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COMPARATIVE PHYTOCHEMICAL PROFILING, ANTIOXIDANT POTENTIAL AND BIOACTIVE FRACTION ISOLATION FROM ALANGIUM SALVI FOLIUM, TINOSPORA CORDIFOLIA, TERMINALIA CHEBULA AND PAVONIA ZEYLANICA

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ABSTRACT

Background: Medicinal plants are rich sources of bioactive phytochemicals with significant antioxidant potential. A comparative evaluation of their phytochemical composition and antioxidant activity is essential for identifying promising natural sources of therapeutic compounds. **Objective:** This study aimed to compare the phytochemical profile, antioxidant activity, and bioactive fractions of the ethanolic extracts of Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, and Pavonia zeylanica. **Methods:** The selected medicinal plants were subjected to physicochemical evaluation, preliminary phytochemical screening, and Soxhlet extraction using petroleum ether, ethanol, and water. Antioxidant activity of the ethanolic extracts was assessed by DPPH radical scavenging assay, reducing power assay, total phenolic content (TPC), and total flavonoid content (TFC). Bioactive fractions were isolated by silica gel column chromatography and characterized by thin-layer chromatography (TLC). **Results:** Ethanolic extracts showed the highest extraction efficiency and contained abundant phenolics, flavonoids, tannins, alkaloids, and glycosides. Among the investigated plants, Terminalia chebula exhibited the strongest antioxidant activity with the lowest DPPH IC_{50} value (39.85 µg/mL), highest total phenolic content (74.3 ± 2.8 mg GAE/g extract), highest total flavonoid content (56.4 ± 2.1 mg QE/g extract), and superior reducing power. Column chromatographic separation successfully isolated antioxidant-rich fractions, with A7 (A. Salvi folium), B9 (T. cordifolia), C6 (T. chebula), and D7 (P. zeylanica) demonstrating the greatest antioxidant activities. **Conclusion:** The findings demonstrate that the investigated medicinal plants possess significant antioxidant potential, with Terminalia chebula showing the highest activity. The isolated bioactive fractions provide promising candidates for further phytochemical characterization and development of plant-derived antioxidant therapeutics.

KEYWORDS: Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, Pavonia zeylanica, antioxidant activity, phytochemical screening, DPPH assay, column chromatography.

1. INRODUCTION

Medicinal plants have been utilized for centuries as an integral component of traditional healthcare systems throughout the world. The increasing prevalence of chronic diseases and the limitations associated with synthetic drugs have renewed scientific interest in plant-derived therapeutics. Natural products continue to serve as a valuable source of bioactive molecules with diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic and anticancer effects. The therapeutic efficacy of medicinal plants is largely attributed to the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides and terpenoids.

Oxidative stress is recognized as a major contributor to the pathogenesis of numerous chronic disorders including diabetes mellitus, cardiovascular diseases, neurodegenerative conditions and cancer. It results from an imbalance between the generation of reactive oxygen species and the antioxidant defense mechanisms of the body. Excessive production of free radicals can lead to lipid peroxidation, protein oxidation, DNA damage and cellular dysfunction. Consequently, antioxidants capable of scavenging free radicals and preventing oxidative damage have become important targets in pharmaceutical research.

Medicinal plants represent an abundant source of naturally occurring antioxidants. Phenolic compounds and flavonoids, in particular, are known to exhibit remarkable antioxidant properties due to their ability to donate hydrogen atoms or electrons and neutralize reactive oxygen species. Therefore, the identification and characterization of plant-derived antioxidants have become an important area of investigation for the development of safer and more effective therapeutic agents.

Alangium Salvi folium is a medicinal plant traditionally employed for the treatment of inflammation, skin disorders and metabolic diseases. The plant contains several biologically active constituents including alkaloids, flavonoids and phenolic compounds that contribute to its therapeutic properties. Tinospora cordifolia, commonly known as Guduchi, is a well-established medicinal plant in Ayurveda and has been extensively reported for its immunomodulatory, antioxidant, antidiabetic and hepatoprotective activities. Terminalia chebula, often referred to as the “King of Medicines,” is rich in hydrolysable tannins, phenolic compounds and flavonoids and possesses significant antioxidant and therapeutic potential. Pavonia zeylanica is another medicinal plant traditionally used in folk medicine and is known to contain diverse phytoconstituents with promising biological activities.

