Phytochemical Screening, GC-MS Analysis, Antioxidant, Cytotoxicity, and Anti- Inflammatory Studies of Medicinal Plant Persicaria glabra Growing in Bangladesh

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Phytochemical Screening, GC-MS Analysis, Antioxidant, Cytotoxicity, and Anti-Inflammatory Studies of Medicinal Plant *Persicaria glabra* Growing in Bangladesh

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Abstract

Persicaria glabra (Willd.) M.Gómez (Polygonaceae), commonly referred to as Lal Kukri, is an aquatic to semi-aquatic plant that is extensively used in traditional medicine. In this study, the phytochemical profile and biological activities of different solvent extracts of aerial and root part of *P. glabra* were evaluated. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, terpenoids, quinones, and steroids. GC-MS analysis identified 13 compounds in the aerial n-hexane extract, and 25 and 12 compounds in the root n-hexane and chloroform extracts, respectively, with (Z)-13-docosenamide, progesterone, and 1,3,5-tris(trimethylsiloxy)benzene as the primary constituents. The biological assays showed that extracts exhibited significant bioactivity, with the aerial chloroform extract having the highest DPPH radical scavenging activity (IC_{50} =11.29 µg/mL) and the highest total phenolic content, and the root ethyl acetate extract having the highest root antioxidant activity (IC_{50} =16.90 µg/mL). Ethyl acetate root extract and aerial extract showed moderate cytotoxicity in brine shrimp lethality assay with LC_{50} values of 17.73 µg/mL and 34.77 µg/mL respectively. Furthermore, both parts of the plant showed strong *in vitro* anti-inflammatory activity similar to the standard drug diclofenac sodium. In general, *P. glabra* is a rich source of therapeutic phytochemicals with high antioxidant, cytotoxic and anti-inflammatory potential.

Keywords: *Persicaria glabra*, phytochemical profiling, GC-MS Analysis, Antioxidant activity, Cytotoxicity, *In-vitro* anti-inflammatory activity.

1. Introduction

Natural vegetation still constitutes an invaluable source of variety of chemical structures of great therapeutic importance [1]. Traditional medicine systems, such as Ayurvedic Medicine, Unani Medicine and Traditional Chinese Medicine (TCM) have long been dependent on herbal treatments for curing ailments and maintaining health [2, 3]. Here are some of these ancient methods that have greatly influenced the development of medicines today [4, 5]. The World Health Organization (WHO) claims that, the majority of people in developing countries are still relying on the local herbal medicines for basic treatment of health problems [6]. This persistent preference has been attributed to the variety of chemical compounds, therapeutic efficacy and lesser side effects of natural products as compared with synthetic pharmaceuticals [7, 8]. Secondary

metabolites are the main factor contributing to the healing potential in plants [9]. Plants act as bio-factories to produce various compounds such as phenolics, flavonoids, alkaloids and terpenoids [10]. These include polyphenols and flavonoids, both of which are renowned for having strong antioxidant activity [11], which aids in the neutralization of reactive oxygen species (ROS) and free radicals [12] which protect cells. Furthermore, antioxidants present in plants can exhibit selective cytotoxicity towards cancerous cells, which renders them useful for cancer treatment [13,14]. Due to the adverse effects of synthetic anti-inflammatory medications, researchers are now more interested in natural alternatives based on traditional knowledge [15].

Persicaria is a genus of Polygonaceae family of flowering herbs with ~132 species commonly referred to as smartweeds throughout the world [16]. *P. barbata*, *P. hydropiper* and *P. glabra* are plentifully present in the wetlands of Bangladesh and are extensively used in folk medicine in the country [17]. These traditional claims have been partially confirmed by initial studies in the genus, which demonstrate potent anti-inflammatory, analgesic and gastroprotective activity [18]. Out of these, *Persicaria glabra* (also known as Bihagni or Lal Kukri) is a perennial erect herb that is distributed throughout the floodplains in Bangladesh, mainly in marshy floodplains. In ethnomedicine of South Asia, its leaves, stems and roots are used as medicine in skin infections and as antiseptic and carminative for treating internal inflammation and digestive ailments. Some important flavonoids (e.g., quercetin, kaempferol, rutin), phenolic acids and sterol glycosides have been identified in the plant through previous screening [19]. Although the aerial parts (leaves and stems) of *P. glabra* have received much study, systematic studies of its root system are still quite limited. Roots are in constant contact with waterlogged soil and environmental stresses, and the unique highly concentrated defensive metabolites resulting from such interactions have been little explored. In order to fill this gap, the aerial part and roots of *Persicaria glabra* are comparatively evaluated in this study. This study comprises of the following: Qualitative phytochemical screening for the identification of baseline metabolite classes. An examination using gas chromatography-mass spectrometry (GC-MS) for the identification of the volatile, non-polar constituents [20]. Antioxidant, Cytotoxic and Anti-inflammatory Activities Assays In Vitro. Finally, this study seeks to scientifically substantiate the folkloric applications of this plant and to give new scientific data for natural product drug discovery.

2. Materials & Methods

2.1 Collection and Identification of the Plant *Persicaria glabra*

The plant of *Persicaria glabra* were collected in November 2024 from Lawachara National Park, Moulvibazar, Bangladesh. The collected plant material was subsequently authenticated and designated by the National Herbarium of Bangladesh (NHB), located in Dhaka's Mirpur, through the submission of a voucher specimen. The voucher specimen number was 94756.

2.2 Extraction of Aerial parts and Roots of the *Persicaria glabra*

The aerial portions (695 g) and roots (920 g) of *Persicaria glabra* were shade dried and powdered. Cold maceration (96 h x 2 cycles per solvent) with successive use of n-hexane, chloroform, ethyl acetate, and methanol at ambient temperature was used to extract the powdered plant material. Each extract was filtered before being concentrated utilizing a rotary evaporator under lower pressure. The aerial parts produced n-hexane (5.29 g), chloroform (17.16 g), ethyl acetate (3.22 g), and methanol (8.45 g) extracts, whereas the roots produced n-hexane (2.20 g), chloroform (3.20 g), ethyl acetate (2.78 g), and methanol (26.10 g) extracts.

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical investigations were performed on different crude extracts of aerial and root parts of *Persicaria glabra* [21]. Screening was targeted towards the key classes of bioactive compounds (steroids, terpenoids, coumarins, flavonoids, alkaloids, quinones, carbs, tannins, and saponins). All qualitative colorimetric and precipitation tests were carried out following the established procedures and standard laboratory handbooks as reported by Trease and Evans [22], Harborne [23] and Sofowora [24].

2.3.1. Test for Steroids and Terpenoids (*Liebermann-Burchard Test*)

Steroidal and terpenoidal nuclei of aerial and root extracts of *P. glabra* were detected using the Liebermann-Burchard test [25]. The dry crude extract (ca. 10 mg) was dissolved in chloroform ($CHCl_3$), and a few drops of acetic anhydride were incorporated. Next, 1 mL of conc. Sulphuric acid (H_2SO_4) was gently added down the side of the reaction vessel. The appearance of the blue color in the chloroform layer, which gradually changed to green, indicated the presence of steroids. Terpenoids were confirmed by the appearance of a distinct pink layer within the chloroform phase.

2.3.2. Flavonoids Test (*Shinoda's Test*)

Shinoda's reaction was used for identification of flavone derivatives in the plant parts [26]. A quantity of 10 mg of the extract of the sample was dissolved totally in methanol of analytical grade. Small pieces of Magnesium turnings were added to the solution, and concentrated Hydrochloric Acid (HCl) was added drop by drop. Positive results for flavonoids were indicated by a pink or magenta red colour within a short time.

2.3.3. Phenolic Compound (*Ferric Chloride Test*)

Test A colorimetric ferric chloride assay was used to test the existence of phenolic compounds [27]. In each case, 10 mg of the aerial and root extracts dissolved in methanol were added to a few drops of a 2.5% aqueous solution of ferric chloride ($FeCl_3$). The change of solution color to a characteristic reddish brown was indicative of the presence of phenolic fractions.

2.3.4. Test of Coumarins

The coumarin constituents were evaluated by an alkaline reactivity test [28]. 10 mg of the extract was solubilized in methanol, and the solution was added to alcoholic potassium hydroxide (KOH). The presence of coumarins was validated by the prompt emergence of a yellow hue which decolorized systematically on the addition of concentrated HCl in the next step.

2.3.5. Test of quinone

The screening for quinoid structures was performed by adding a small volume of concentrated sulphuric acid to 10 mg of the dissolved extract in methanol [29]. The presence of quinones was verified by obtaining a clear, deep coloration in the reaction medium.

2.3.6. Alkaloid Detection Test (*Mayer's Test*)

The alkaloid profiling was done by preparing the fresh Mayer's reagent by mixing 5.00 g of potassium iodide (KI) with 1.36 g of mercuric chloride ($HgCl_2$) with 100 mL of purified water [30]. For the test, A tiny quantity of strong HCl was used to dissolve 10 mg of each extract. A clean watch glass was filled with a few drops of this acid solution, and Mayer's reagent was added along the sidewalls with a glass rod. The appearance of white gelatinous precipitate at the junction of the fluids indicated he occurrence of alkaloids.

2.3.7. Test for Saponins (*Foam Test*)

The occurrence of saponins was followed by a standardized agitation technique to assess their surfactant properties [31]. In a test vessel, a small quantity of the extract was diluted with distilled water and shaken vigorously for about 15 s. A positive saponin test was specified by the formation of a stable, persistent column of foam or froth that persisted on standing.

2.3.8. Carbohydrate test (*Molisch's test*)

The carbohydrates in *P. glabra* extracts were identified using the Molisch reaction [32]. After thoroughly shaking the extract with distilled water, it was filtered. A few drops of Molisch's reagent (5% alpha-naphthol in 95% ethanol) were introduced to the aqueous filtrate. Next, the aqueous phase was carefully covered with 1 mL of concentrated H_2SO_4. The positive carbohydrate test was confirmed by the formation of the characteristic brown or purple ring at the liquid-liquid interface.

2.3.9. Test for Tannins (*Lead Acetate Test*)

For identification of tannic compounds, 5 mL of the aqueous extract solution (preheated on a boiling water bath) was transferred into a test tube [33]. A few drops of 1% lead acetate solution were added to the solution. The appearance of a yellow or reddish precipitate immediately confirmed the presence of tannin.

2.4. GC-MS Examination

GC-MS analysis of the n-hexane and chloroform extracts of the aerial parts and root parts of *Persicaria glabra* was carried out at BCSIR Laboratories with a Shimadzu GC-17A gas chromatograph coupled to a QP-2010 Plus mass spectrometer (EI, 70 eV). Separation was conducted using a Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 μm) using helium as the carrier gas. Samples dissolved in chloroform were injected (1.0 μL; split ratio 1:10) and Oven temperature set to increase from 40 °C to 240 °C at a rate of 5 °C/min, followed by a final hold of 10 minutes. The identification of the compounds was performed by searching the mass spectra (m/z 40-550) against the NIST and Wiley spectral libraries [34].

