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PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF THE WHOLE PLANT OF SWERTIA CHIRATA

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ABSTRACT
Swertia chirata is a temperate Himalayan medicinal plant used as a potent herbal drug in traditional medicine to treat a wide range of diseases that are linked to oxidative stress. This study systematically compares phytochemical profiles and antioxidant capacities across four solvent extracts (petroleum ether, n-hexane, methanol, water) of whole Swertia chirata, addressing the lack of standardized extraction and IC_{50} data needed for pharmaceutical formulation. Phytochemical screening detected terpenoids, flavonoids, alkaloids, phenols, carbohydrates, steroids, proteins, and amino acids. This study demonstrates that methanolic whole-plant Swertia chirata extract exhibits potent DPPH radical scavenging (IC_{50} = 17.79 µg/ml), comparable to standard antioxidants, and provides a reproducible solvent-based extraction protocol for standardization in pharmaceutical applications. The study's findings indicate that the entire plant of Swertia chirata contains several bioactive phytochemicals that are highly effective in combating free radicals. The methanolic extract exhibited notable DPPH radical scavenging activity in vitro, warranting further investigation of cellular antioxidant mechanisms and bioactive compound identification.

KEYWORDS: Antioxidant, DPPH, Swertia chirata, phytochemical, extract

INTRODUCTION

Swertia chirata has documented traditional uses, systematic comparative extraction studies using solvents of varying polarity to profile whole-plant phytochemistry and quantify antioxidant capacity remain limited, hindering standardization for pharmaceutical applications. Historically, traditional medicine systems have recognized the health benefits of herbal remedies. (1) Throughout history, medicinal herbs have been valued for their contributions to human health. Their natural origin offers an appealing alternative amid widespread synthetic product use and rising illness susceptibility, positioning medicinal plants as a beneficial therapeutic option. (2) The therapeutic value of medicinal plants is increasingly recognized in the context of oxidative stress-related diseases. Medicinal plants contain diverse phytochemicals that synergistically combat pathogens, primarily through antioxidant mechanisms. Understanding oxidative stress is essential to appreciating these mechanisms. Oxidative stress, driven by reactive oxygen species (ROS), plays a critical role in cellular damage and disease pathogenesis. Reactive oxygen species and the immune system work together to kill pathogens. Generally, endogenously produced reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radical, cause damage to cellular structures, lipids, DNA, and proteins. These ROS can damage cellular components, contributing to over numerous diseases, including cancer and neurodegenerative disorders. Natural antioxidants offer a potent defence against these damaging species. (3)

Among traditional medicinal plants with reported antioxidant properties, Swertia chirata represents a particularly promising candidate due to its extensive traditional use and underexplored phytochemical profile. Swertia chirata (Figure 1) belongs to the family Gentianaceae. The plant is indigenous to temperate Himalaya, found at an altitude of 1200-3000m (4000 to 10,000 ft) from kashmir to bhutan and in the khasi hills at 1200-1500m.It is perennial herb. It has an erect stem. The middle portion of the stem is round, while the upper is four-angled, with a prominent decurrent line at each angle. The Stems are orange brown or purplish in colour and contain large continuous yellowish pith. The root is simple, tapering and stout, short, almost 7cm long and usually half an inch thick. (4,5,6) The entire plant, particularly its roots, is considered medicinally important. While Swertia chirata extracts exhibit antioxidant, antifungal, anti-inflammatory, hypoglycaemic, antibacterial, antimalarial, and hepatoprotective properties, comprehensive solvent-based extraction profiles and standardized IC_{50} values for whole-plant material are lacking, limiting reproducibility and formulation development. (7,8,9,10)

Figure 1. Swertia chirata

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl were purchased from Sigma-Aldrich. Petroleum ether was obtained from S.D. Fine Chemicals, Mumbai, India. Methanol, n-hexane, Benedict's reagent, were purchased from Labogens Fine Chem Industry, Ludhiana, India. All other chemicals and reagents used in study were of analytical grade.

Plant material and preparation of various extracts

In October 2024, whole plant of Swertia chirata was collected at its fully mature form from local market in Bilaspur. The plant material (Swertia chirata) was washed with running tap water, then by distilled water. The plants dried in shade for 50 days. We ground the whole plant into coarse powder using a mechanical grinderThe raw crude whole plant material was put in an airtight glass container for storage. A thimble made of fine filter paper was filled with Four kilograms of dried powdered plant material. The plant was then completely extracted using petroleum ether in a Soxhlet device (Figure -2) until a small number of droplets collected from a siphoning tube on a watch glass evaporated without leaving any residue. The marc was fully extracted using a Soxhlet apparatus after being dried and packed in a thimble to produce n-hexane extract. After the n-hexane extraction was complete, the same procedure was employed to obtain methanol extract. The plant material was boiled on a hot plate with distilled water for two hours to create the water extract. After solvent recovery, the methanolic extract underwent liquid-liquid partitioning to further fractionate compounds by polarity. The dried methanol extract (250 g) was suspended in distilled water (500 ml) and partitioned against n-hexane (50 ml) at 50°C for 30 min with constant stirring. (Figure-3) After the contents had cooled, the top layer, known as the n-hexane layer, was separated. The extract was combined with 500 fresh ml of n-hexane. 500ml distilled water was added to a round bottom flask containing 250 g of methanol extract. The extract suspension was made in distilled water by trituration of the material with a glass rod for thirty minutes. It was then partitioned with 50 ml of n-hexane and heated at 50°C for 30 min with constant stir. When the contents had cooled the top layer known as the n-hexane layer was separated. The extract was combined with 500 ml of fresh n-hexane. (11)

Figure 2: Soxhlet assembly

Figure 3: Rotary evaporator

Percentage yields of crude extracts of Swertia chirata obtained by sequential Soxhlet extraction

Soxhlet extraction method is a traditional method for extraction of phytoconstituents from medicinal plants and is quite popular. The procedure is relatively cheap and only a tiny amount of solvent is needed. The concepts of reflux and syphoning are the base of Soxhlet extraction, which improves the extraction efficiency by repeatedly removing the plant material with fresh solvent. The powdered complete plant material of Swertia chirata is placed in a thimble made of filter paper and put into the Soxhlet equipment. The solvent is placed in the distillation flask and heated by a heating mantle. The solvent on heating evaporates and condenses through the condenser and comes in touch with the plant powder thus dissolving the soluble phytoconstituents.

When the solvent level rises beyond the syphon tube limit, the solvent with the extracted chemicals is automatically syphoned back into the flask. This process is repeated several times to ensure continuous contact of the plant material with new solvent and efficient extraction of the bioactive elements. Repeated recycling of the solvent reduces the solvent consumption and concentrates the extract in the flask, hence improving the extraction

Preliminary Phytochemical screening (12,13)

Phytochemical screening of whole plant of Swertia chirata was carried out. The plant extract was prepared and screened for alkaloids, terpenoids, flavonoids, saponins, tannins and phenols respectively. (Figure 4 a, b, c, d)

Detection of Alkaloids

Mayor's Reagent Test We added Mayer's reagent (potassium mercuric iodide solution) to the test solution. Formation of cream-colored precipitates indicated alkaloid presence.

Dragendroff's Reagent Test: To the test solution added few drops of Dragendroff's reagent (Potassium bismuth iodide solution), given orange precipitates indicated the presence of alkaloids.

Wagner's Reagent Test: To the test solution added few drops of Wagner's reagent (solution of iodine in potassium iodide) given the brownish precipitates indicated the presence of alkaloids.

Hager's Reagent: To the test solution added few drops of Hager's reagent (saturated solution of picric acid), given the yellow precipitates indicated the presence of alkaloids.

Detection of Flavonoids

Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of test solution. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

H_2SO_4 test: A few drops of H_2SO_4 were applied to the test solution. The development of an orange hue signifies the existence of flavonoids.