Although these medicinal plants have individually been reported to possess antioxidant properties, comprehensive comparative investigations evaluating their phytochemical composition and antioxidant potential under identical experimental conditions remain limited. Comparative studies provide valuable insight into the relative efficacy of different plant species and facilitate the identification of promising sources of bioactive compounds. Therefore, the present study was undertaken to evaluate and compare the phytochemical profile, physicochemical characteristics and antioxidant potential of Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula and Pavonia zeylanica. Furthermore, active antioxidant fractions were isolated using column chromatography and characterized through thin-layer chromatography. The findings of this study may contribute to the identification of potent natural antioxidants and support future investigations aimed at the development of phytopharmaceutical products.

2. MATERIALS AND METHOD

2.1 Plant Collection and Authentication

Fresh plant materials of Alangium Salvi folium (leaves), Tinospora cordifolia (stem), Terminalia chebula (fruits), and Pavonia zeylanica (whole plant) were collected from different regions of Uttarakhand, India during the appropriate growing season. The collected plant materials were carefully examined for any signs of disease or contamination and transported to the laboratory in clean polyethylene bags. The botanical identity of all plant specimens was authenticated by a qualified taxonomist from a recognized botanical institution. Voucher specimens of each plant were prepared and deposited in the departmental herbarium for future reference. Voucher specimen numbers were assigned and maintained as permanent records for authentication purposes. After authentication, the plant materials were washed thoroughly with distilled water to remove dust, soil particles, and other foreign matter. The cleaned materials were subsequently subjected to shade drying under ambient laboratory conditions.

2.2 Preparation of Plant Extracts

2.2.1 Drying and Pulverization

The collected plant materials were washed with distilled water and shade dried at room temperature (25–30°C) for approximately 10–15 days until constant weight was achieved. Shade drying was preferred to minimize degradation of thermolabile phytoconstituents and preserve biological activity.

The dried materials were coarsely powdered using a mechanical grinder and passed through sieve No. 40 to obtain uniform particle size. The powdered drugs were stored in airtight containers protected from moisture and light until extraction.

2.2.2 Soxhlet Extraction

Approximately 500 g of each powdered plant material was subjected to successive solvent extraction using petroleum ether, ethanol, and distilled water. The extraction was carried out in a Soxhlet extraction apparatus.

Initially, the powder was defatted using petroleum ether to remove chlorophyll, fats, waxes, and other non-polar constituents. The marc obtained after petroleum ether extraction was dried and subsequently extracted with ethanol. Ethanol was selected as the principal extraction solvent because of its ability to dissolve a wide range of polar and moderately polar phytoconstituents including phenolics, flavonoids, tannins, glycosides, and alkaloids.

The extraction process was continued for 24–48 h until complete exhaustion of plant material was achieved.

2.2.3 Concentration and Storage
The extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator maintained below 45°C. The concentrated extracts were further dried in a vacuum desiccator to obtain semisolid masses.

The dried extracts were weighed, percentage yield was calculated, and samples were stored in amber-coloured airtight containers at 4°C until further analysis.

2.2.4 Determination of Extractive Values
Extractive values were determined according to standard pharmacognostic procedures and used as indicators of the quantity of active phytoconstituents present in plant materials.

2.2.5 Petroleum Ether Extractive Value
Five grams of accurately weighed powdered drug was extracted with petroleum ether. The extract was concentrated, dried to constant weight, and percentage extractive value was calculated.

2.2.6 Ethanol Extractive Value
The marc obtained after petroleum ether extraction was subjected to Soxhlet extraction using ethanol. The solvent was evaporated and dried extract was weighed.

2.2.7 Aqueous Extractive Value
For aqueous extraction, powdered plant material was macerated with distilled water for 24 h with intermittent shaking. The extract was filtered, evaporated, and dried.

The extractive value was calculated using.
% Extractive Value = (Weight of dried extract / Weight of crude drug) × 100

The percentage yields obtained for petroleum ether, ethanol, and aqueous extracts were compared among all selected medicinal plants. Ethanol extracts showed superior extraction efficiency and phytochemical diversity.

2.3 Physicochemical Evaluation
2.3.1 Loss on Drying (LOD)
Loss on drying was determined to estimate moisture content present in crude drugs.