2.5 Bioassays

2.5.1 *Tests for Determination of Antioxidant Activity*

2.5.1.1 *DPPH Free Radical Scavenging Assay*

The antioxidant activity of the aerial and root extracts *of Persicaria glabra* was tested by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using a standard method [35]. Serial concentrations of each extract and reference standard (6.25-200 µg/mL) were prepared. An aliquot of each sample (300 µL) was mixed with 0.004% methanolic DPPH solution and a UV-Visible spectrophotometer was employed to quantify the absorbance at 517 nm subsequent to the sample being incubated in the dark at room temperature for 30 minutes. Using the following formula, radical scavenging activity was measured [36]:

$$\% \text{ Inhibition} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100$$

where the absorbance of the test and control samples is denoted by A_{sample} and $A_{control}$, respectively. The IC_{50} values were obtained from the linear regression of the percentage inhibition against concentration [37, 38]. All studies were performed in triplicate and the results are given as mean values.

2.5.1.2 *Evaluation of Total Phenolic Concentration*

The total phenolic content (TPC) in the root and aerial extracts of *Persicaria glabra* was assessed using the Folin-Ciocalteu colorimetric technique [39]. In brief, 300 µL of each extract or gallic acid standard (6.25-200 µg/mL) was mixed with diluted Folin-Ciocalteu reagent (1:10), and the mixture was incubated for 3-5 min, and then 7.5% sodium carbonate was added. 765 nm was used to measure absorbance using a UV-visible spectrophotometer following incubation in the dark for 30 min at room temperature. Total phenolic content was calculated as mg of gallic acid equivalents (GAE) per g of dry extract (mg GAE/g) using the following equation [40, 41]:

$$\boldsymbol{C = \frac{c \times V}{m}}$$

Where, C symbolizes the absolute phenolic content expressed in mg GAE/g of dry extract, c denotes the absolute concentration of gallic acid (mg/mL) extrapolated directly from the standard calibration curve, V signifies the total volume (mL) of the sample solution. m indicates the exact starting weight (g) of the dry crude plant extract.

2.5.1.3 *Estimation of Total Flavonoid Content (TFC)*

The total flavonoid content (TFC) of aerial and root extracts of *Persicaria glabra* was determined by the aluminum chloride ($AlCl_3$) colorimetric method as described by Wang and Jiao [42]. In brief, 0.5 mL of extracts or quercetin standard (6.25-200 µg/mL) was mixed with ethanol, 10% $AlCl_3$, 1 M of potassium acetate and distilled water. Following a 40-minute incubation at ambient temperature, absorbance was assessed at 415 nm with a UV-visible spectrophotometer. A blank was simultaneously prepared where the sample was substituted with the extraction solvent. The results were reported in quercetin equivalents (QE) by the standard calibration curve. The absolute flavonoid yield was quantified as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g) using the following mathematical formulation [43]:

$$\boldsymbol{C = \frac{c \times V}{m}}$$

Where C is the total flavonoid content expressed as mg QE/g of dry raw extract, c is the concentration of quercetin (mg/mL) obtained from a standard linear calibration curve, V is the total volume (mL) of the operational extract solution, m is the dry mass (g) of the crude plant extract used.

2.5.1.4 Total Antioxidant Capacity (TAC) Determination

The total antioxidant capacity of the aerial and root extracts of *Persicaria glabra* was determined by the phosphomolybdenum assay of Prieto *et al*. [44]. In brief, each extract or standard (3.125-200 µg/mL) was combined with 3.0 mL of phosphomolybdenum reagent and incubated at 95 °C for 90 min. The absorbance was measured at 695 nm relative to a reagent blank using a UV-Visible spectrophotometer after cooling to ambient temperature [45]. The cumulative antioxidant capacity was calibrated and reported as mg of ascorbic acid equivalents (AAE) per g of dry extract (mg AAE/g) according to the following mathematical model:

$$C = \frac{c \times V}{m}$$

C indicates the absolute total antioxidant capacity (mg AAE/g of dry extract), c represents the equivalent conc. of ascorbic acid derived from the linear calibration curve (mg/mL), V denotes the operational volume of the plant extract matrix (mL), m signifies the initial dry weight of the crude plant extract utilized (g).

2.5.2 *Cytotoxicity Assay*

Brine Shrimp Lethality Bioassay

The cytotoxic activity of the aerial and root extract of *Persicaria glabra* was evaluated using the *Artemia salina* (brine shrimp) lethality test [46]. *A. salina* cysts were incubated in artificial seawater (38 g/L sea salt) for 48 h with constant aeration and illumination. Test concentrations were obtained by serial dilution of stock extract solutions (1600 µg/mL) prepared in DMSO. Ten active nauplii were exposed to each concentration for 24 h. The positive control was Vincristine sulfate (0.313-10 µg/mL), and the negative control was 50 µL DMSO in seawater. After the incubation, the number of surviving nauplii was determined, and the cytotoxicity was expressed as the LC_{50} value. The mortality rate was computed using the following formula:

$$\%\ Mortality = \frac{No.of\ nauplii\ taken - No.of\ nauplii\ alive}{No.of\ nauplii\ \ taken} \times 100$$

Median lethal concentration (LC_{50}) values were resolved via linear regression analysis by illustrating the % mortality vs the corresponding log concentration values of the plant extracts [47].

2.5. 3 *Estimation of Anti-inflammatory Activity* (*Inhibition of Egg Albumin Denaturation*)

Assessment of anti-inflammatory activity of aerial and root extracts of *Persicaria glabra* was done using egg albumin denaturation test with minimal modifications [48]. In brief, 1.0 mL of either extract or diclofenac sodium (100-800 µg/mL) was combined with 1.0 mL of 1 mM egg albumin, incubated at 27 ± 1 °C for 15 minutes, and subsequently heated at 70 °C for 10 minutes. The inhibition of protein denaturation was ascertained through measurement the absorbance at 660 nm in a UV-Visible spectrophotometer after cooling down. The capacity of the samples to inhibit protein denaturation was computed using the subsequent equation:

$$\% \, inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where, $A_{control}$ signifies the control solution's absorbance (with all reagents except the plant fractions) and A_{sample}represents absorbance of sample under test or standard. All trials were conducted in triplicates (n = 3), and the values are documented as the average results.

3. Results & discussion

3.1 *Preliminary Phytochemical Screening of Extracts*

Table-3.1: Phytochemical Profiling of *Persicaria glabra* Aerial Extracts

Tests for	n-Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1. Steroids	+	+	+	+
2. Terpenoids	+	+	+	+
3. Flavonoids	+	+	+	+
4. Phenolic Compounds	+	+	+	+
5. Coumarins	-	+	+	+
6. Quinones	-	+	+	+
7. Alkaloids	-	+	+	+
8. Saponines	-	-	-	+
9. Carbohydrates	-	-	-	+
10. Tannins	-	-	-	+

Table-3.2 Phytochemical Characterization of *P. glabra* Root Fractions

Tests for	n-Hexane extract	$CHCl_3$ extract	Ethyl acetate extract	Methanol extract
1. Steroids	+	+	+	+
2. Terpenoids	+	+	+	+
3. Flavonoids	-	+	+	+
4. Phenolic compounds	-	+	+	+
5. Coumarins	-	+	+	+
6. Quinones	-	+	+	+
7. Alkaloids	-	+	+	+
8. Saponines	-	-	-	+
9. Anthra-quinones	-	+	+	+
10. Carbohydrates	-	-	-	+
11. Tannins	-	-	-	+

Note: Presence and absence are indicated by (+) and (-) respectively.

Qualitative screening of *Persicaria glabra* extracts showed that steroids and terpenoids were ubiquitous across all fractions, while saponins, tannins, and carbohydrates were strictly restricted to methanol. Mid-polarity compounds (quinones, coumarins, alkaloids, and root polyphenols) were present in all phases except n-hexane, although aerial phenolics/flavonoids extended into all fractions. Notably, anthraquinones appeared as a tissue-specific trait, detected exclusively within the mid-to-high polarity root extracts.

3.2 GC-MS Study of the Plant Extracts

Characterization of the extract constituents involved a comparative spectral analysis using the NIST mass spectral library (>62,000 entries). By correlating the fragmentation patterns of the unidentified compounds with the library's standard reference spectra, the specific chemical entities were successfully identified.

3.2.1 GC-MS Evaluation of *Persicaria glabra* Aerial Part *n*-Hexane Extract

A total of 13 chemical constituents were successfully characterized and quantified within the n-hexane extract of *Persicaria glabra* aerial parts via GC-MS analysis. The corresponding chromatographic and mass spectral data, including retention times, molecular formulas, molecular weights, and relative percentage compositions, are summarized in Table–3.3.

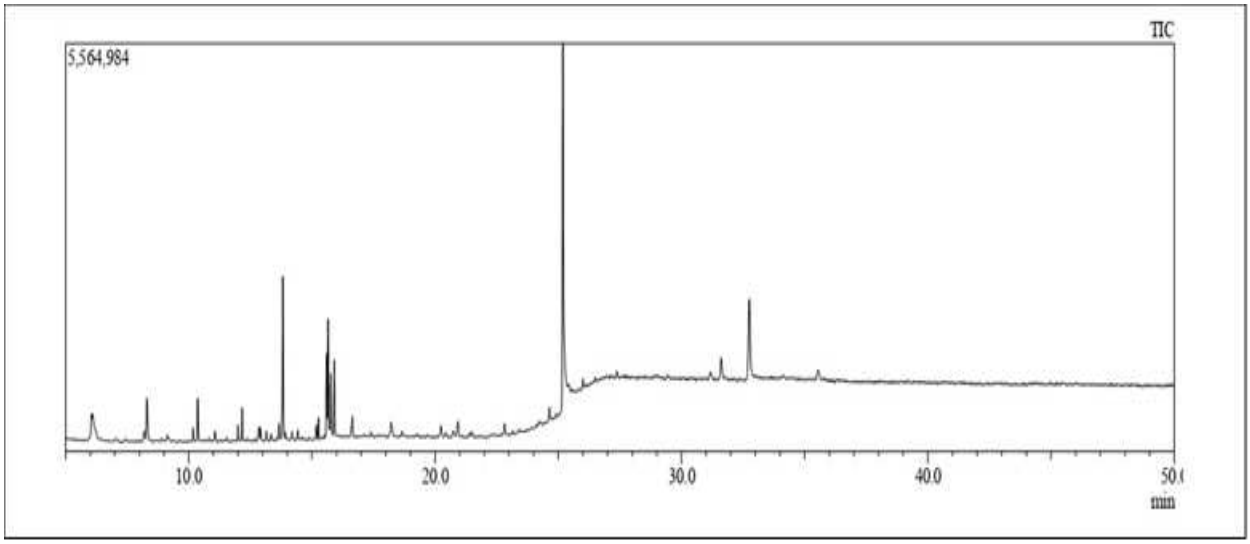


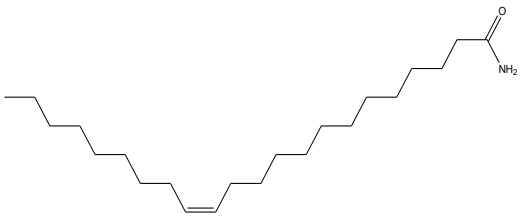
Figure 3.1: TIC of the n-Hexane Extract of Aerial Parts of *P. glabra*