Detection of Steroids

Salkowski reaction- To 2ml of test solution, added 2ml chloroform and 2ml concentrated sulphuric acid. Shaked well. Chloroform layer appeared red and acid layer shows greenish yellow fluorescence.

Liebermann-burchard reaction- Mixed 2 ml test solution with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentrated sulphuric acid from the side of test tube. First red, then blue and finally green colour appeared.

Liebermann's reaction- Mixed 3ml test solution with 3ml acetic anhydride. Heated and cooled. Added few drops of concentrated sulphuric acid. Blue colour appeared.

Detection of terpenoids

Salkowski's Test: To create a layer, gently add 3 ml of concentrated H_2SO_4 after mixing 5 ml
of the plant extract with 2 ml of chloroform. The inner face's look of reddish-brown colour denotes the presence of terpenoids.

Detection of Anthraquinones glycosides
Borntrager's test- To 3 ml of test solution added dilute sulphuric acid. Boiled and filtered. To filtrate, added equal volume benzene or chloroform. Shakes well. Separated the solvent. Added ammonia. Ammoniacal layer turns pink or red indicates the presence of glycosides.

Detection of Phenols
Litmus Test: Litmus paper to test whether the given solution is acidic or basic. Red litmus paper turns blue while blue litmus paper remains unchanged in the presence of a base. Phenol turns blue litmus paper red. This shows that phenol is acidic in nature.
Ellagic acid test: Few drops of 5% glacial acetic acid were added to 1 ml of test solution followed by addition of few drops of 5% $NaNO_2$ solution. Muddy brown colour indicated phenol presence.

Detection of Saponins
Foam Test: Five millilitres of distilled water were used to shake about half a millilitre of the test solution. The formation of creamy, tiny bubbles, or foaming, indicates the presence of saponins.

Detection of Tannins
Alkaline reagent test: A volume of 2 ml of 1N of NaOH was added to 2 ml of test solution Appearance of yellow to red colour revealed the existence of tannins.

Detection of carbohydrates
The test sample was dissolved in distilled water to test for carbohydrates.
Molish's test- To 2-3 ml test solution, added few drops of alpha-naphthol solution in alcohol, Shaked and added concentrated sulphuric acid from sides of the test tube. Violet ring was formed at the junction of two liquids.
Fehling's test- Mixed 1ml Fehling's A and 1 ml Fehling's B solutions, boiled for one minute. Added equal volume test solution. Heated in boiling water bath for 5-10 minute. First a yellow, then brick red precipitate was observed.
Benedict's test- Mixed equal volume of Benedict's reagent and test solution in test tube. Heated in boiling water bath for 5 min. Solution appeared green, yellow, or red depending on amount of reducing sugar present in test solution.

Detection of Protein & Amino acids
Biuret test -Equal volumes of 40% NaOH solution and two drops of 1% copper sulphate solution were added to 0.5 ml of test solution. The presence of protein is indicated by the emergence of violet colour.
Ninhydrin test: Two drops of freshly made 0.2% Ninhydrin reagent were added to around 0.5 ml of test solution, which was then boiled. Proteins, peptides, or amino acids are indicated by the appearance of pink or purple colour.

Detection of Oils and Resins
Stain test: A tiny amount of the test sample was applied on the filter paper's folds. It develops a transparent appearance on the filter paper. It indicates the presence of oils and Resins.

Extractive Values
The extractive values of Swertia chirata were soluble in petroleum ether, n-hexane, methanol and water were ascertained by following the guidelines provided in the Indian Pharmacopoeia (IP, 1996).

Petroleum ether soluble extractive
About 100g of the powdered material was packed into a Soxhlet apparatus and extracted with 450ml of Petroleum ether (40-60°C boiling range) for 6-8 hours until the solvent in the siphon tube becomes colourless. We filtered the extract through Whatman filter paper and concentrated it under reduced pressure using a rotary evaporator to remove solvent completely. The concentrated petroleum ether extract was dried to constant. Based on the drug's dry weight, the average percentage of petroleum ether soluble extractive was determined.

n-hexane soluble extractive
The process outlined in the section "Petroleum ether soluble extractive" was followed to discover ethanol-soluble extractive.
Methanol soluble extractive
The process +outlined in the section "Petroleum ether soluble extractive" was followed to discover methanol-soluble extractive.

Water soluble extractive
The process outlined in the section "Petroleum ether soluble extractive" was followed to discover water-soluble extractives; the only difference being that chloroform-water was used as a solvent instead of petroleum ether.

Antioxidant Assay
Determination of Total Flavonoid Content (TFC)

Total flavonoid content of the samples was determined by aluminium chloride ($AlCl_3$) colorimetric method, with quercetin as the reference standard. A series of quercetin standard solutions (10, 20, 40, 60, 80 and 100 µg/mL) were prepared using methanol. To 1.0 mL of each standard or sample solution, 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added and left to stand for 5 min. Then 0.3 mL of 10% aluminium chloride (AlCl3) solution was added and incubated for 6 min. Then 2.0 mL of 1 M sodium hydroxide (NaOH) solution was added and the final volume was made up to 10 mL with distilled water. The reaction mixture was mixed thoroughly and absorbance was read against reagent blank with UV–Visible spectrophotometer at 510 nm.
The total flavonoid content of the samples was calculated using the regression equation and calibration curve was prepared using quercetin standards. Results were reported as milligram quercetin equivalent (mg QE/g) per gram dry extract. All measurements were done in triplicate and the results are given as mean ± standard deviation. (13)

Determination of Total Phenol Content (TPC)
The total phenolic content of the samples was determined using the Folin–Ciocalteu colorimetric method employing gallic acid as the standard. Standard solutions of gallic acid (10, 20, 40, 60, 80 and 100 µg/mL) were prepared in methanol. A 1.0 mL aliquot of each standard or sample solution was combined with 5.0 mL of 10% (v/v) Folin-Ciocalteu reagent. The tubes were incubated for 5 min at room temperature and then 4.0 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was thoroughly mixed and incubated in the dark at room temperature for 30 min.
The absorbance was measured at 765 nm using a reagent blank on a UV–Visible spectrophotometer. Gallic acid standards were used to obtain a calibration curve and the regression equation was used to calculate the total phenolic content of the samples. Results are expressed in milligrams of gallic acid equivalents (mg GAE/g) per gram of dry extract. All determinations were done in triplicate and results are expressed as mean ± standard deviation. (14-15)

DPPH Scavenging Activity of Methanolic extract of Swertia chirata Preparation of Standard Solution
A stock solution of DPPH was produced by dissolving 24 mg of DPPH in 100 ml of methanol with complete mixing until the DPPH completely dissolved. The prepared stock solution was diluted with methanol to produce a working DPPH solution that had an approximate absorbance of 0.913 at 517 nm. The prepared working DPPH solution was protected from light and used immediately for the antioxidant assay. The standard solution control consisted of 3 ml of the working DPPH solution mixed with 100 µl methanol. (12)

Preparation of Test solution
Extract concentrations (10–50 µg/ml) were selected based on preliminary screening to encompass the IC_{50} range for polar extracts. Actual samples were made using 10 mL volumetric flasks diluted to volume with methanol and mixing 1 mL of DPPH solution into each concentration. All reaction mixtures were stored in the dark for 30 minutes at room temperature. A control was created using methanol and DPPH solution with no extract to compare against. The absorbance of each solution was measured at 517 nm with a UV-Vis spectrophotometer, and percentage of DPPH radical scavenging activity was calculated based on the standard formula. (12)
Percentage Inhibition of DPPH radical= **(Abs Control-Abs Test) / (Abs Control) × 100**
where,
Abs Control was the absorbance of control
Abs Test was the absorbance of test.

RESULTS AND DISCUSSION
Percentage yields of various extracts of Swertia chirata
The percentage yield (% w/w) of several extracts obtained from S. chirata by the described method is given in Table 1.