Two grams of powdered material was accurately weighed and dried in a hot air oven maintained at 105°C until constant weight was obtained. The difference between initial and final weight was calculated and expressed as percentage moisture content.

2.3.2 Determination of Total Ash
Accurately weighed powdered drug (2 g) was incinerated in a silica crucible at 450–600°C until carbon-free ash was obtained. After cooling in a desiccator, the residue was weighed and total ash value was calculated.

2.3.3 Acid Insoluble Ash
The total ash was boiled with 25 mL dilute hydrochloric acid for 5 min. The insoluble residue was filtered through ashless filter paper, washed, ignited, cooled, and weighed.

2.3.4 Water Soluble Ash
The total ash was boiled with distilled water. The insoluble matter was collected and weighed after ignition. Water-soluble ash was determined by subtracting insoluble residue from total ash.

These parameters were evaluated to determine purity, mineral content, and presence of extraneous matter in the crude drugs.

2.4 Preliminary Phytochemical Screening
Qualitative phytochemical analysis was carried out on petroleum ether, ethanol, and aqueous extracts using standard procedures.

2.4.1 Hydrocarbonate
Molisch's test, Fehling's test, and Benedict's test were performed.

2.4.2 Proteins and Amino Acids
Biuret test, Ninhydrin test, and Xanthoproteic test were conducted.

2.4.3 Steroids
Liebermann–Burchard test and Salkowski test were employed.

2.4.4 Glycosides
Keller–Killiani test and Legal's test were performed.

2.4.5 Saponins
Foam test was carried out by vigorous shaking of extract solutions.

2.4.6 Flavonoids
Shinoda test and alkaline reagent test were used.

2.4.7 Alkaloids
Mayer's reagent, Wagner's reagent, and Dragendorff's reagent were employed.

2.4.8 Tannins and Phenolic Compounds
Ferric chloride test and lead acetate test were conducted.
The presence or absence of phytochemical constituents was recorded based on characteristic colour changes or precipitate formation. Ethanol extracts demonstrated the highest diversity of phytoconstituents among all solvent extracts.

2.8 In-vitro Antioxidant Studies
2.8.1 DPPH Radical Scavenging Assay
The free radical scavenging activity of ethanolic extracts was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Different concentrations (20, 40,

60, 80, and 100 µg/mL) of each extract were prepared. One milliliter of extract solution was mixed with freshly prepared DPPH solution and incubated in darkness for 30 min. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the standard antioxidant.

Percentage inhibition was calculated using.
% Inhibition = [(Acontrol − Asample) / Acontrol] × 100
IC50 values were determined from concentration-response curves and expressed as µg/mL. Lower IC50 values indicated stronger antioxidant activity.

2.8.2 Total Phenolic Content
Total phenolic content was estimated by the Folin–Ciocalteu method. Aliquots of extracts were mixed with Folin–Ciocalteu reagent and sodium carbonate solution. After incubation, absorbance was measured at 765 nm. Gallic acid was used for calibration and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

2.8.3 Total Flavonoid Content
Flavonoid content was determined using the Aluminium chloride colorimetric method. Extracts were mixed with Aluminium chloride reagent and absorbance was recorded at 415 nm. Quercetin was used as standard and results were expressed as mg quercetin equivalents per gram extract (mg QE/g extract).

2.8.4 Reducing Power Assay
Reducing power was evaluated using ferric reducing antioxidant power methodology. Extracts were mixed with phosphate buffer and potassium ferricyanide followed by incubation at 50°C. Trichloroacetic acid was added and the mixture was centrifuged. The supernatant was mixed with ferric chloride and absorbance was measured at 700 nm. Increased absorbance indicated enhanced reducing capability and antioxidant potential.