Table 3.3: GC-MS Analysis of the n-Hexane Extract of the Aerial Parts of *P. glabra*

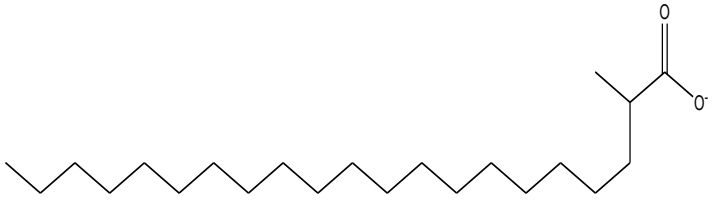
Symbol	Retention Time	Name of the Compound	Molecular Weight (g/ml)	Molecular Formula	Conc.% ug/mL
P-1	6.098	N, N-dimethyl-methanamide	73.093	C_3H_7NO	2.12
P-2	8.303	Tetradecamethyl-cycloheptasiloxane	519.078	$C_{14}H_{42}O_7Si_7$	2.33
P-3	9.165	D-Alanine	89.093	$C_3H_7NO_2$	0.58
P-4	10.365	Hexadecamethyl-cyclooctasiloxane	593.232	$C_{16}H_{48}O_8Si_8$	1.47
P-5	11.030	Octodrine	129.243	$C_8H_{19}N$	0.71
P-6	11.345	3-Hydroxybutyrolactone	102.088	$C_4H_6O_3$	0.73

P-7	13.003	Arginine	174.201	$C_6H_{14}N_4O_2$	1.26
P-8	13.823	Methyl-heneicosanoate	340.584	$C_{22}H_{44}O_2$	19.61
P-9	15.657	Dihomo-γ-linolenic acid (DGLA)	306.483	$C_{20}H_{34}O_2$	5.92
P-10	15.765	Phytol	296.531	$C_{20}H_{40}O$	3.34
P-11	15.906	Methyl stearate	298.504	$C_{19}H_{38}O_2$	3.03
P-12	25.188	(Z)-13-Docosenamide	337.583	$C_{22}H_{43}NO$	42.78
P-13	32.281	Campesterol	400.680	$C_{28}H_{48}O$	1.46

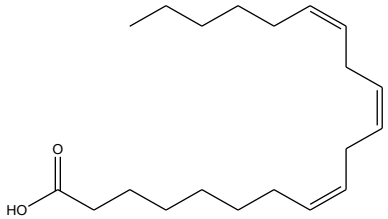
The total number of detected components can be seen from Table-3.3. The total amount of recognized substance was around 85% while the composition of about 15% was unidentifiable. The main chemicals identified here were (Z)-13-Docosenamide (P-12, 42.78%), Methyl-heneicosanoate (P-8, 19.61%), Dihomo-γ-linolenic acid (DGLA) (P-9, 5.92%), Phytol (P- 10, 3.34%), Methyl stearate (P-11, 3.04%).



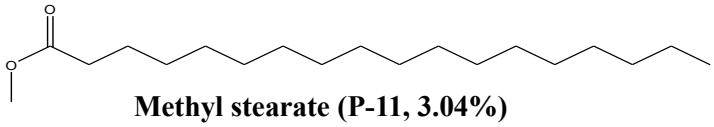
(Z)-13-Docosenamide (P-12,



Methyl-heneicosanoate (P-8, 19.61%)



Dihomo-γ-linolenic acid (DGLA) (P-9, 5.92%)



Methyl stearate (P-11, 3.04%)

Figure 3.2: Chemical Structures of the Predominant Constituents Identified in the *n*-Hexane Extract from *P. glabra* Aerial Parts.

3.2.2 GC-MS Evaluation of *Persicaria glabra* Root Part *n*-Hexane Extract

GC–MS analysis allowed us to identify and quantify 25 chemicals found in the n-hexane extract of *Persicaria glabra* roots. Table 3.4 lists the retention time, chemical formula, molecular weight, and % composition of the compounds identified in the sample of *P. glabra*.

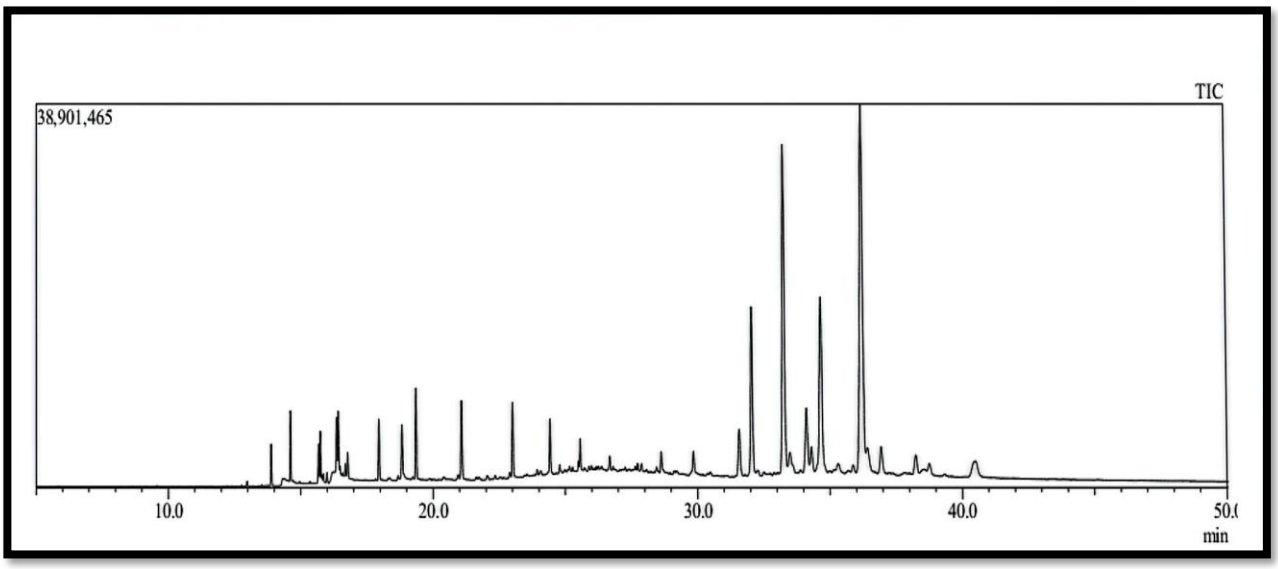


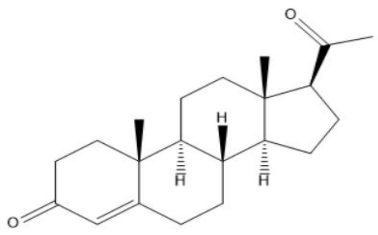
Figure 3.3: TIC of the n-hexane Extract of Root Parts of *Persicaria glabra*

Table 3.4: GC-MS Analysis of the n-hexane Extract of the Roots of *Persicaria glabra*

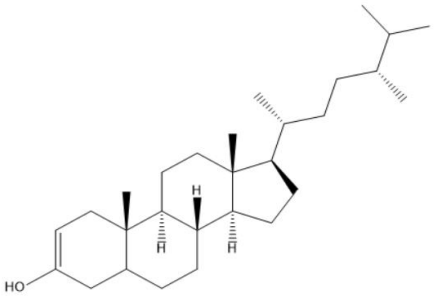
Symbol	Retention Time	Name of the Compound	Molecular Weight (g/mol)	Molecular Formula	Conc.%
P-1	13.887	Methyl stearate	298.503	$C_{19}H_{38}O_2$	2.63
P-2	14.616	Ethyl Octadecanoate	312.530	$C_{20}H_{40}O_2$	4.10
P-3	15.677	Methyl (11E,14E)-11,14-octadecadienoate	294.5	$C_{19}H_{34}O_2$	0.98
P-4	15.742	Cis-9-Hexadecenal	238.408	$C_{16}H_{30}O$	1.20
P-5	16.42	(8Z)-Oxacycloheptadec-8-en-2-one	252.392	$C_{16}H_{28}O_2$	1.61
P-6	16.687	Oleic acid	282.47	$C_{18}H_{34}O_2$	0.79

P-7	16.775	Tetracosane	338.653	$C_{24}H_{50}$	0.82
P-8	17.961	Nonacosane	408.7867	$C_{29}H_{60}$	2.02
P-9	18.829	Cyclohexanecarboxamide	127.184	$C_7H_{13}NO$	1.98
P-10	19.358	9-Methyl-nonadecane	282.547	$C_{20}H_{42}$	1.31
P-11	21.077	Pentatriacontane	492.946	$C_{35}H_{72}$	4.12
P-12	23.014	Heptacosane	380.733	$C_{27}H_{56}$	3.12
P-13	24.429	Oxirane	44.052	C_2H_4O	4.18
P-14	25.562	Phytol	296.531	$C_{20}H_{40}O$	5.08
P-15	26.679	Methyl 2-oxooctadecanoate	312.487	$C_{19}H_{36}O_3$	1.30
P-16	27.727	9-Dodecyltetradecahydro-anthracene	360.659	$C_{26}H_{48}$	2.11
P-17	27.884	1-Heptacosanol	396.732	$C_{27}H_{56}O$	0.46
P-18	28.626	1H-3a,6-Epoxyazulen-7-ol	238.3657	$C_{15}H_{26}O_2$	2.55
P-19	29.847	Cholest-4-ene-3,6-dione	386.653	$C_{27}H_{42}O_2$	0.90
P-20	31.576	Stigmasterol	412.6907	$C_{29}H_{48}O$	0.87
P-21	32.056	5,6-Dihydroergosterol	398.675	$C_{28}H_{46}O$	5.89
P-22	33.278	Campesterol	400.680	$C_{28}H_{48}O$	10.52
P-23	34.128	17α-Methyltestosterone	302.451	$C_{20}H_{30}O_2$	3.21
P-24	34.681	17β-Hydroxy-17α-methyl-5α-androstan-3-one	304.466	$C_{20}H_{32}O$	8.77
P-25	36.244	Progesterone	314.461	$C_{21}H_{30}O_2$	24.18

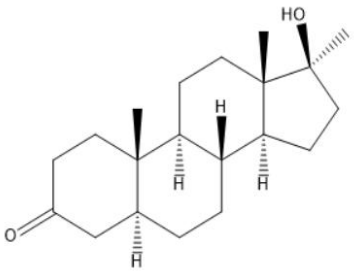
Table 3.4 shows that 25 compounds, accounting for 94.43% of the extract, were identified, while about 5.40% remained unidentified. The major compounds identified were Progesterone (P-25, 24.18 %), Campesterol (P-22, 10.52 %), 17β-Hydroxy-17α-methyl-5α-androstan-3-one (P-24, 8.77 %), 5,6-Dihydroergosterol (P-21, 5.89 %) and Phytol (P-14, 5.08 %).