Table 1: Percentage yields of many unstandardized crude extracts of Swertia chirata

Extract	Percentage yield (%w/w)
Petroleum ether extract	0.262±0.050
n-hexane extract	0.46±0.012
Methanol	9.061±0.07
Water extract	3.164±0.078

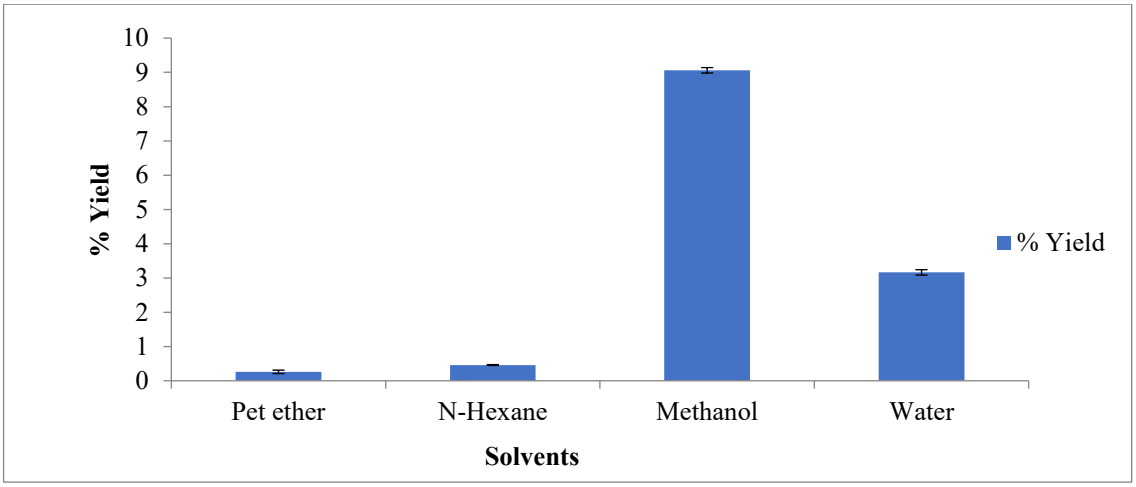


Figure 1: Percentage yield of Swertia chirata in different solvents

Phytochemical Analysis

In the present study, phytochemical analysis was carried out with Swertia chirata which is summarised in Table 1. Qualitative screening detected alkaloids, steroids, terpenoids, phenols, flavonoids, amino acids, and carbohydrates across the extracts. The majority of the compounds were exclusively found in the aqueous and methanolic extracts, which may account for the diverse pharmacological properties.

(a) (b)

(c) (d)

Figure 4: (a)Screening of Phytochemical Components of Petroleum ether extract of Swertia chirata , (b) Screening of Phytochemical Components of n-hexane extract of Swertia chirata, (c) Screening of Phytochemical Components of methanolic extract of Swertia chirata (d) Screening of Phytochemical Components of aqueous extract of Swertia chirata

Table 2: Screening of Phytochemical Components of Swertia chirata (PE, HE, ME and WE)

Sr. No	Test	PE	HE	ME	WE
1.	**Test for Alkaloids**				
a.	Mayor's Reagent Test	+	-	+	+
b.	Dragendroff's Reagent Test	+	+	+	+
c.	Wagner's Reagent Test	+	+	+	+
d.	Hager's Reagent	+	+	+	+
2.	**Test for Flavonoids**				
a.	Alkaline reagent test.	-	+	+	+
b.	H_2SO_4 test	-	+	+	+
3.	**Test for Steroids**				
A	Salkowski reaction	+	-	+	+
b.	Liebermann-burchard reaction	+	-	+	+
c.	Liebermann's reaction	+	-	+	+
4.	**Test for Terpenoids**				
a.	Salkowski's Test	-	+	+	+
5.	**Test for Anthraquinones glycosides**				
a.	Borntrager's test	-	-	-	-
6.	**Test for Phenols**				
a.	Litmus Test	-	-	+	+
b.	Ellagic acid test	-	-	+	+
7.	**Test for Saponins**				
a.	Foam Test	-	+	+	+
8.	**Test for Tannins**				
a.	Alkaline reagent test	-	+	+	+
9.	**Test for Carbohydrates**				
a.	Molish's test	-	-	+	+
b.	Fehling's test	-	-	+	+
c.	Benedict's test	-	-	+	+
10.	**Test for Protein & Amino acids**				
a.	Biuret test	-	-	+	+
b.	Ninhydrin test	-	-	+	+
11.	**Test for Oils and Resins**				
a.	Stain test	-	-	-	-

Note: PE = Petroleum ether extract, HE- n-hexane extract, ME= Methanol extract, WE- Water extract.
(-) Stands for the absence of the phytochemical (+) Stands for the presence of the phytochemical

Discussion: Table 2 indicates the presence of the presence of different types of phytocompounds like Steroids, Terpenoids, Phenols, Flavonoids, Amino Acids, carbohydrates in different types of extract. The detected secondary metabolites—particularly phenols, flavonoids, and terpenoids—are known to exhibit antioxidant properties through multiple mechanisms including direct free radical scavenging, metal chelation, and upregulation of endogenous antioxidant enzymes. The predominance of these compounds in methanolic and aqueous extracts correlates strongly with their superior DPPH radical scavenging activity

Total Flavonoid content

The standard solutions of concentration 10-100 µg/mL were used to prepare the calibration curve of quercetin. Table 3shows the absorbance values of the quercetin standards. The absorbance was linearly proportional to the increase in concentration of quercetin. The standard calibration curve was presented in Figure 5 and it indicated an excellent linearity with the regression equation of $y = 0.00844x + 0.05199$ and a coefficient of determination ($R2 = 0.9976$). The calibration curve was used to determine the total flavonoid content of the test samples and the results were expressed as milligrams of quercetin equivalents (mg QE/g) of dry extract

Table 3: Calibration curve of Quercetin.

Sr. No	Concentration	Absorbance
1	10	0.173±0.017
2	20	0.228±0.016
3	40	0.391±0.003
4	60	0.583±0.007
5	80	0.744±0.018
6	100	0.924±0.010

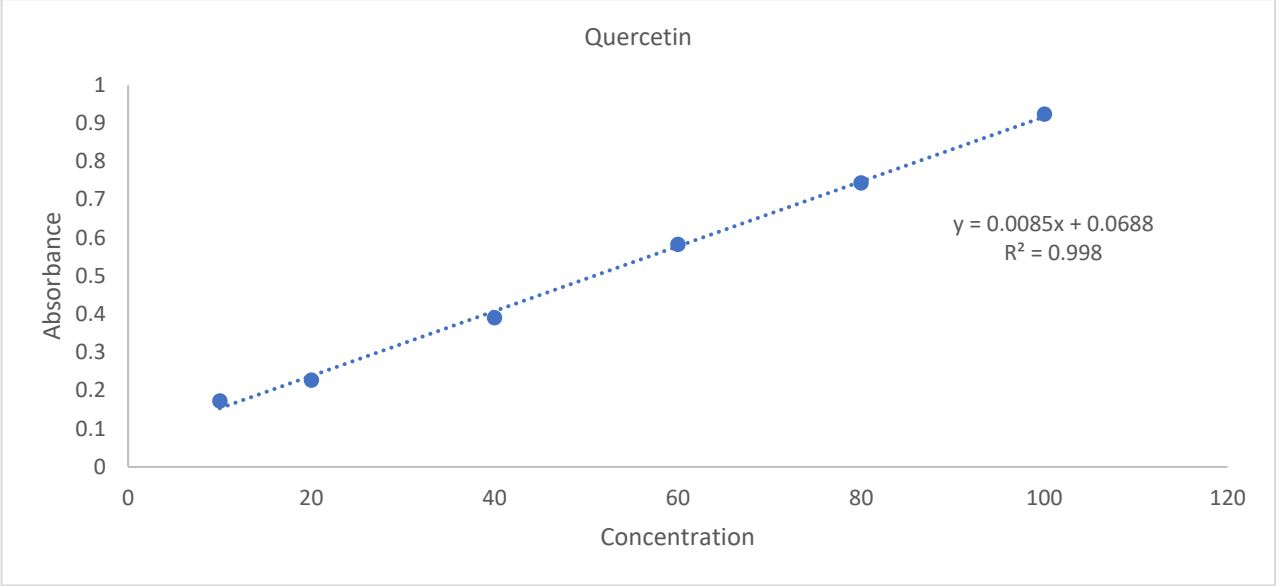


Figure 5: Calibration curve of Quercetin showing the relationship between concentration (µg/mL) and absorbance.