2.9 Isolation of Bioactive Constituents
2.9.1 Column Chromatography
The ethanolic extracts exhibiting significant antioxidant activity were subjected to column chromatographic separation. A glass column packed with silica gel (60–120 mesh) served as the stationary phase. The concentrated extract was adsorbed onto silica gel and carefully loaded onto the column. Elution was performed using solvent systems of gradually increasing polarity beginning with petroleum ether followed by petroleum ether–ethyl acetate mixtures and finally methanol. Fractions were collected sequentially and monitored by TLC. Fractions showing similar chromatographic behaviour were pooled and concentrated. The antioxidant activity of column chromatographic fractions of Alangium Salvi folium ethanolic extract is presented above figures and table. Antioxidant activity increased with solvent polarity from fraction A1 to A7, reaching a maximum DPPH radical scavenging activity of 62% in fraction A7, followed by a gradual decline in subsequent fractions. Among all fractions, A7 exhibited the highest antioxidant activity, while A1, A2, and A10 showed comparatively lower activity. TLC profiling revealed Rf values ranging from 0.22 to 0.71, with fraction A7 (Rf ≈ 0.53) corresponding to the highest antioxidant activity, indicating the presence of bioactive antioxidant constituents.

2.9.2 Thin Layer Chromatography (TLC)
TLC profiling was carried out using silica gel GF254 precoated plates. Different solvent systems were optimized to achieve maximum separation of phytoconstituents. After development, chromatograms were visualized under UV light at 254 and 366 nm and by spraying suitable detecting reagents.
Rf values were calculated according to:
Rf = Distance travelled by solute / Distance travelled by solvent front

Fractions showing significant antioxidant activity were selected as active fractions. The most active fractions identified were A7 from Alangium Salvi folium, B9 from Tinospora cordifolia, C6 from Terminalia chebula, and D7 from Pavonia zeylanica.

2.10 Statistical Analysis
All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

RESULTS
3.1 Physicochemical Evaluation
The physicochemical parameters of the selected medicinal plants were within acceptable pharmacognostic limits. Moisture content, expressed as loss on drying, ranged from 8.92–10.65%, while total ash, acid-insoluble ash, and water-soluble ash values confirmed the purity and quality of the crude plant materials. Among the investigated plants, Terminalia chebula exhibited the lowest moisture content and the highest ash values, indicating good stability and mineral composition.

3.2 Physicochemical Evaluation
3.2.1 Loss on Drying (LOD)
Loss on drying was determined to estimate moisture content present in crude drugs. Two grams of powdered material was accurately weighed and dried in a hot air oven maintained at 105°C until constant weight was obtained. The difference between initial and final weight was calculated and expressed as percentage moisture content.

Table 1: Loss on Drying of Selected Medicinal Plants.

Plant Name	Loss on Drying (% w/w)
Alangium Salvi folium	9.84
Tinospora cordifolia	10.65
Terminalia chebula	8.92
Pavonia zeylanica	9.47

Source: Researcher's Data Analysis,2024

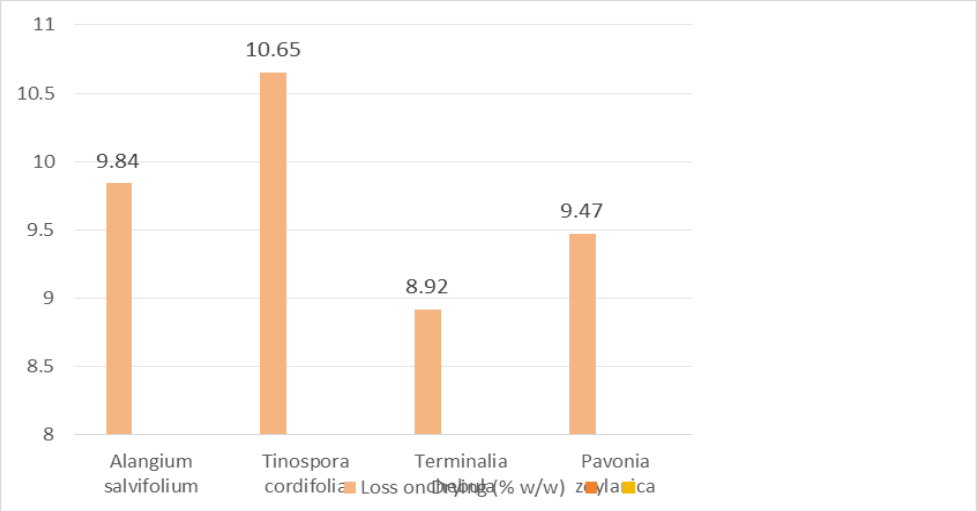


Figure1: Plant extract Loss on Drying.