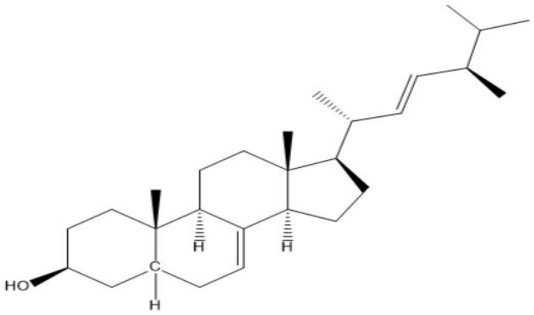
Progesterone (P-25, 24.18 %)



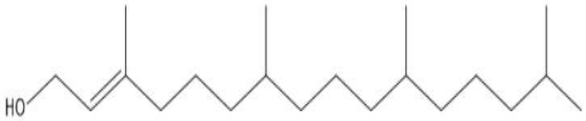
Campesterol (P-22, 10.52 %)



17β-Hydroxy-17α-methyl-5α-androstan-3-one (P-24, 8.77 %)



5,6-Dihydroergosterol (P-21, 5.89 %)



Phytol (P-14, 5.08 %)

Figure 3.4: Structure of Major Identified Compounds from n-hexane Extract of Root Parts

3.2.3 GC-MS Analysis of the Chloroform Extract of Root of *Persicaria glabra*

The GC-MS analysis made it possible to identify and quantify 12 chemicals in the chloroform extract produced from Persicaria glabra roots. Table 3.5 summarizes the retention duration, molecular formula, molecular weight, and relative % content of identified chemicals in *P. glabra.*

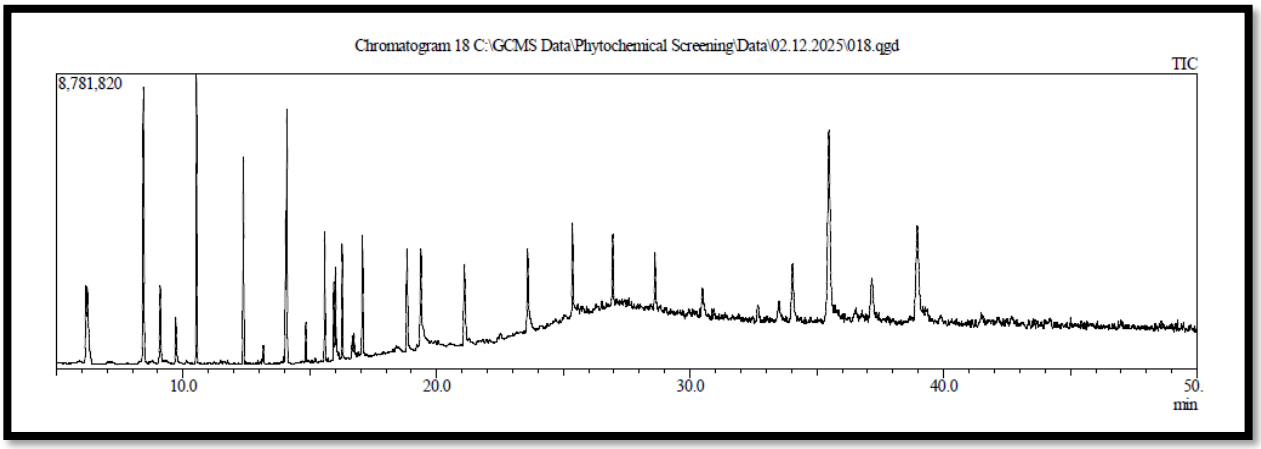
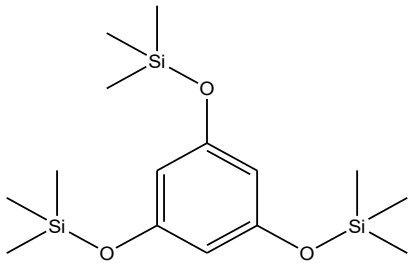


Figure 3.5: TIC of the Chloroform Extract of Root Parts of *Persicaria glabra*

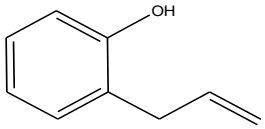
Table 3.5: GC-MS Analysis of the Chloroform Extract of the Roots of *Persicaria glabra*

Symbol	Retention Time	Name of the Compound	Molecular Weight (g/mol)	Molecular Formula	Conc.%
PG-1	6.214	1,3,5-Tris(trimethylsiloxy)benzene	342.6534	$C_{15}H_{30}O_3Si_3$	34.31
PG-2	9.707	2-Allylphenol	134.175	$C_9H_{10}O$	6.23
PG-3	12.378	7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one	285.612	$C_{16}H_{14}ClN_2O$	11.17
PG-4	14.046	Methyl tridecanoate	228.370	$C_{14}H_{28}O_2$	10.57
PG-5	15.946	(E, E)-9,12-Octadecadienoate	294.472	$C_{19}H_{34}O_2$	3.94
PG-6	16.013	cis-9-Hexadecenal	238.408	$C_{16}H_{30}O$	2.82
PG-7	16.677	cis-8, 11, 14-Eicosatrienoic Acid	306.482	$C_{20}H_{34}O_2$	1.16
PG-8	19.378	Cyclohexanecarboxamide	127.184	$C_7H_{13}NO$	1.90
PG-9	23.608	Benzeneethanamine	121.179	$C_8H_{11}N$	1.46
PG-10	35.476	Cholest-5-en-3-ol (3β)-, acetate	428.690	$C_{29}H_{42}O_2$	5.90
PG-11	37.341	Cyclobarbital	236.267	$C_{12}H_{16}N_2O_3$	2.06
PG-12	39.191	Testosterone enanthate	400.594	$C_{26}H_{40}O_3$	1.26

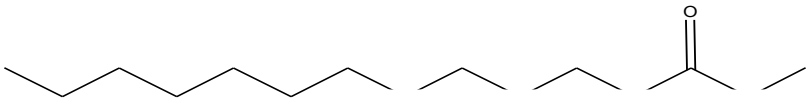
Table 3.5 shows the total number of discovered components. The total number of discovered compounds was around 82.78 %, while nearly 17.22 % of the composition remained unidentified. The major compounds identified were 1,3,5-Tris (trimethylsiloxy) benzene (PG-1, 34.31 %), 2H-1,4-Benzodiazepin-2-one, 7-Chloro-1,3-dihydro-1-methyl-5-phenyl (PG-3, 11.17 %), Methyl tridecanoate (PG-4, 10.57 %), 2-Allylphenol (PG-2, 6.23 %) and Cholest-5-en-3-ol (3β)-, acetate (PG-10, 5.90 %).



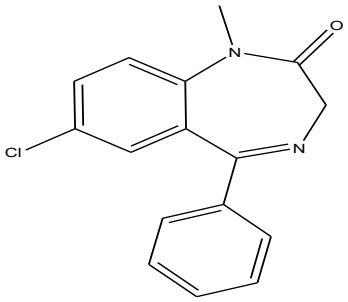
1,3,5-Tris(trimethylsiloxy)benzene (PG-1, 34.31 %)



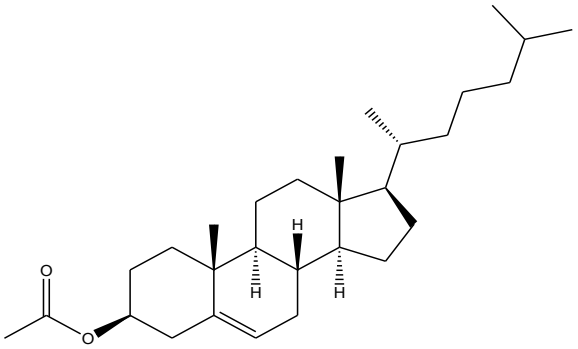
2-Allylphenol (PG-2, 6.23 %)



Methyl tridecanoate (PG-4, 10.57 %)



7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (PG-3, 11.17 %)



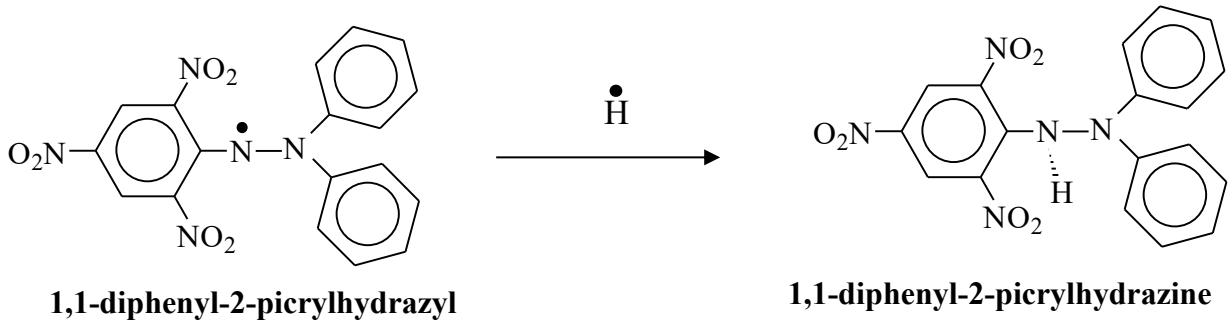
Cholest-5-en-3-ol (3β)-, acetate (PG-10, 5.90 %)

Figure 3.6: Structure of Major Identified Compounds from $CHCl_3$ Extract of Root Parts of *Persicaria glabra*