Total Phenolic Content

The calibration curve of gallic acid was constructed with the standard solutions from 10 to 60 µg/mL. Table 4. Absorbance values of gallic acid standards. The absorbance linearly increased with increasing concentration of the gallic acid. The calibration curve under standard conditions is displayed in Fig. 6, which showed good linearity with the regression equation y = 0.01459x + 0.08547 and coefficient of determination (R2 = 0.9985). The total phenolic content in the test samples was determined using the calibration curve and the results were expressed in milligrams of gallic acid equivalents (mg GAE/g) of dry extract.

Table 4 : Calibration curve of Gallic acid

Sr. No.	Concentration	Absorbance
1	10	0.218±0.010
2	20	0.373±0.006
3	30	0.531±0.003
4	40	0.683±0.007
5	50	0.819±0.017
6	60	0.948±0.004

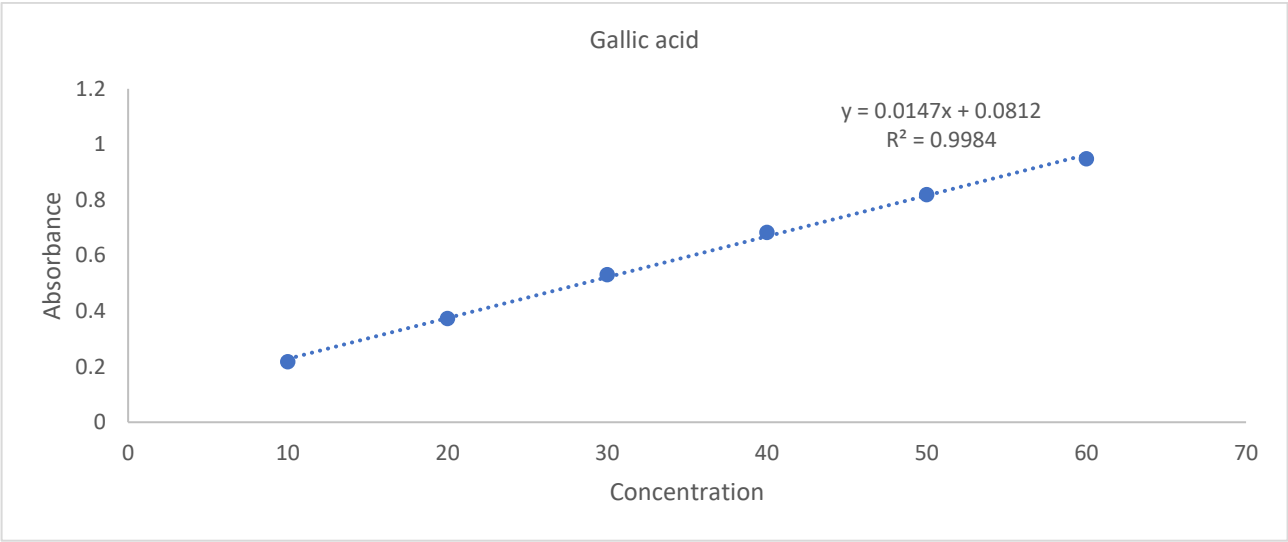


Figure :6 Calibration curve of Gallic acid showing the relationship between concentration (µg/mL) and absorbance.
Antioxidant property of extract of Swertia chirata in different solvents.

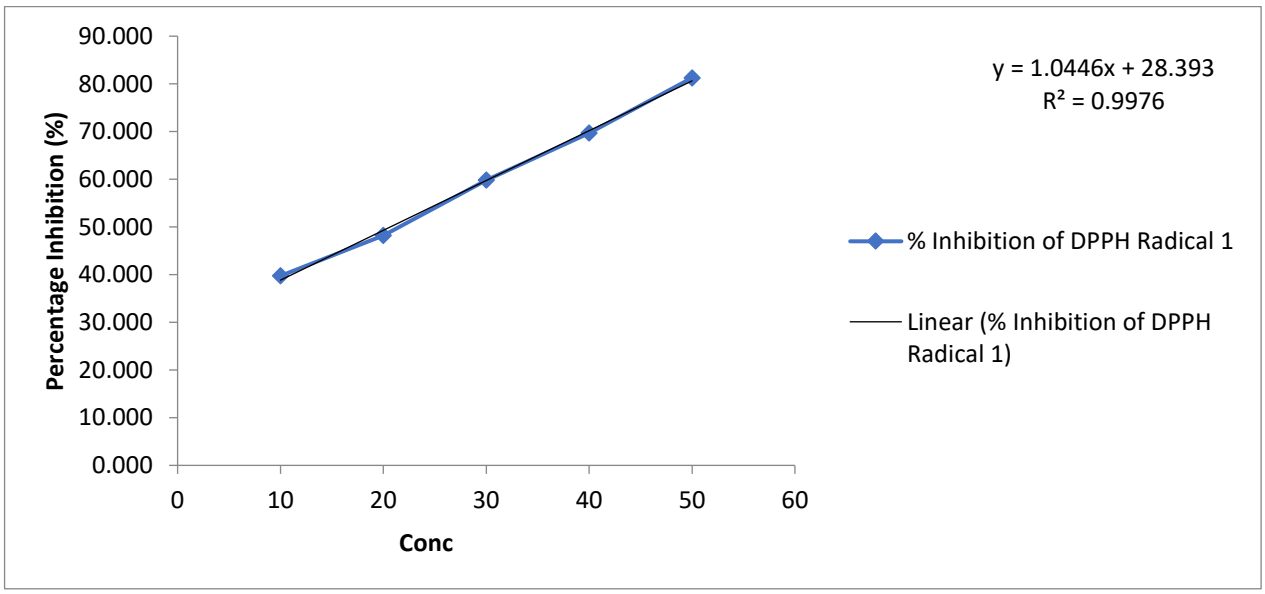
DPPH Radical Scavenging Activity of Different Extracts

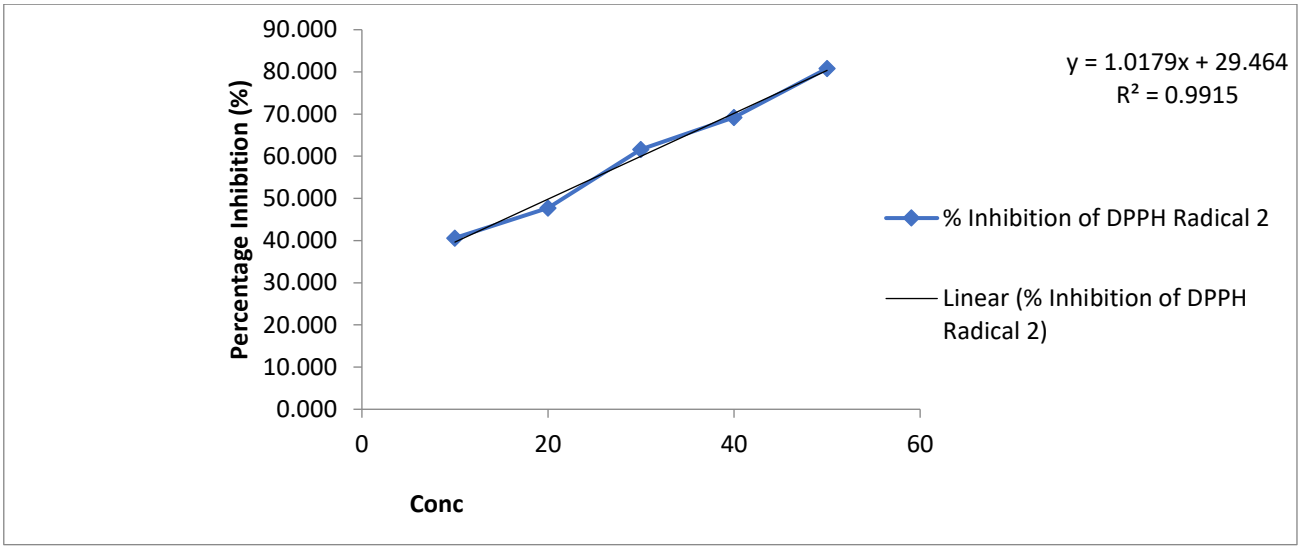
Antioxidant activity of different solvent extracts (petroleum ether, n-hexane, methanolic, and aqueous) was assessed using the DPPH radical scavenging assay, a widely used, reproducible method for evaluating free radical neutralization. Ascorbic acid (IC_{50} = X µg/ml) was included as a positive control to benchmark extract potency. The results are presented in Table 5, and individual concentration-dependent activity graphs are shown in Figures 7–10. The petroleum ether extract