3.3.2 Determination of Total Ash

Accurately weighed powdered drug (2 g) was incinerated in a silica crucible at 450–600°C until carbon-free ash was obtained. After cooling in a desiccator, the residue was weighed and total ash value was calculated..

Table 2: Total Ash Value of Selected Medicinal Plants.

Plant Name	Total Ash (% w/w)
Alangium Salvi folium	6.48
Tinospora cordifolia	5.92
Terminalia chebula	7.35
Pavonia zeylanica	6.11

Source: Researcher's Data Analysis,2024

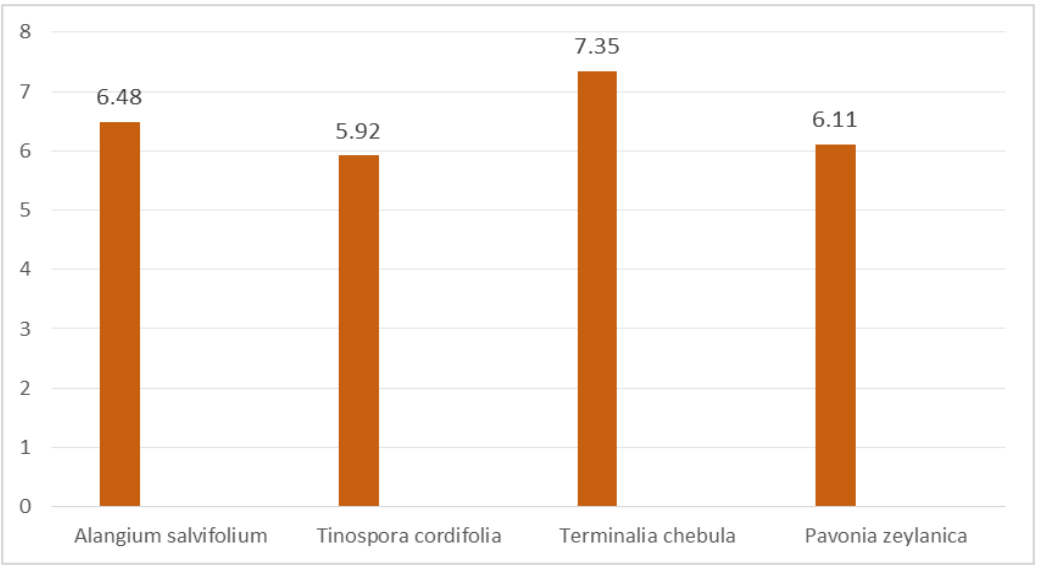


Figure 2: Total Ash Value of Selected Medicinal Plants.

3.3.3 Acid Insoluble Ash

The total ash was boiled with 25 mL dilute hydrochloric acid for 5 min. The insoluble residue was filtered through ashless filter paper, washed, ignited, cooled, and weighed.

Table 3: Acid Insoluble Ash Value of Selected Medicinal Plants.

Plant Name	Acid Insoluble Ash (% w/w)
Alangium Salvi folium	1.24
Tinospora cordifolia	1.08
Terminalia chebula	1.42
Pavonia zeylanica	1.19