3.3 Bioassays of Extracts from the Roots and Aerial Parts of *Persicaria glabra*

3.3.1.1 *DPPH Radical Scavenging Activity*

The antioxidant efficiency of the various fractions obtained from both the roots and aerial parts of *Persicaria glabra* was examined by stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. The radical scavenging profiles and the corresponding IC_{50} values for the distinct root and aerial part extracts are detailed in the figure below. **[Table SM.1-11 & Figure SM. 1-9]**



1,1-diphenyl-2-picrylhydrazyl **1,1-diphenyl-2-picrylhydrazine**

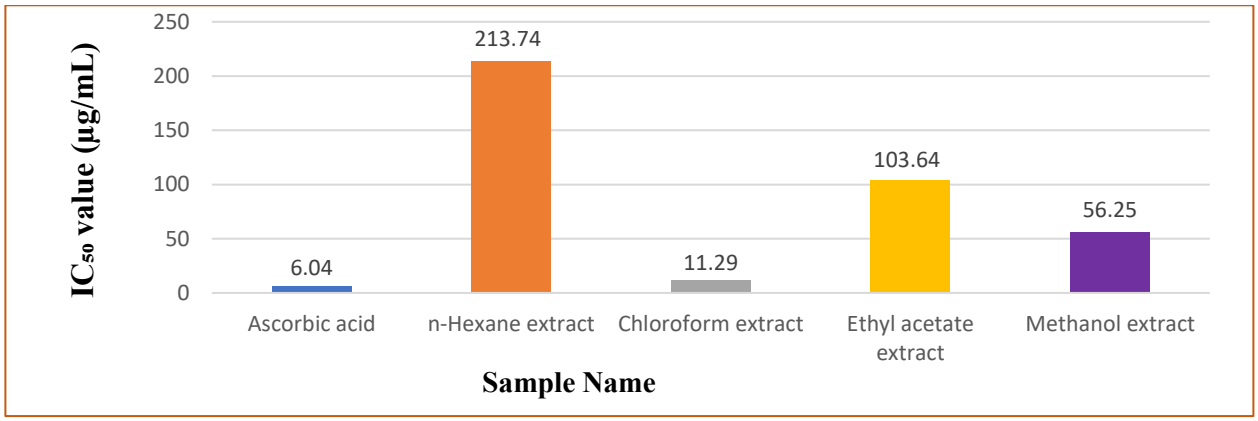


Figure 3.7: IC_{50} values for standard and other extracts of aerial parts of *Persicaria glabra*

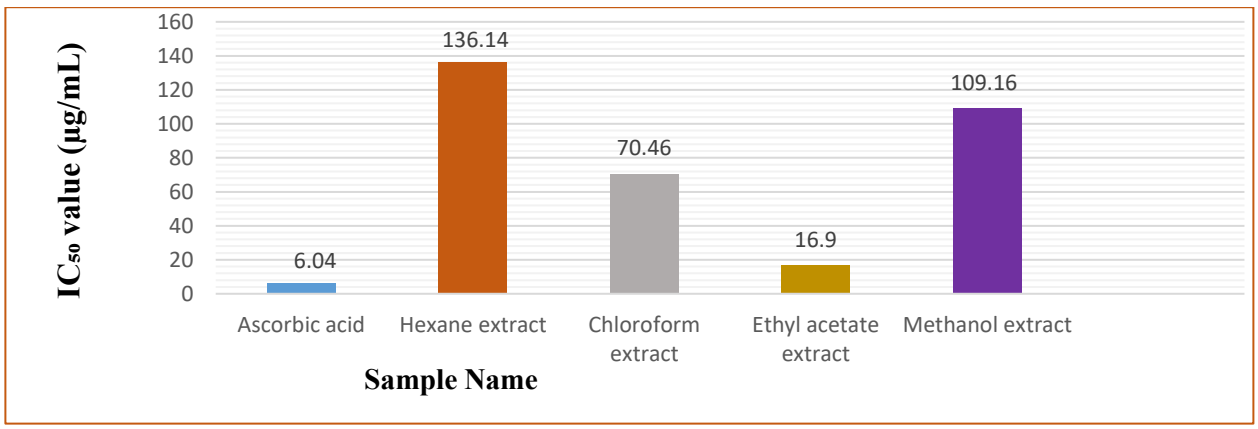


Figure 3.8: IC_{50} Values of Standard and Different Extracts of Root Parts of *Persicaria glabra*

The DPPH radical scavenging reaction is a regularly used method to measure plant antioxidant activity [49,50]. This discoloration will be more or less depending on the scavenging efficiency of the sample and a lower IC_{50} will indicate higher antioxidant activity. The comparative performance of the antioxidant activity of *Persicaria glabra* extracts was found to be different with the ascorbic acid as standard with IC_{50} values from 6.04 µg/mL to 67.45 µg/mL. In the aerial parts the chloroform fraction exhibited the highest radical scavenging activity with IC_{50} value of 11.29 µg/mL, Whereas, the methanol extract showed IC_{50} value of 56.25 µg/mL, the ethyl acetate and n-hexane fractions were less effective. On the other side, the ethyl acetate fraction exhibited the highest activity (IC_{50} = 16.9 µg/mL), chloroform fraction moderate activity (IC_{50} = 76.46 µg/mL) and methanol and n-hexane fractions showed the least activity. The results obtained indicate that the medium polar solvents were able to concentrate the most important antioxidants polyphenols and flavonoids from *P. glabra*.

3.3.1.2 Total Phenolic Content Determination

The total phenolic content (TPC) of different root extracts of *Persicaria glabra* was evaluated by Folin-Ciocalteu technique. Phenolic content was measured and represented as mg GAE/g of extract [51]. The quantity of phenolic compounds in each extract was determined by utilizing the calibration curve of gallic acid. The obtained results are illustrated in Figure 3.9 **[Table SM.12]**

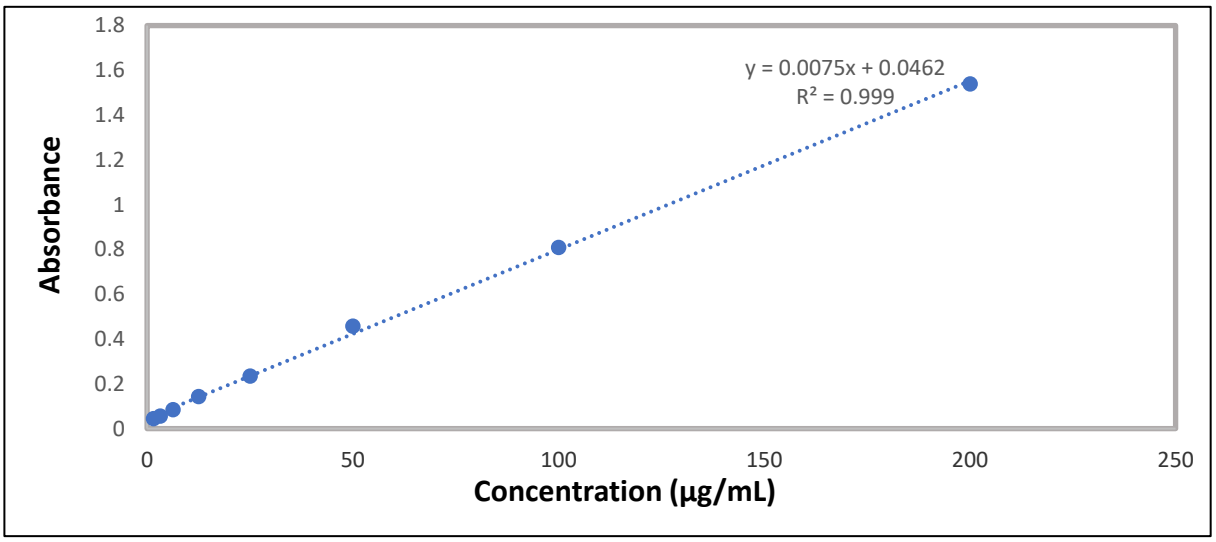


Figure 3.9: Calibration Curve of Gallic Acid (Both Aerial & Root extracts)

Table 3.6: Total Phenolic Contents Present in Extracts of the Aerial Parts of *Persicaria glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	GAE conc. (C) (µg/ml)	GAE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TPC as GAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.418	0.0002	5.707	0.006	1	0.006	247.87	250.20±3.30
	0.425	0.0002	7.040	0.007	1	0.007	252.53	
Chloroform extract	0.565	0.0002	16.907	0.017	1	0.017	345.87	348.87±4.24
	0.574	0.0002	17.307	0.017	1	0.017	351.87	
Ethyl acetate extract	0.496	0.0002	35.707	0.036	1	0.036	299.87	296.53±4.71
	0.486	0.0002	34.507	0.035	1	0.035	293.20	
Methanol extract	0.509	0.0002	26.107	0.026	1	0.026	308.53	304.20±6.13
	0.496	0.0002	24.907	0.025	1	0.025	299.87	

Table 3.7: Total Phenolic Contents Present in Extracts of the Root Parts of *Persicaria glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	GAE conc. (C) (µg/mL)	GAE conc. (C) (mg/mL)	V (mL)	c*V (mg)	TPC as GAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.077	0.0002	4.107	0.004	1	0.004	20.53	21.87±1.89
	0.081	0.0002	4.640	0.005	1	0.005	23.20	
Chloroform extract	0.137	0.0002	12.107	0.012	1	0.012	60.53	62.20±2.36
	0.142	0.0002	12.773	0.013	1	0.013	63.87	
Ethyl acetate extract	0.129	0.0002	11.040	0.011	1	0.011	55.20	57.87±3.77
	0.137	0.0002	12.107	0.012	1	0.012	60.53	
Methanol extract	0.169	0.0002	16.373	0.016	1	0.016	81.87	84.87±4.24
	0.178	0.0002	17.573	0.018	1	0.018	87.87	