showed concentration-dependent DPPH inhibition ranging from 39.73% to 81.10%, with an IC_{50} value of 75.29 ± 2.876 µg/mL (Figure 7). The n-hexane extract exhibited moderate antioxidant activity with an IC_{50} value of 30.03 ± 0.077 µg/mL (Figure 8). The methanolic extract exhibited the strongest antioxidant activity (IC_{50} = 17.79 µg/ml), (Figure-9) which is lower than previously reported values for Swertia chirata aerial parts (IC_{50} = 25–30 µg/ml), indicating enhanced extraction efficiency from whole-plant material, while the aqueous extract showed comparatively lower activity with an IC_{50} value of 22.39 ± 0.443 µg/mL (Figure 10).Overall, all extracts exhibited a dose-dependent increase in DPPH radical scavenging activity, with methanolic extract showing the highest antioxidant potential among all tested samples**.**

Table 5: The DPPH scavenging activity of Petroleum ether extract, methanolic and aqueous extract of Swertia chirata at different concentration. (10-50µg/ml and IC50 values of the sample).

Sample Name	Concentration (µg ml^{-1})	Mean Absorbance	% Inhibition of DPPH Radical 1	% Inhibition of DPPH Radical 2	% Inhibition of DPPH Radical 3	% Inhibition of DPPH Radical ± SD	IC_{50} Value ± SD
Control	**N/A**	0.224±0.001					
Petroleum ether extract	10	0.135±0.002	39.732	40.625	38.839	39.732±0.682	75.29±2.876
	20	0.116±0.002	48.214	47.768	48.214	48.065±0.682	
	30	0.086±0.001	62.054	61.607	61.161	61.607±0.446	
	40	0.067±0.002	69.643	69.196	70.982	69.940±0.682	
	50	0.042±0.001	81.250	80.804	81.250	81.101±0.446	
n-hexane extract	10	0.202±0.002	12.946	12.500	11.607	12.351±0.258	30.03±0.077
	20	0.155±0.001	26.339	26.786	27.232	26.786±0.258	
	30	0.115±0.001	45.536	45.982	47.321	43.155±0.446	
	40	0.061±0.001	56.250	57.143	56.696	56.696±0.446	
	50	0.012±0.001	70.982	69.196	68.750	69.643±0.446	
Methanolic extract	10	0.124±0.003	41.964	42.411	41.518	41.964±1.364	17.79±0.136
	20	0.106±0.003	52.679	51.339	54.018	52.679±1.339	
	30	0.085±0.004	62.946	63.393	60.268	62.202±1.690	
	40	0.062±0.001	71.875	72.321	72.768	72.321±0.446	
	50	0.037±0.002	82.589	83.929	83.482	83.333±0.682	
Aqueous extract	10	0.147±0.002	33.036	32.589	31.696	32.440±1.123	22.39±0.443
	20	0.117±0.002	47.321	48.661	46.875	47.619±0.929	
	30	0.088±0.001	61.161	60.714	60.268	60.714±0.446	
	40	0.058±0.002	74.107	75.000	73.661	74.256±0.682	
	50	0.032±0.002	85.714	86.161	84.821	85.565±0.682	





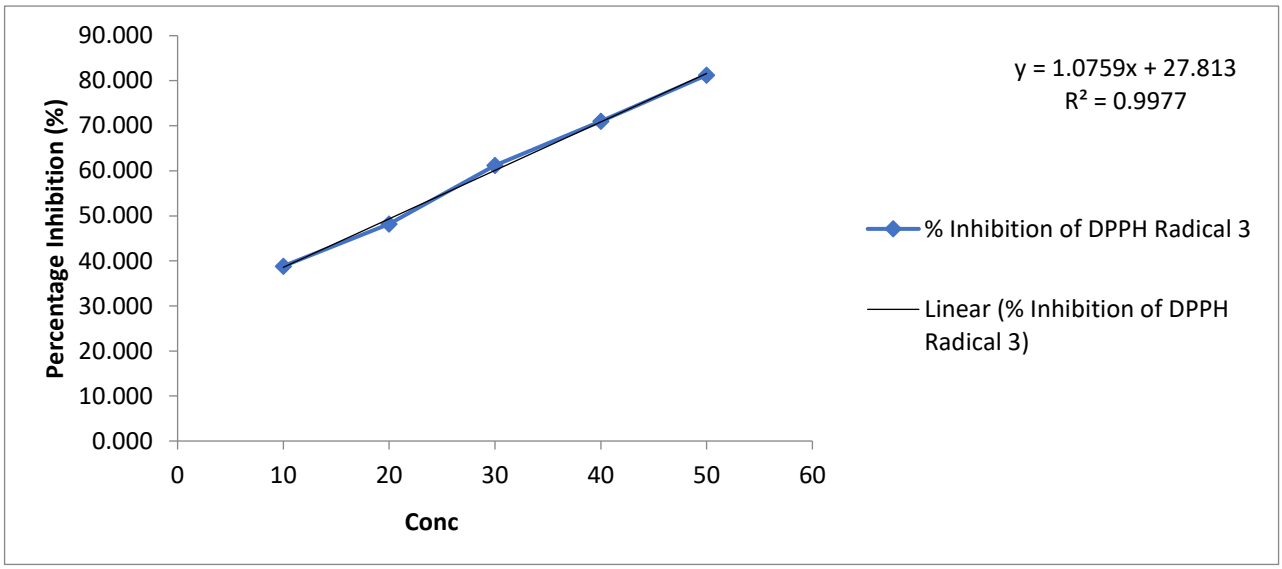
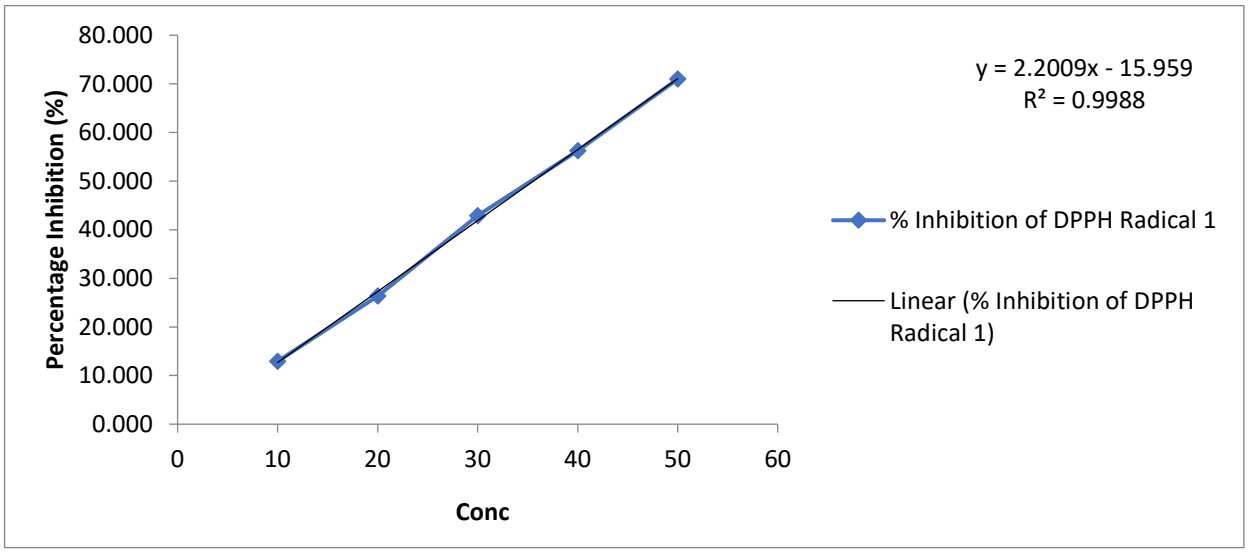
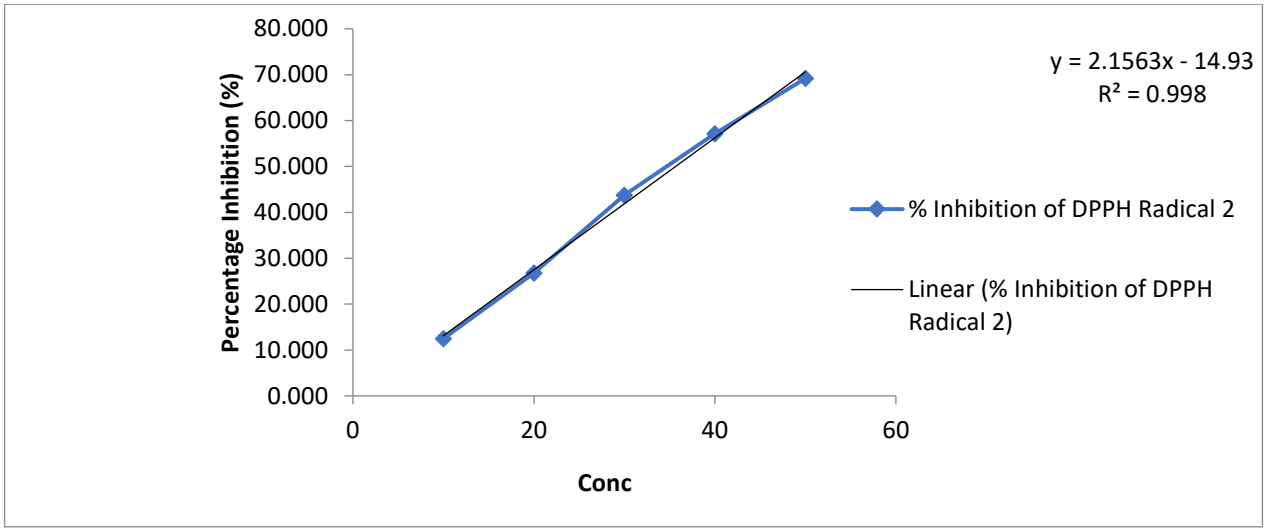