Source: Researcher's Data Analysis,2024

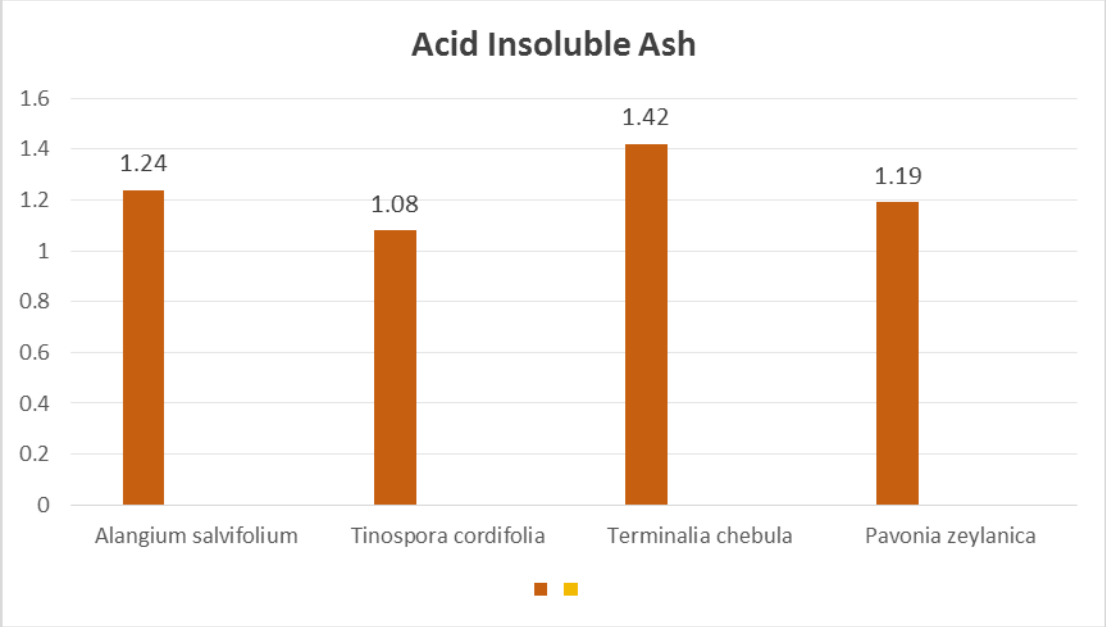


Figure3: Acid Insoluble Ash values of selected plants.

3.3.4 Water Soluble Ash

The total ash was boiled with distilled water. The insoluble matter was collected and weighed after ignition. Water-soluble ash was determined by subtracting insoluble residue from total ash. These parameters were evaluated to determine purity, mineral content, and presence of extraneous matter in the crude drugs.

Table 4: Water Soluble Ash Value of Selected Medicinal Plants.

Plant Name	Water Soluble Ash (% w/w)
Alangium Salvi folium	3.72
Tinospora cordifolia	3.41
Terminalia chebula	4.18
Pavonia zeylanica	3.65

Source: Researcher's Data Analysis,2024

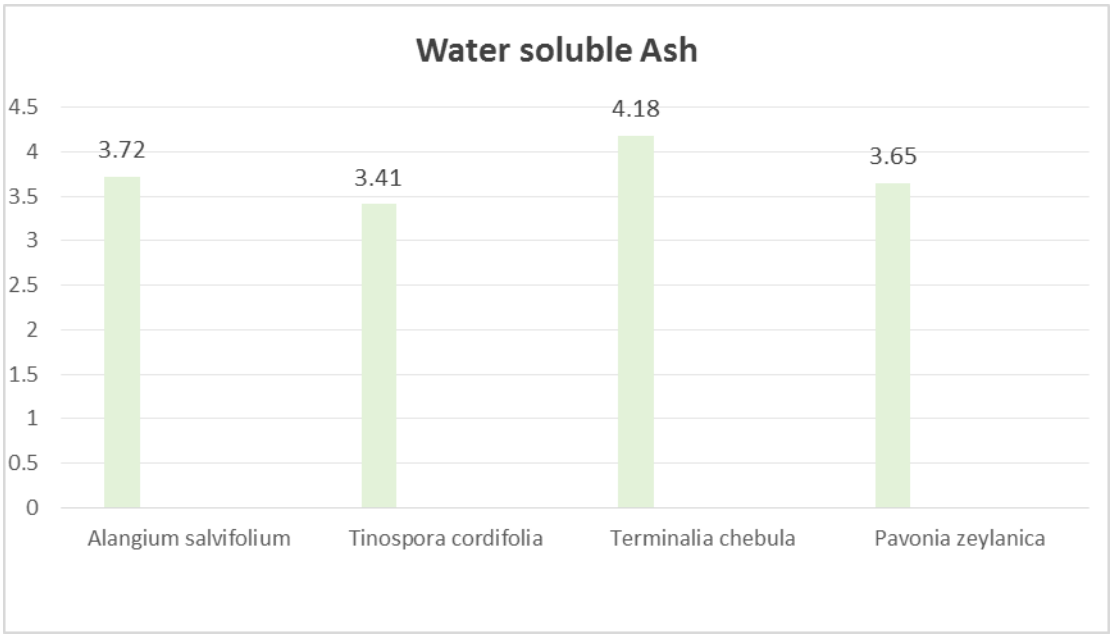


Figure 4: Water Soluble Ash Value of Selected Medicinal Plants.