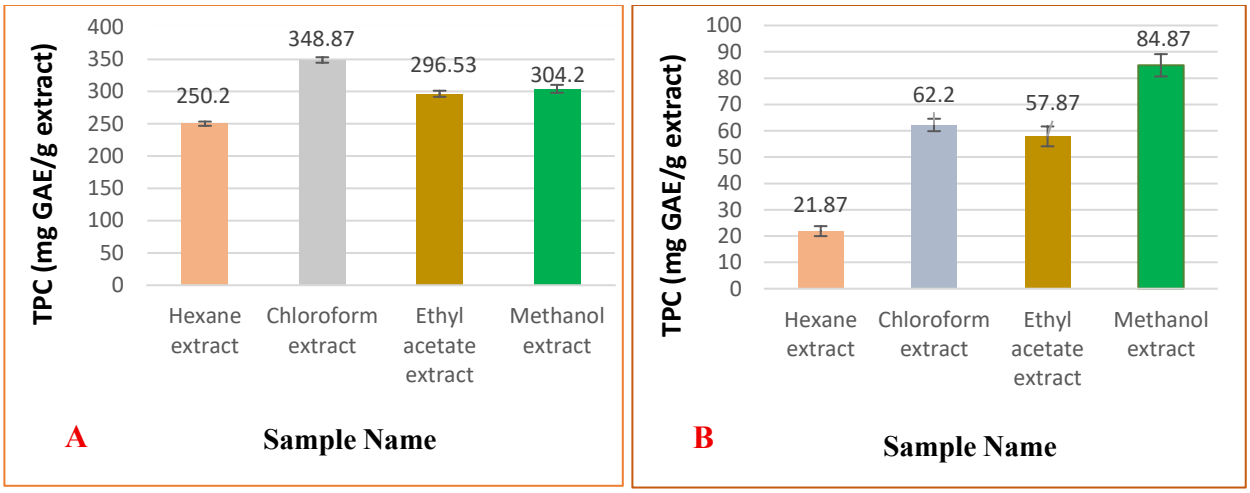


Figure 3.10: Comparative Bar Chart of Total Phenolic Contents (TPC) Present in Various Extracts of *P. glabra* :(A) Aerial Part Extracts and (B) Root Part Extracts

The results of the present study within the aerial parts, the non-polar to mid-polar chloroform extract demonstrated the highest phenolic accumulation (348.87±4.24 mg/g), surpassing the methanol (304.20±6.13 mg/g), ethyl acetate (296.53±4.71 mg/g), and n-hexane extracts (250.20±3.30 mg/g). Conversely, the root parts exhibited an entirely different trend, where the highly polar methanol extract yielded the maximum TPC (84.87±4.24 mg GAE/g), followed by chloroform (62.20±2.36 mg GAE/g), ethyl acetate (57.87±3.77 mg GAE/g), and n-hexane (21.87±1.89 mg GAE/g). It is noteworthy that the overall phenolic concentration in the aerial parts is drastically higher than that of the root parts across all corresponding solvents.

3.3.1.3 *Determination of Total Flavonoid Content*

Aerial and root extracts of *Persicaria glabra* were tested for total flavonoid concentration (TFC) using an aluminum chloride colorimetric assay. Flavonoids were estimated using the quercetin calibration curve and reported as milligrams of quercetin equivalents (QE) per gram of extract. Flavonoids are important antioxidant compounds, and their content is often associated with the antioxidant potential of plant extracts. In the $AlCl_3$ colorimetric assay, flavonoids form a yellow-colored complex with aluminum chloride, and the resulting absorbance is measured spectrophotometrically to estimate the flavonoid content**. [Table SM.13]**

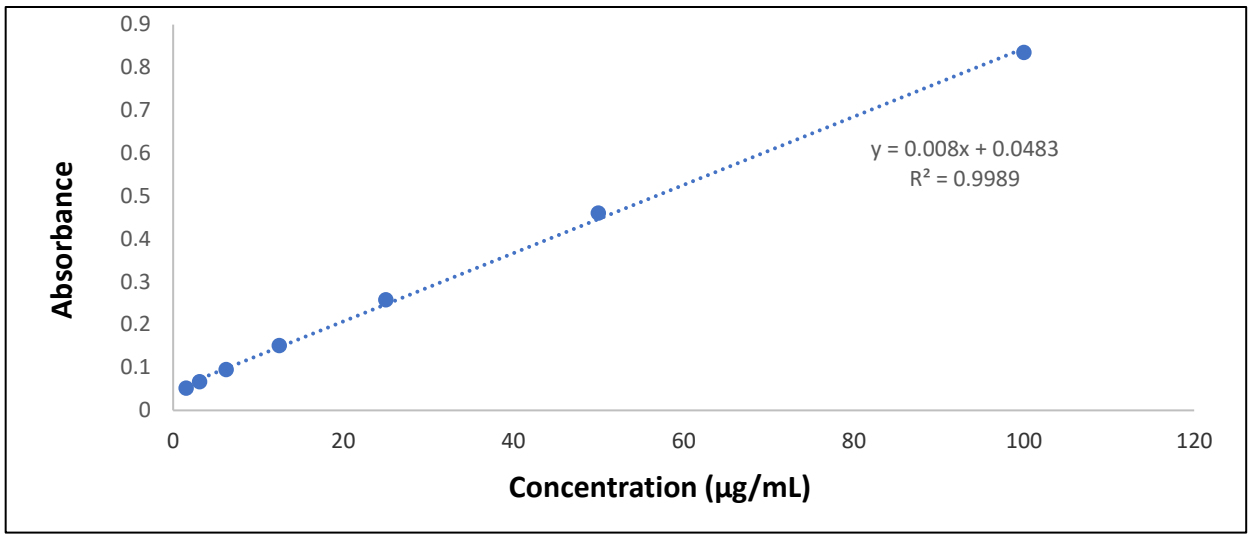


Figure 3.11: **Calibration Curve of Quercetin (Both Aerial & Root extracts)**

Table 3.8: Total Flavonoid Contents of the Extracts of the Aerial Parts of *Persicaria glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE conc. (C) (µg/ml)	QE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TFC as QE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.170	0.0002	6.838	0.007	1	0.007	76.06	75.44±0.88
	0.168	0.0002	8.963	0.009	1	0.009	74.81	
Chloroform extract	0.189	0.0002	4.838	0.005	1	0.005	87.94	89.81±2.65
	0.195	0.0002	4.463	0.004	1	0.004	91.69	
Ethyl acetate extract	0.166	0.0002	64.213	0.064	1	0.064	73.56	77.31±5.30
	0.178	0.0002	63.463	0.063	1	0.063	81.06	
Methanol extract	0.192	0.0002	0.838	0.001	1	0.001	89.81	92.63±3.98
	0.201	0.0002	0.963	0.001	1	0.001	95.44	

Table 3.9: Total Flavonoid Contents of the Extracts of the Root Parts of *Persicaria glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE conc. (C) (µg/mL)	QE conc. (C) (mg/mL)	V (mL)	c*V (mg)	TFC as QE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.131	0.0002	10.338	0.010	1	0.010	51.69	50.44±1.77
	0.127	0.0002	9.838	0.010	1	0.010	49.19	
Chloroform extract	0.189	0.0002	4.838	0.005	1	0.005	87.94	89.81±2.65
	0.195	0.0002	4.463	0.004	1	0.004	91.69	
Ethyl acetate extract	0.166	0.0002	64.213	0.064	1	0.064	73.56	77.31±5.30
	0.178	0.0002	63.463	0.063	1	0.063	81.06	
Methanol extract	0.178	0.0002	16.213	0.016	1	0.016	81.06	81.89±0.88
	0.18	0.0002	16.463	0.016	1	0.016	82.31	

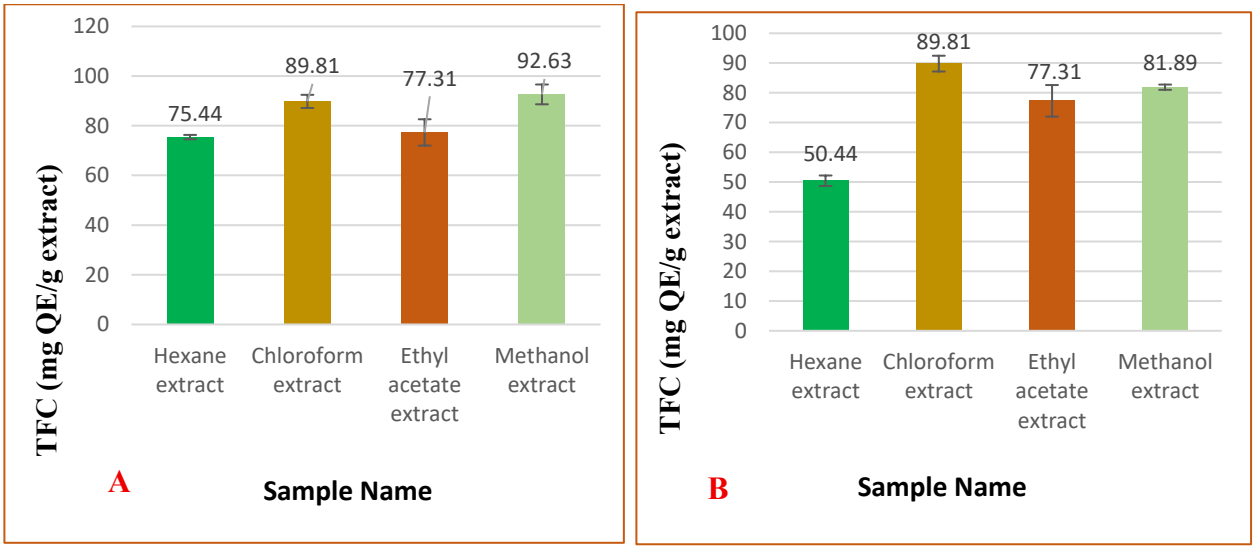


Figure 3.12. Total flavonoid Contents (TFC) of *Persicaria glabra* Extracts: (A) Aerial Part Extracts and (B) Root Part Extracts.

The total flavonoid content of *Persicaria glabra* varied among the different extracts. In the aerial parts, methanol extract exhibited the highest flavonoid content (92.63 ± 3.98 mg QE/g), followed

by chloroform (89.81 ± 2.65 mg QE/g), ethyl acetate (77.31 ± 5.30 mg QE/g), and n-hexane extracts (75.44 ± 0.88 mg QE/g). Similarly, in the root extracts, the highest flavonoid content was recorded in the chloroform extract (89.81 ± 2.65 mg QE/g), followed by methanol (81.89 ± 0.88 mg QE/g), ethyl acetate (77.31 ± 5.30 mg QE/g), and n-hexane extracts (50.44 ± 1.77 mg QE/g). The comparatively higher flavonoid contents in methanol and chloroform extracts may account for their enhanced antioxidant activities.