Figure 7: DPPH activity of Petroleum ether extract





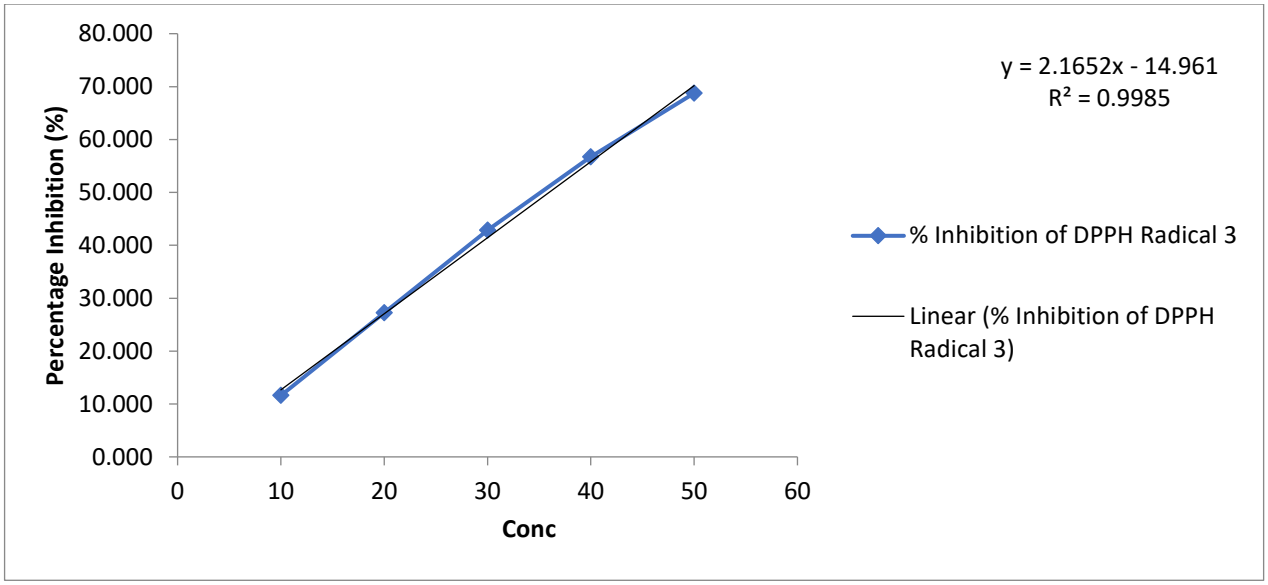
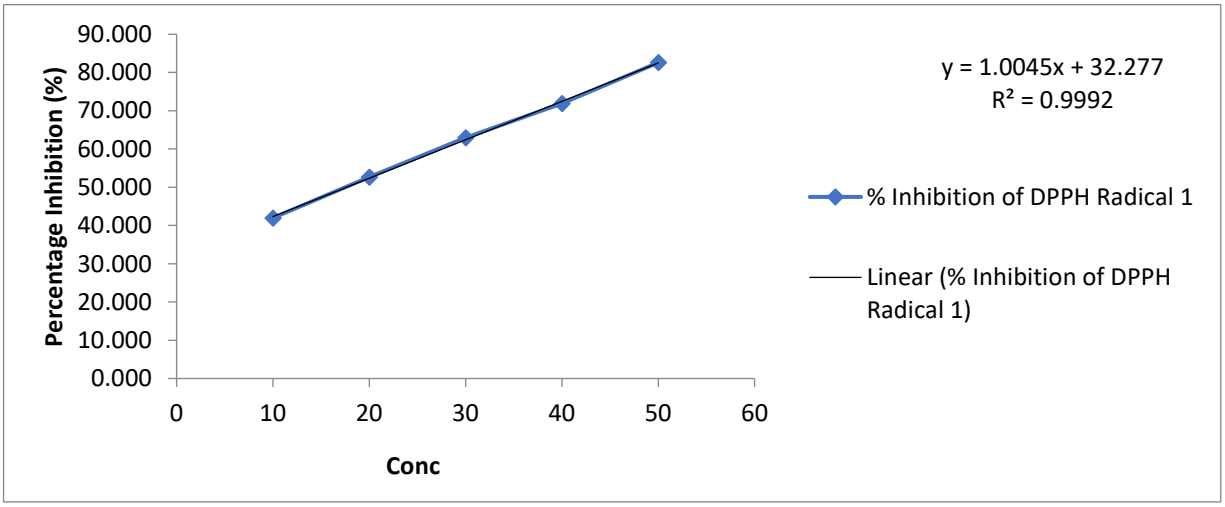
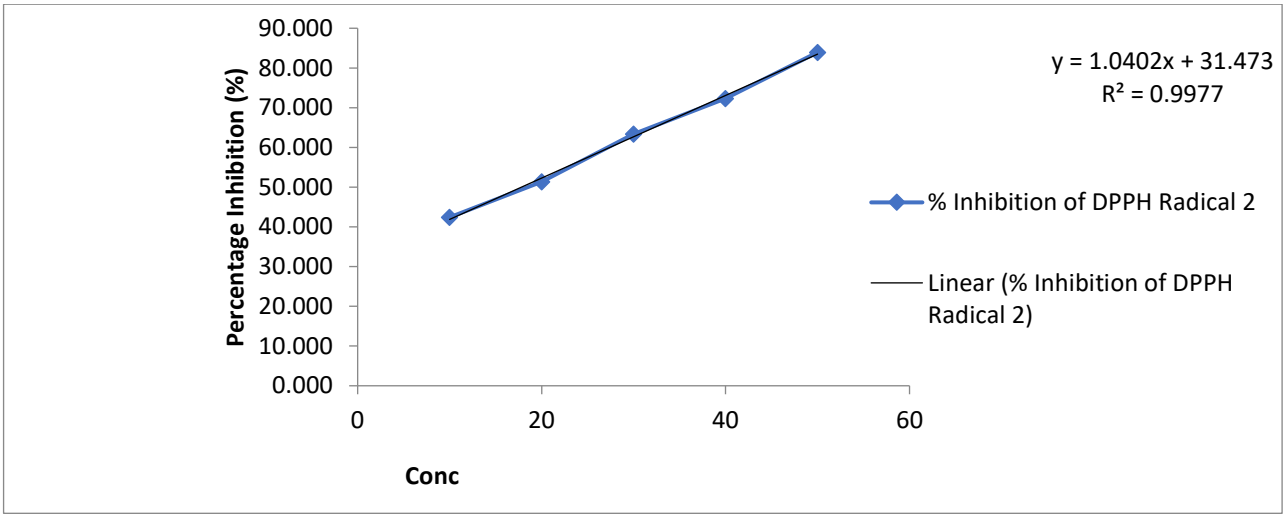