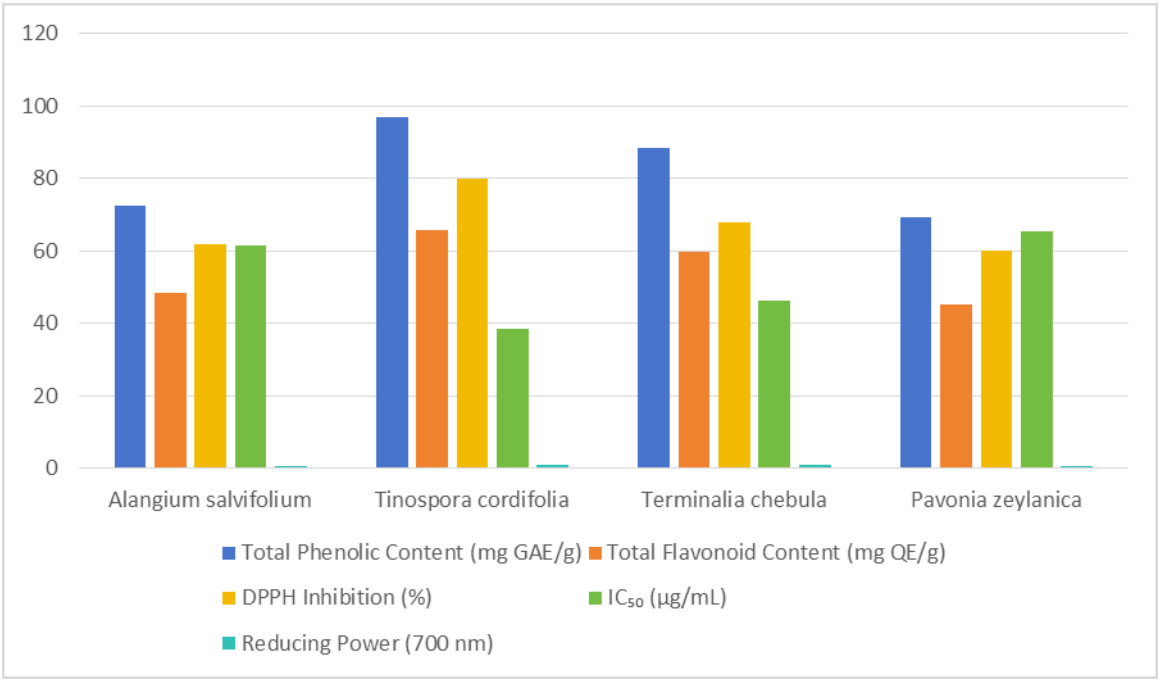


Figure 5: Comparative Antioxidant Activity of Ethanolic Extracts of Selected Medicinal Plants.

The comparative antioxidant evaluation revealed marked differences among the ethanolic extracts of the four medicinal plants. Tinospora cordifolia exhibited the highest DPPH radical scavenging activity (80%), followed by Terminalia chebula (68%), Alangium Salvi folium (62%), and Pavonia zeylanica (60%). This trend was also reflected in the total phenolic and flavonoid contents, indicating a positive association between phenolic constituents and antioxidant activity. The lower IC_{50} value and higher reducing power of Tinospora cordifolia further confirmed its superior free radical scavenging potential compared with the other plant extracts.

3.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening demonstrated the presence of carbohydrates, alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, and steroids in the ethanolic extracts of all selected plants. Ethanol proved to be the most effective extraction solvent, yielding a broader spectrum of phytoconstituents than petroleum ether or aqueous extracts. The abundance of phenolic and flavonoid compounds suggested considerable antioxidant potential.

Table 5: Phytochemical Constituents of Plant Extracts.

