iodide, Iodine in potassium iodide, Dragendorff's reagent, Saturated picric acid, Tannic acid, Neutral ferric chloride, Sodium nitroprusside, Glacial acetic acid, Concentrated $H_2SO_4$, Molish's reagents, $CuSO_4$, Alcoholic KOH, Basic lead acetate, Acetic anhydride, NaOH, Chloroform, $NaNO_2$, Methanol, Folin-Ciocalteu reagent, $Na_2CO_3$ were used.

**Preparation of plant extract**
After the washing the leaves with clean water the leaves were shade dried for several days and were grounded finely as a granular particle with a high power grinding machine. About 400gm of grounded leave powder of *Persicaria glabra* which was drenched in 2L of methanol for 14 days period in a room temperature (22-25$^oC$) with random agitation. After 14 days of soaking, the substances of the bottle were emptied out first to filter them by using Whatman filter paper (pore size 100nm). The filtrate was concentrated by using rotary evaporator (Heidolph) at 30$^oC$ temperature with a rotation speed of 100rpm up to form the concentrated methanolic extract.

**Phytochemical screening**
The crude extract of *Persicaria glabra* was used for phytochemical screening to identify its chemical compound present in its leaves.

**Procedure of extract preparation for screening**
2-3 grams of dried methanol extract was mixed with 50ml methanol in a 100ml of conical flask. After that, that flask was labeled properly closing with cotton plugs and kept still for 1 to 2 hours. Later, the mixture was filtered through Whatman filter paper. Collected filtrates were used for phytochemical screening by following the standard process [6, 7]. The following qualitative tests were performed sequentially:

**Tests for Alkaloids**
**Mayer's test:** A few drops of Mayer's reagent (Potassium mercuric iodide solution) was added in 1ml of plant extract. If cream color precipitation form then it will contain the presence of alkaloids.

**Wagner's test:** In 1ml of plant extract was added with the same amount of Wagner's reagent (Iodine in potassium iodide). If reddish brown color precipitation form then it will point to the presence of alkaloids.

**Dragendorff's reagent test:** 2ml of Dragendorff's reagent was added in 1ml of plant extract and later dilute HCL of 2ml was added in that solution. If orange color precipitation forms then it will confirm the presence of alkaloids.

**Hager's test:** A few drops of Hager's reagent (Saturated picric acid solution) was added in 2ml of plant extract. If bright yellow color precipitation form then it will point to the presence of alkaloids.

**Tannic acid test:** A few drops of tannic acids was added in 1ml of plant extract. If yellow-brown colored precipitation form then it will point to the presence of alkaloids.

**$FeCl_3$ test:** About 1-2 ml extract was mixed with a little amount of neutral ferric chloride solution in dropwise. If cream yellow precipitation forms then it will point to the presence of alkaloids.

**Tests for glycosides**
**Legal's Test:** Addition of alkaline sodium nitroprusside and pyridine in extract solution results in the formation of cherry red color then it will confirm to the presence of glycosides.

**Keller Killiani test:** At first 1ml of glacial acetic acid was mixed-up with 1 ml of extract and cooled. After that 2-3 drops of ferric chloride was mixed and 2ml of concentrated $H_2SO_4$ was added carefully in sideways of test tube walls. If reddish brown colored ring at the junction of two layers form then it will point to the presence of glycosides.

**Concentrated $H_2SO_4$ test:** 1ml of Concentrated $H_2SO_4$ was added in 1ml of plant extract and kept still for 2 minutes. If reddish color precipitate form then it will point to the presence of glycosides.

**Molish's test:** In plant extract around 2-3 drops of Molish's reagents was added. Later, a few drops of concentrated $H_2SO_4$ was added properly. If reddish purple colored ring at the junction of two layers form then it will point to the presence of glycosides.

**Test for phlobatannins** At first 2-3ml of 10% HCl was added in 10ml of plant extract in a boiling test tube which was boiled for 5-6 minutes. If red color precipitate occurs then it will point to the presence of phlobatannins.

**Test for resins** 3-4ml of the $CuSO_4$ solution was mixed-up with plant extract which was shaken vigorously for 1-2 minutes and allowed to discrete. If green color precipitate occurs then it will point to the presence of resins.

**Test for quinones:** Alcoholic KOH solution was added in plant extract. If color ranging from red to blue occur then it will point to the presence of quinones.