Figure-8: DPPH activity of n-hexane extract





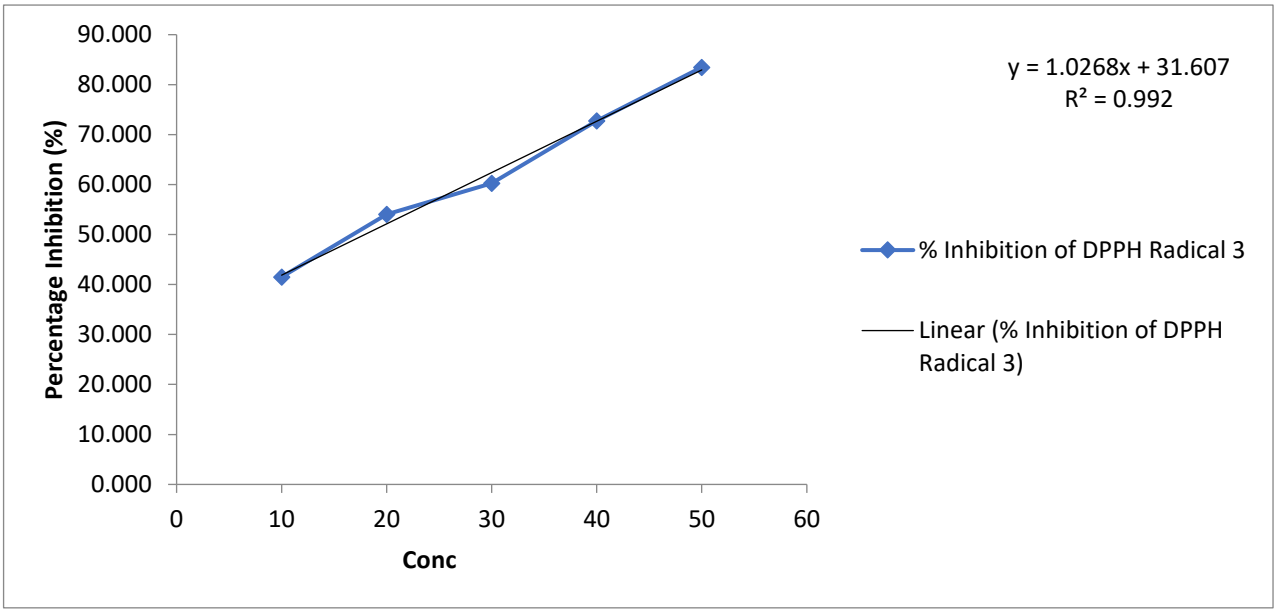
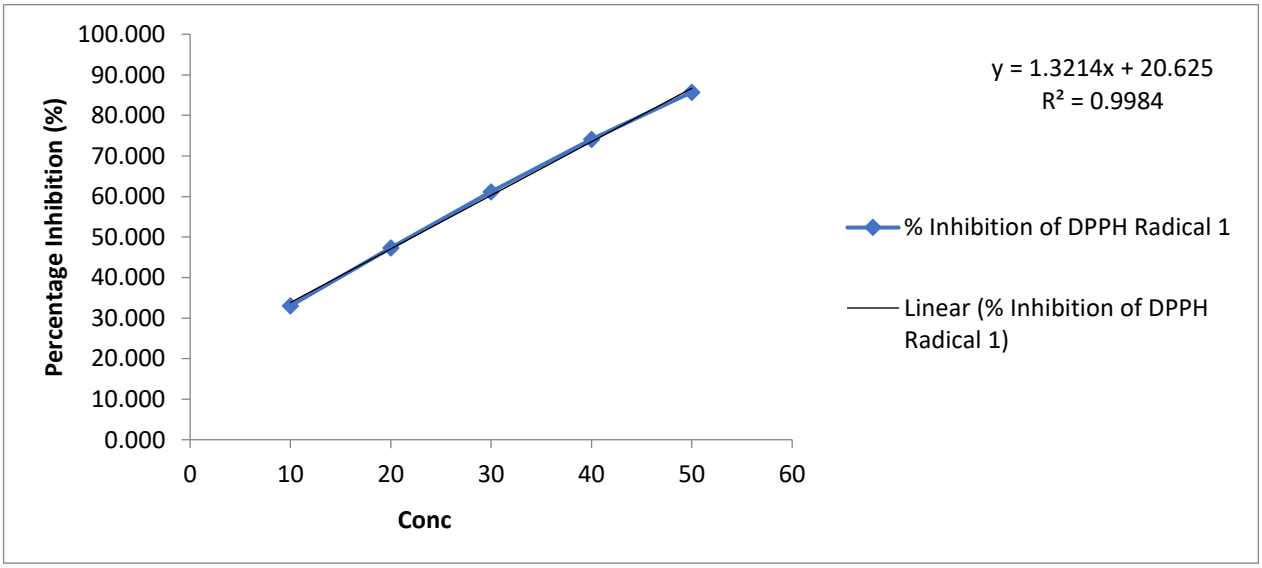
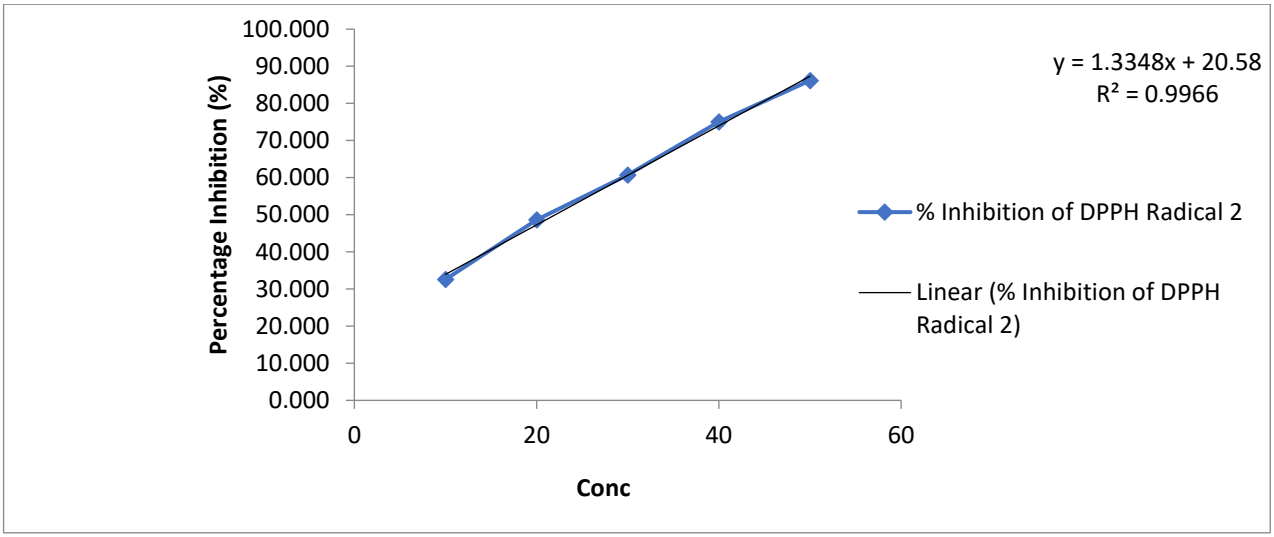