3.2.1.4 *Determination of Total Antioxidant Capacity*

Total antioxidant activity (TAC) of the extracts was estimated by phosphomolybdenum method. This approach involves the addition of antioxidants to reduce Mo (VI) to Mo (V) and form a green complex of phosphate/Mo (V) that has a maximum absorbance at 695 nm. The antioxidant capacity was calculated from the calibration curve using ascorbic acid and given as ascorbic acid equivalents (AAE). Phenolics, flavonoids, carotenoids, vitamins and volatile constituents are accountable for the antioxidant properties of plant extracts. Phenolic compounds are especially known for their redox characteristics, which make them act as reducing agents, hydrogen donors and free radical scavengers [52] and hence play significant role in overall antioxidant activity of plant extracts. **[Table SM.14]**

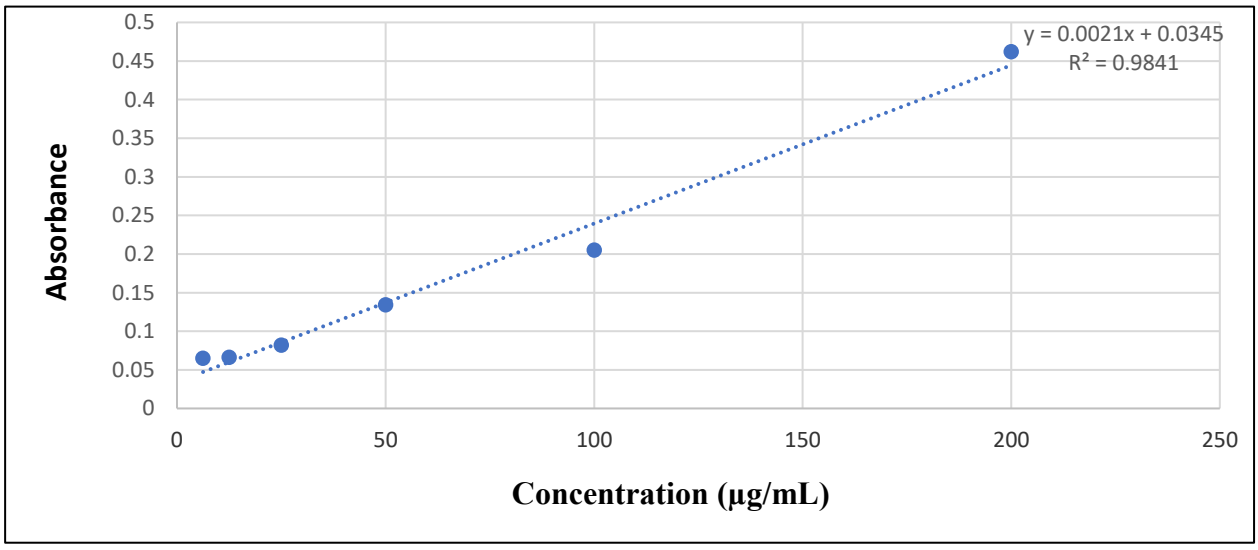


Figure 3.13: Calibration Curve for Ascorbic Acid (Both Aerial & Root extracts)

Table 3.10: Total Antioxidant Capacity of the Extracts of the Aerial Parts of *P. glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	AAE conc. (C) (µg/ml)	AAE conc. (C) (mg/ml)	V (mL)	c*V (mg)	TAC as AAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.061	0.0002	12.619	0.013	1	0.013	63.10	60.71±3.37
	0.059	0.0002	11.667	0.012	1	0.012	58.33	
Chloroform extract	0.263	0.0002	108.810	0.109	1	0.109	544.05	548.81±6.73
	0.267	0.0002	110.714	0.111	1	0.111	553.57	
Ethyl acetate extract	0.119	0.0002	40.238	0.040	1	0.040	201.19	204.76±5.05
	0.122	0.0002	41.667	0.042	1	0.042	208.33	
Methanol extract	0.21	0.0002	83.571	0.084	1	0.084	417.86	415.48±3.37
	0.208	0.0002	82.619	0.083	1	0.083	413.10	

Table 3.11: Total Antioxidant Capacity of the Extracts of the root Parts of *P. glabra*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	AAE conc. (C) (µg/mL)	AAE conc. (C) (mg/mL)	V (mL)	c*V (mg)	TAC as AAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
n-Hexane extract	0.062	0.0002	13.095	0.013	1	0.013	65.48	64.29±1.68
	0.061	0.0002	12.619	0.013	1	0.013	63.10	
Chloroform extract	0.067	0.0002	15.476	0.015	1	0.015	77.38	75.00±3.37
	0.065	0.0002	14.524	0.015	1	0.015	72.62	
Ethyl acetate extract	0.116	0.0002	38.810	0.039	1	0.039	194.05	197.62±5.05
	0.119	0.0002	40.238	0.040	1	0.040	201.19	
Methanol extract	0.081	0.0002	22.143	0.022	1	0.022	110.71	108.33±3.37
	0.079	0.0002	21.190	0.021	1	0.021	105.95	

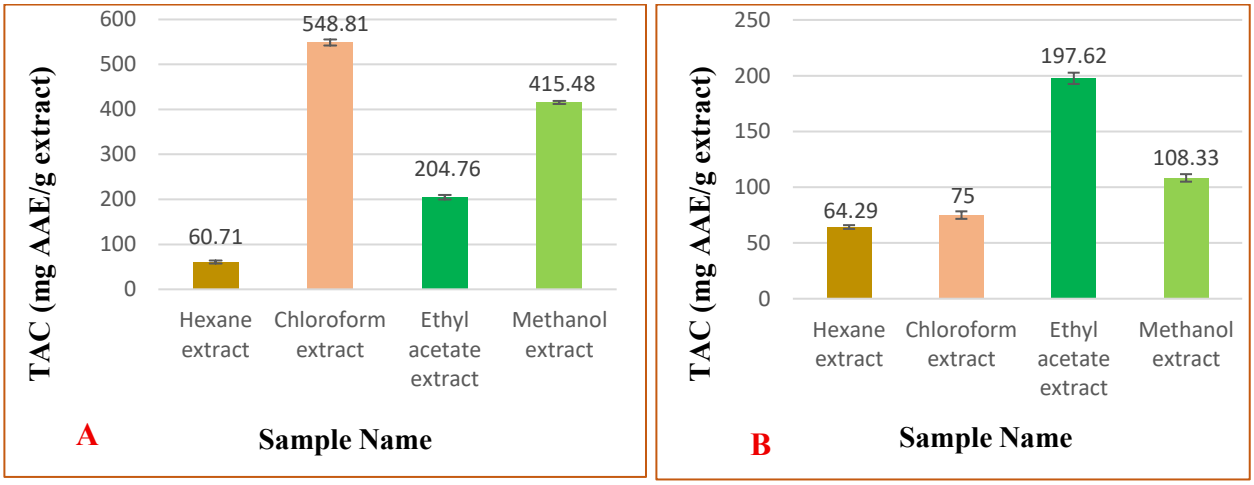


Figure 3.14: Total Antioxidant Capacity of *Persicaria glabra* Extracts: (A) Aerial Parts, and (B) Root Parts

The total antioxidant capacity of *Persicaria glabra* varied considerably among the different extracts of both aerial and root parts. Among the aerial part extracts, the chloroform extract exhibited the highest antioxidant activity (548.81 ± 6.73 mg AAE/g), followed by methanol, ethyl acetate, and n-hexane extracts. In contrast, the root extracts showed the greatest antioxidant capacity in the ethyl acetate extract (197.62 ± 5.05 mg AAE/g), followed by methanol, chloroform, and n-hexane extracts. The variations noticed in antioxidant activity may be claimed by differences in the type and concentration of antioxidant phytochemicals present in the extracts. These findings suggest that chloroform and ethyl acetate are effective solvents for extracting antioxidant constituents from the aerial and root parts of *P. glabra*, respectively.

3.3.2 *Cytotoxicity*

Brine Shrimp Lethality Bioassay

The Brine Shrimp Lethality Bioassay (BSLT) is a strong and simple bioassay that is widely used for screening various bioactivities in crude plant extracts, with good predictability of general toxicity and pesticides [53]. In this study, the toxicity of the different extracts was determined using *Artemia salina* nauplii. After 24 hours exposure the number of surviving nauplii in the test tubes was recorded after visual inspection. The percentage mortality was then determined for each concentration group. For both the experimental extracts and the standard reference drug vincristine

sulfate, a linear regression analysis was carried out to obtain the correlation between the percent lethality and the log of the concentration. The cytotoxic effects and median lethal concentrations are described in the following figures 3.15 & 3.16. **[Table SM.15-25 & Figure SM.10-18]**

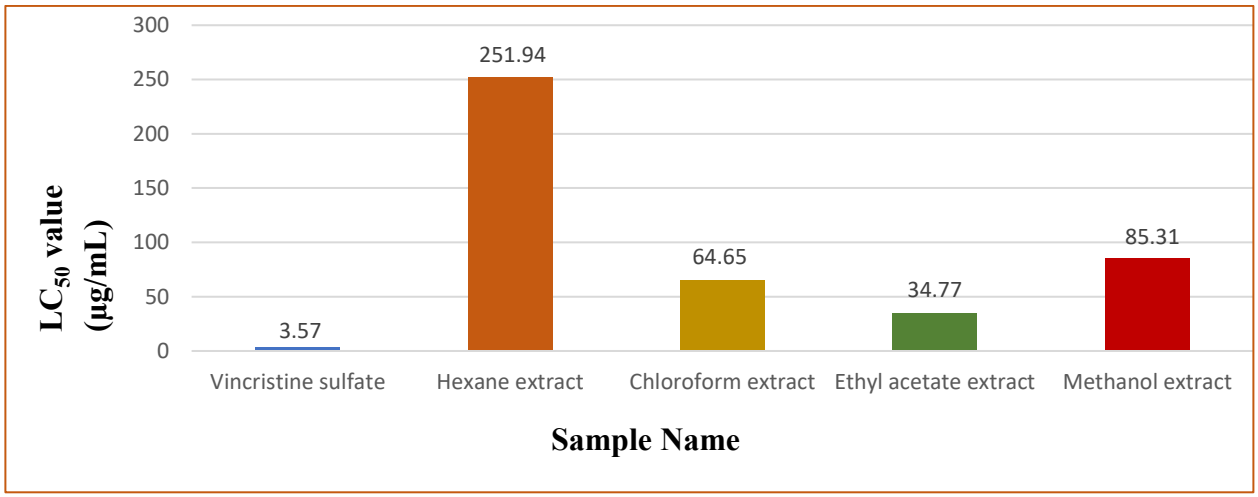


Figure 3.15: LC_{50} Values of Aerial Part Extracts and Standard of *Persicaria glabra*