**Test for Saponins:** In the test tube 5ml of the extract was taken and shaked vigorously to get a stable froth. 5-6 drops of olive oil were added into frothing solution. If the emulsion is formed then it will point to the presence of saponins.

**Tests for phenols**
**Ellagic acid test:** A few drops of 5% (w/v) glacial acetic acid was added in plant extract. After that 5% (w/v) $NaNO_2$ solution was added. If muddy brown color form then it will point to the presence of phenols.

**Phenol test:** 1ml of the $FeCl_3$ solution was added in 2ml of plant extract. If the development of intense color form then it will point to the presence of phenols.

**Tests for Tannins**
**Ferric chloride test:** A few drops of $FeCl_3$ was added in plant extract. If blackish color precipitate form then it will point to the presence of tannins.

**Lead acetate test:** A few drops of basic lead acetate was added in 1-2ml of plant extract. If bulky red color precipitate form then it will point to the presence of tannins.

**Alkaline reagent test:** A few drops of sodium hydroxide

solution was added in plant extract. If red color form then it will point to the presence of tannins.

**Tests for flavonoids**

**Lead-acetate test:** A few drops of basic lead acetate solution was added in 1-2ml of plant extract. If reddish brown color precipitate form then it will point to the presence of flavonoids.

**$FeCl_3$ test:** A few drops of neutral ferric chloride solution was added in 1-2ml of plant extract. If the deposition of blackish red color precipitate form then it will point to the presence of flavonoids.

**Alkaline reagent test:** A few drops of sodium hydroxide was added in 1-2ml of plant extract. If yellowish red color occurs then it will point to the presence of flavonoids.

**Test for sterols**

**Libermann-Burchard test:** A few drops of acetic anhydride solution was mixed with 1-2ml of plant extract. After that, a few drops of concentrated $H_2SO_4$ was given beside the test tube walls in the mixture. If reddish brown color ring at the junction of two layers occur then it will point to the presence of sterols.

**Salkowski test:** 5ml of chloroform was added in 1-2ml of plant extract. After that, 1ml of concentrated $H_2SO_4$ was put beside the test tube walls. If the reddish color in the lower layer occurs then it will point to the presence of sterols.

**Evaluation of Antioxidant activity**

**Determination of total phenolic content**

Generally, the antioxidative action is shown by phenolics, phenolic acid, phenolic diterpenes, and flavonoids. Chemical properties of the phenolic compounds show that they exert their antioxidative properties by redox reaction [8]. Researches show that various amount of the phytochemicals retain antioxidant capacities which might be related to lower mortality rate and lower incidence human cancer [9]. Phenols get ionized in an alkaline condition which is why the Folin-Ciocalteu reagent is used which readily gets ionized in phenolic solution. Oxidized reagent turns blue from yellow. Color change intensity is measured as absorbance at 760 nm by UV spectrophotometer. Absorbance indicates the TPC (Total Phenolic Content) of particular test compound [10]. Total phenolic content of leaves of the Total phenolic content of leaves of *Persicaria glabra* extract was measured by using the method which was designed [11] involving Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as standard [12].

**Determination of DPPH radical scavenging activity**

DPPH assay is simple and fast procedure to evaluate antioxidant activity of extract sample where its stability in the radical form is good [13]. Basic law of this assay is color change of DPPH solution from purple to yellow as the radical is quenched by antioxidant [14]. Based on the method described by Brand-Williams [15] the free radical scavenging activity or antioxidant property of plant extracts was measured using DPPH (1, 1-diphenyl-2-picrylhydrazyl) reagent. Above mentioned procedure follows the addition of extract's methanol solution (2 ml) with DPPH methanol solution (3 ml, conc. 20µg/ml). Decoloration of purple colored DPPH methanol solution by the test plant extract is compared with a standard ascorbic acid and BHT. DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants [16, 17]

3ml of methanolic solution of DPPH was mixed with 2ml of methanolic sample (extract or control) solution at a various concentration which was ranging from 500 to 0.977µg/ml. The prepared solution was kept in dark for 30 minutes at room temperature for reaction. After that, prepared solution absorbance was measured against methanol as blank by UV spectrometer at 517nm.

Percent of inhibition of free radical DPPH (I%) was calculated by given equation:

$(I\%) = (1 - A_{sample}/B_{blank}) \times 100$

In the given equation, $B_{lank}$ is denoted as absorbance of the control reaction (contain all chemical reagents except the test material).