Figure:9 DPPH activity of Methanolic extract





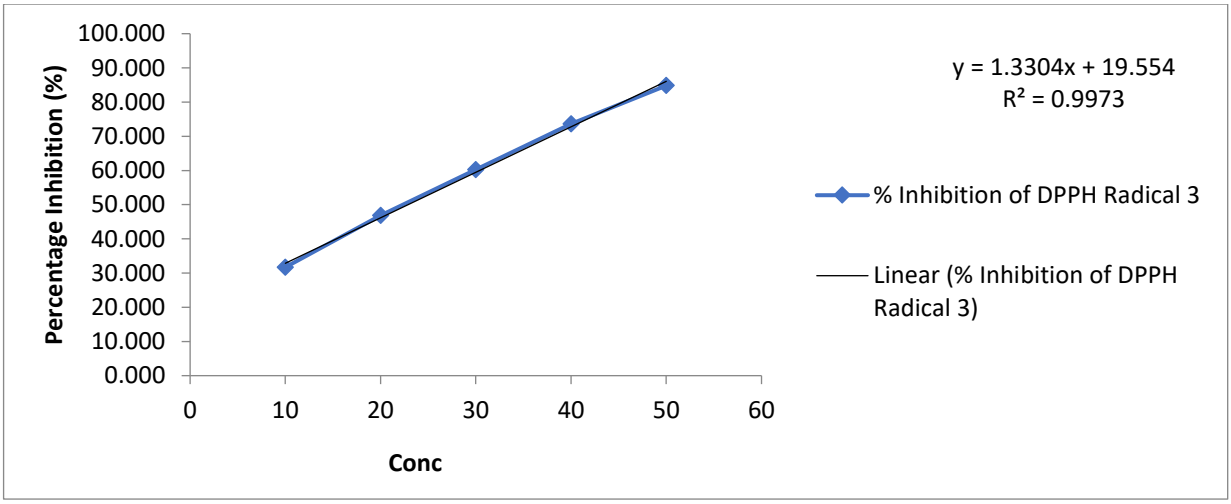


Figure-10: DPPH activity of Aqueous extract

CONCLUSION

Antioxidant activity of petroleum ether, n-hexane, methanolic and aqueous extracts was assessed by DPPH radical scavenging test in 10-50 µg/ml dosages. The methanolic extract showed greatest antioxidant activity among the studied extract with low IC_{50} value (17.79 ± 0.136 µg/ml) showing its potent DPPH radical scavenging activity. The aqueous extract also displayed good antioxidant potential with IC_{50} value of 22.39±0.443 µg/ml while n-hexane extract revealed moderate activity with IC_{50} value of 30.03±0.077 µg/ml. The petroleum ether extract showed lowest antioxidant activity with the highest IC_{50} value of 75.29±2.876 µg/ml. The enhanced antioxidant activity of methanolic extract, attributed to efficient extraction of phenolic and flavonoid components, supports its potential development as a standardized botanical antioxidant for pharmaceutical formulations. Future studies should evaluate in vivo efficacy, identify and quantify specific bioactive compounds, and assess stability under formulation conditions

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Khan, M. A., Zia, M., Arfan, M., Nazir, A., Fatima, N., Naseer, M., Khan, S. A., Ismail, T., Alkahraman, Y. M. S. A., Murtaza, G., & Mannan, A. (2018). Antioxidants, antimicrobial and cytotoxic of Swertia chirata. Biomedical Research, 29(13), 2722–2726.
2. Hossain, M. S., Chowdhury, M. E. H., Das, S., & Chowdhury, I. U. (2012). In vitro thrombolytic and anti-inflammatory activity of Swertia chirata ethanolic extract. Journal of Pharmacognosy and Phytochemistry, 1(4), 99–104.
3. Naqvi, S. A. R., Qurat-Ul-Ain, Khan, Z. A., Hussain, Z., Shaizad, S. A., Yar, M., Ghuffar, A., Mahmood, N., & Kousar, S. (2013). Antioxidant, antibacterial and antiproliferative activities of aerial parts of Swertia chirata (Bush Ham) plant extracts using in vitro models. Asian Journal of Chemistry, 25(10), 5448–5452.
4. Clarke, C. B. (1885). In The flora of British India (p. 124). Reeve and Co.
5. Kirtikar, K. R., & Basu, B. D. (1984). Indian medicinal plants (p. 1664). Allahabad.
6. Bentley, R., & Trimen, H. (1880). Medicinal plants (p. 183). J. & A. Churchill.

7. Chandrasekar, B., Bajpai, M. D., & Mukerjee, S. K. (1990). Hypoglycaemic activity of Swertia chirata Karst. Indian Journal of Experimental Biology, 28, 616–618.
8. Chowdhary, N. I., Bandyopadhyay, S. K., Banerjee, S. N., Dutta, M. K., & Das, P. C. (1995). Preliminary studies on the anti-inflammatory effects of Swertia chirata in albino rats. Indian Journal of Pharmacology, 27, 37–39.
9. Chakravarty, A. K., Mukhopadhyay, S., Moitra, S. K., & Das, B. (1994). Syringaresinol, a hepatoprotective agent and other constituents from Swertia chirata. Indian Journal of Chemistry, 33(8), 405–408.
10. Bhat, G. P., & Surolia, N. (2001). In vitro antimalarial activity of extracts of three plants used in the traditional medicine of India. American Journal of Tropical Medicine and Hygiene, 65, 304–308.
11. Khandelwal, K. R. (2005). Practical pharmacognosy: Techniques and experiments (14th ed., pp. 149–156). Nirali Prakashan.
12. Chen, Y., Huang, B., He, J., Han, L., Zhan, Y., & Wang, Y. (2011). In vitro and in vivo antioxidant effects of the ethanolic extract of Swertia chirayita. Journal of Ethnopharmacology, 136(2), 309–315. https://doi.org/10.1016/j.jep.2011.03.056
13. Khanal, S., Shakya, N., Thapa, K., & Pant, D. R. (2015). Phytochemical investigation of crude methanol extracts of different species of Swertia from Nepal. BMC Research Notes, 8, 821. https://doi.org/10.1186/s13104-015-1753-0
14. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16(3), 144–158. This is the original paper describing the Folin–Ciocalteu assay for total phenolics.
15. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178. This is the most widely cited reference for the method.
16. Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, 2(4), 875–877. This paper provides a practical protocol for plant extracts.