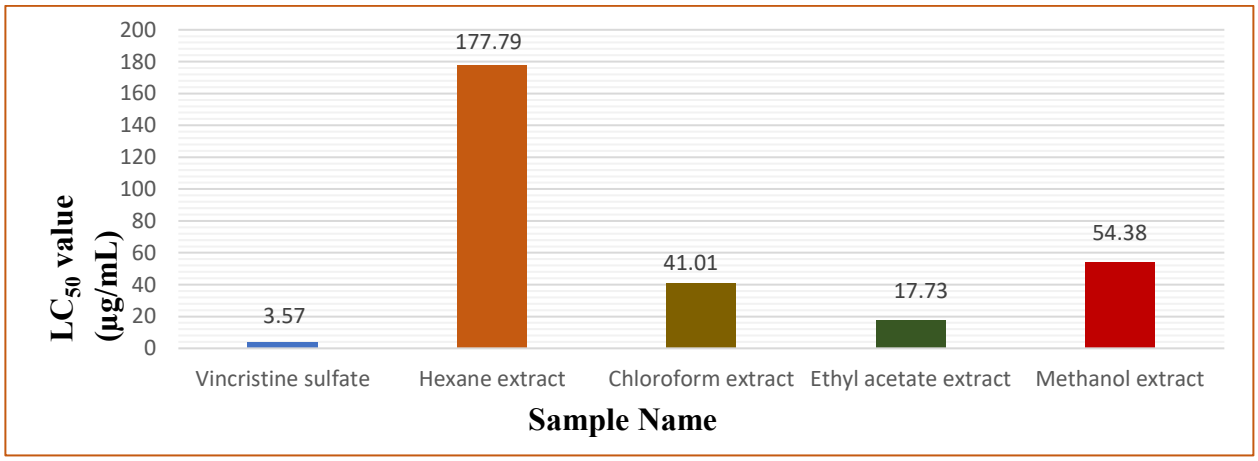


Figure 3.16: LC_{50} Values of Root Part Extracts and Standard of *Persicaria glabra*

The extracts from the different parts of *Persicaria glabra* were cytotoxic to different extents depending on the solvent used for extraction. The ethyl acetate fractions of the tested extracts showed highest cytotoxic activity with LC_{50} of 34.77 and 17.73 µg/mL for aerial and root parts respectively, although less potent than vincristine sulfate (LC_{50}= 3.57 µg/mL). Methyl and chloroform extracts and comparatively weak were n-hexane extracts. The ethyl acetate extracts were more cytotoxic, so the effects observed may be due to moderately polar bioactive components.

3.3.3 *In-vitro Anti-inflammatory Assay*

A hallmark of inflammation is protein denaturation, particularly under heat-induced circumstances, which can often result in cellular damage and malfunction. In this paper, the anti-inflammatory potential of *Persicaria glabra* was evaluated by assessing the ability of its extracts to inhibit the denaturation of egg albumin (EA). The protective effects of distinct extracts from both the aerial and root parts of the plant assessed by IC_{50} values (Fig. 3.17 & 3.18), with lower values representing better protein stability. To benchmark their efficacy, the anti-denaturation performance of these extracts was assessed using the standard anti-inflammatory medication, diclofenac sodium [54]. **[Table SM.26-36 & Figure 19-27]**

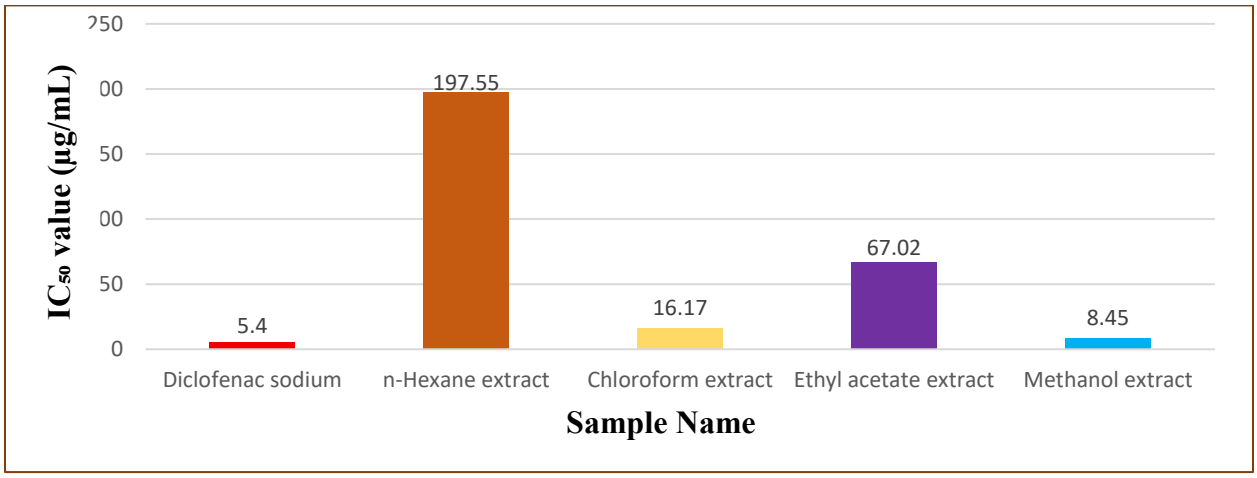


Figure 3.17: IC_{50} Values of Different Extracts of Aerial Parts and standard of *P. glabra*

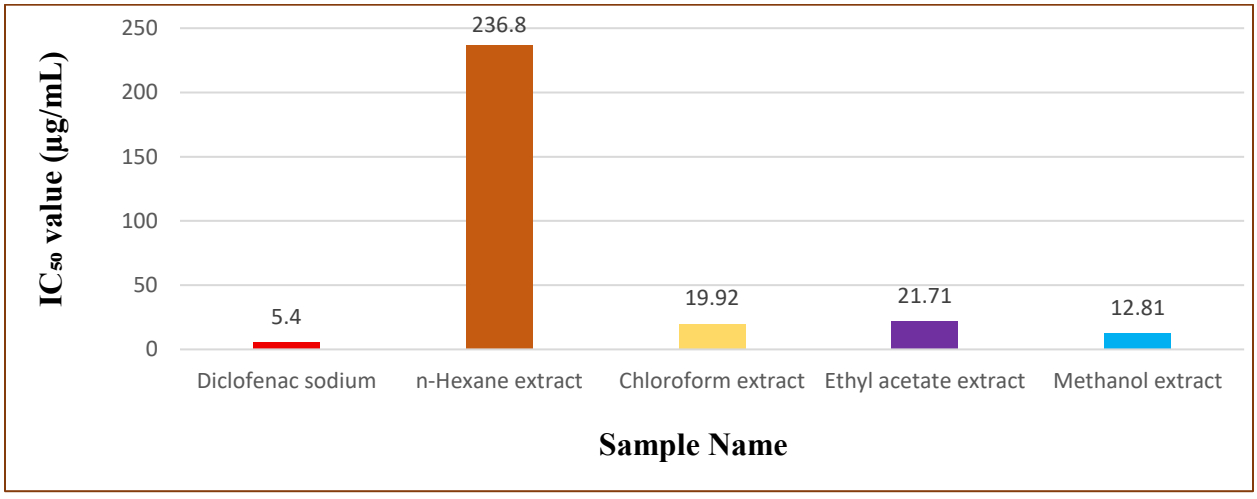


Figure 3.18: IC_{50} Value of Standard and Different Extracts of Root Parts of *Persicaria glabra*

The anti-inflammatory activity of *Persicaria glabra* extracts varied with the plant part and extraction solvent. Among the aerial part extracts, the methanol (IC_{50} = 8.45 µg/mL) and chloroform (IC_{50} = 16.17 µg/mL) extracts exhibited the strongest activity, whereas in the root extracts, methanol (IC_{50} = 12.81 µg/mL), ethyl acetate (IC_{50} = 21.71 µg/mL), and chloroform (IC_{50} = 19.92 µg/mL) showed notable anti-inflammatory effects compared with diclofenac sodium (IC_{50} = 5.40 µg/mL). Conversely, the n-hexane extracts demonstrated the weakest activity in both plant parts. These findings suggest that polar and semi-polar extracts are richer in anti-inflammatory phytoconstituents than the non-polar fraction.

4. Conclusion

In the current investigation, it was observed that both aerial parts and roots of *P. glabra* are good source of bioactive secondary metabolites of significant pharmacological potential. The phytochemical analysis indicated the existence of steroids, terpenoids, flavonoids, phenolic compounds, alkaloids, coumarins, quinones and anthraquinones in the extracts, with saponins, tannins and carbohydrates being mainly found in the methanol extracts. GC-MS analysis also identified some bioactive compounds. The dominant compound in the n-hexane extract of the aerial part was (Z)-13-Docosenamide (42.78%) while the dominant compounds in root extracts were progesterone (24.18%), Campesterol (10.52%), 1,3,5-Tris(trimethylsiloxy)benzene (34.31%) and Methyl tridecanoate (10.57%). The aerial parts chloroform extract showed the highest antioxidant potential (the lowest DPPH IC_{50} value equal 11.29 µg/mL, the highest total phenolic content equal 348.87 ± 4.24 mg GAE/g extract and the highest total antioxidant capacity equal 548.81 ± 6.73 mg AAE/g extract), while the roots chloroform extract showed the highest total phenolic content equal 419.67 ± 3.96 mg GAE/g extract, and the highest total antioxidant capacity equal 358.72 ± 4.56 mg AAE/g extract, the highest antioxidant activity (the lowest DPPH IC_{50} value equal 16.90 µg/mL), and the highest cytotoxic activity (IC_{50} = 17.73 µg/mL). The anti-inflammatory activity of both aerial part and root extracts was the highest when extracted by methanol (IC_{50} = 8.45 and 12.81 µg/mL, respectively). The methanol extract of the aerial parts had the greatest amount of flavonoids (92.63 ± 3.98 mg QE/g extract), followed by the chloroform extract of the roots (89.81 ± 2.65 mg QE/g extract). The findings confirmed the conventional medicinal applications of *P. glabra* and indicate that its aerial parts and roots can be considered a

significant source of bioactive chemicals which can be used for further phytochemical exploration and pharmacological validation.

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Authors contributions

1. Nazmul Hasan: Performed the experiments, contributed to data analysis and wrote the manuscript original draft.
2. Md Aliul Azim Zim: Performed experiments and contributed to data analysis
3. Ananta Kumar Das: Performed experiments, co-supervised the research work and contributed to manuscript revision.
4. Md. Farid Uddin: Performed experiments, contributed to data analysis, supervised the research work & study design, and contributed to manuscript writing and revision.
5. Evena Parvin Lipy: Data curration
6. Hayatullah: Data curration
7. Koushik Saha: Contributed to data analysis, supervised the research work & study design, and contributed to manuscript revision.

All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare no conflict of interest

Declarations

Ethics approval and consent to participate

Persicaria glabra (Willd.) M.Gómez was collected from wild populations in Lawachara National Park, Moulvibazar, Bangladesh, in November 2024 **(Figure SM.28)**, in accordance with the applicable institutional, local, and national guidelines governing the collection of plant materials in Bangladesh. Collection of the plant material was conducted with the necessary permissions from

the relevant authorities, where applicable. The collected specimen was authenticated by Mr. Ahmed Saqee, Scientific Officer, Bangladesh National Herbarium (NHB), Mirpur, Dhaka, Bangladesh. A voucher specimen (No. 94756) has been deposited at the Bangladesh National Herbarium for future reference.

Consent to participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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