The concentration of extract provided 50% inhibition *($IC_{50}$)* which was calculated by using the graph of inhibition percentage vs. extract concentration. In this graph we will use logartmic trendline to get an equation by which we will determine the $IC_{50}$ value for our extract sample.

General equation for calculating $IC_{50}$ is given below –

$y = mln(x) + c$

## Result and Discussion

**Phytochemical Screening**

**Table 1:** Phytochemical screening of *Persicaria glabra*

| Serial Number | Class of compound | Result |
|---|---|---|
| 1 | Alkaloid | ++++++ |
| 2 | Glycoside | + |
| 3 | Phlobatannin | + |
| 4 | Resin | - |
| 5 | Quinone | - |
| 6 | Phenol | ++ |
| 7 | Tannnin | - |
| 8 | Flavonoids | + |
| 9 | Sterol | ++ |

**Note:** (+) = presence in a single method test, (++) = presence experimented in two methods, (+++) = presence experimented in three methods, (++++) = presence experimented in four methods, (+++++) = presence experimented in five methods and (-) = absence.

**Determination of Total Phenolic Content:**

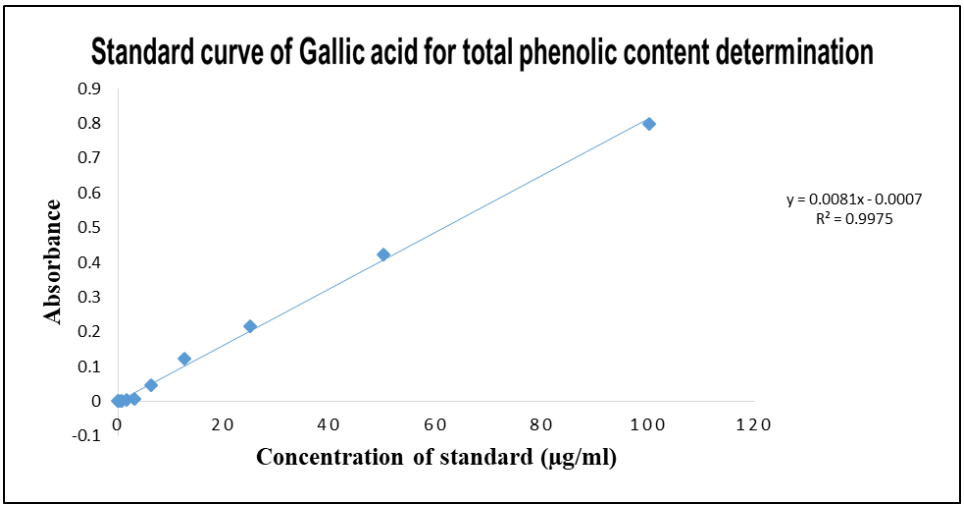


**Fig 1:** Standard curve of Gallic acid for total phenolic content determination

**Table 2:** Test samples for total phenolic content determination

| Plant part | Sample code | Test Sample | Absorbance (Y) | Total phenolic content (mg of GAE / gm of extractives) (X) |
|---|---|---|---|---|
| Leaves of *Persicaria glabra* | ME | Methanolic extract | 0.865 | 106.877 |

## DPPH Free radical scavenging assay

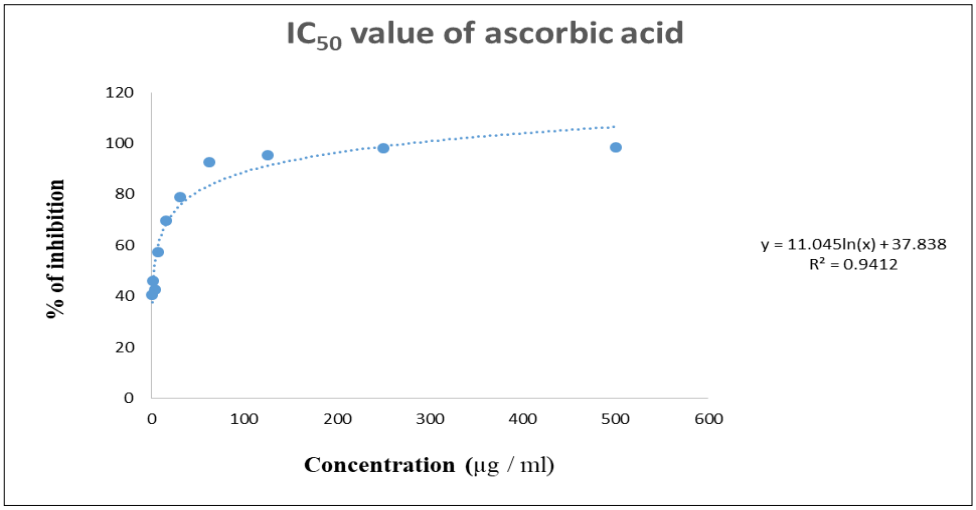


**Fig 2:** $IC_{50}$ value of ascorbic acid

**Table 1:** $IC_{50}$ value of Methanolic extracts

| Absorbance of the blank | Concentration (µg/ml) (X) | Absorbance of the extract | % Inhibition (Y) | $IC_{50}$ |
|---|---|---|---|---|
| 0.325 | 500 | 0.021 | 93.54 | 5.524 |
| | 250 | 0.028 | 91.38 | |
| | 125 | 0.042 | 87.08 | |
| | 62.5 | 0.066 | 79.69 | |
| | 31.25 | 0.083 | 74.46 | |
| | 15.625 | 0.116 | 64.31 | |
| | 7.813 | 0.144 | 55.69 | |
| | 3.906 | 0.193 | 40.62 | |
| | 1.953 | 0.209 | 35.69 | |
| | 0.977 | 0.224 | 31.08 | |

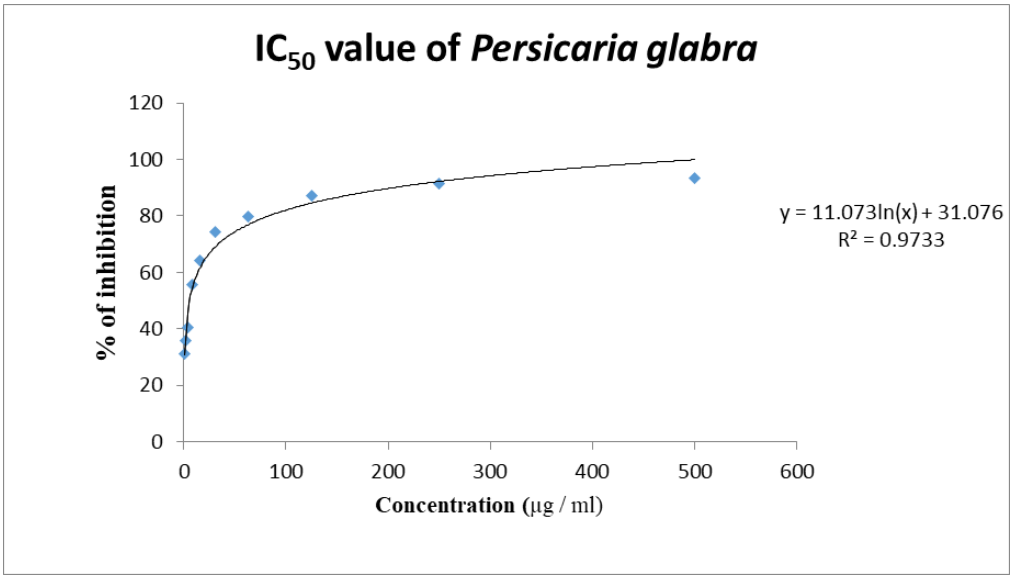


**Fig 3:** $IC_{50}$ value of ME of leaves of *Persicaria glabra*

## Discussion

The phytochemical screening of *Persicaria glabra* showed the presence of alkaloids, flavonoids, glycosides, phenol, phlobatannin, resins, sterol and tannins whereas showing the absence of tannin, resin and quinone.

The total phenolic content of the methanolic extract of the leaves of *Persicaria glabra* was found to be 106.877 (mg of GAE / gm of extractives). The Methanolic extract of the leaves of *Persicaria glabra* was tested to free radical scavenging activity by using the method suggested by Brand-Williams.[15] Reference standard was Ascorbic acid (ASA). Methanolic extract solution presented the notable free radical scavenging activity with an $IC_{50}$ value of 5.524µg/ml which is comparable with the value of standard Ascorbic Acid, which provided an $IC_{50}$ value of 3.01µg/ml.

## Conclusion

The phytochemical analysis showed the presence of several phytochemicals which can be further isolated using compound isolation. The antioxidant analysis showed *Persicaria glabra* has higher level of antioxidant property and can be used as an antioxidative agent.

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