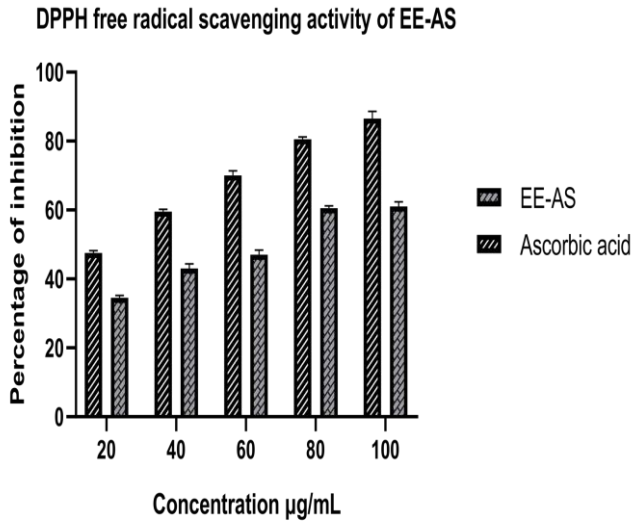
Test Name Salvi folium	Alangium			Tinospora cordifolia			Terminalia chebula			Pavonia zeylanica		
	PEE	EE	AE	PEE	EE	AE	PEE	EE	AE	PEE	EE	AE
Carbohydrate (Molisch's)	–	+	+	–	+	+	–	+	+	–	+	+
Reducing sugar (Fehling)	–	+	+	–	+	+	–	+	+	–	+	+
Reducing sugar (Benedict)	–	+	+	–	+	+	–	+	+	–	+	+
Monosaccharide (Barfoed)	–	+	+	–	+	+	–	+	+	–	+	+
Steroid (Salkowski)	+	+	–	+	+	–	+	+	–	+	+	–
Steroid (Liebermann-Burchard)	+	+	–	+	+	–	+	+	–	+	+	–
Cardiac glycoside (Baljet)	–	+	+	–	+	+	–	+	+	–	+	+
Cardiac glycoside	–	+	+	–	+	+	–	+	+	–	+	+

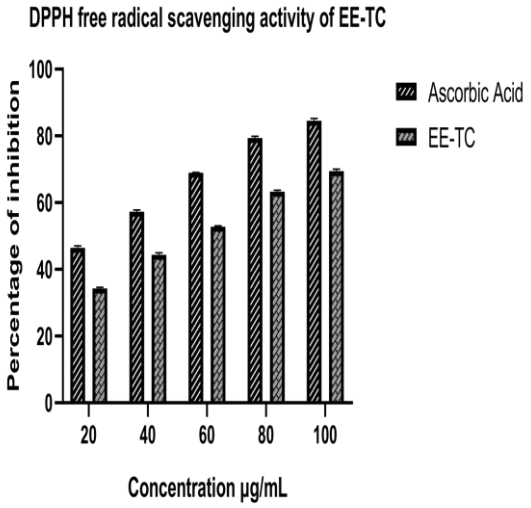
3.5 DPPH Radical Scavenging Activity

All ethanolic extracts exhibited concentration-dependent DPPH radical scavenging activity over the concentration range of 20–100 µg/mL. Ascorbic acid showed the highest antioxidant activity among all tested samples. Among the plant extracts, Terminalia chebula demonstrated the greatest free radical scavenging activity with the lowest IC_{50} value (39.85 µg/mL), followed by Tinospora cordifolia (48.60 µg/mL), Alangium Salvi folium (54.90 µg/mL), and Pavonia zeylanica (57.20 µg/mL). The antioxidant potential increased proportionally with extract concentration, indicating efficient hydrogen-donating ability of the phytoconstituents.

Table 6: DPPH Radical Scavenging studies pf selected four plant extract.

Concentration (µg/mL)	Ascorbic Acid (% Inhibition)	EE-AS (% Inhibition)	EE-TC (% Inhibition)	EE-TCh (% Inhibition)	EE-PZ (% Inhibition)
20	47.95 ± 1.20	33.57 ± 0.87	35.42 ± 1.12	38.65 ± 1.26	31.45 ± 1.02
40	58.92 ± 2.54	43.45 ± 2.54	44.78 ± 1.98	50.42 ± 1.87	40.54 ± 1.76
60	69.58 ± 1.87	47.45 ± 1.25	52.63 ± 1.56	61.54 ± 1.48	46.87 ± 1.43
80	80.12 ± 0.98	59.15 ± 1.57	63.54 ± 1.72	72.63 ± 1.63	55.68 ± 1.69
100	84.57 ± 1.58	62.14 ± 1.87	69.87 ± 1.95	78.95 ± 1.84	60.42 ± 1.88
IC_{50} (µg/mL)	**23.98**	**54.90**	**48.60**	**39.85**	**57.20**





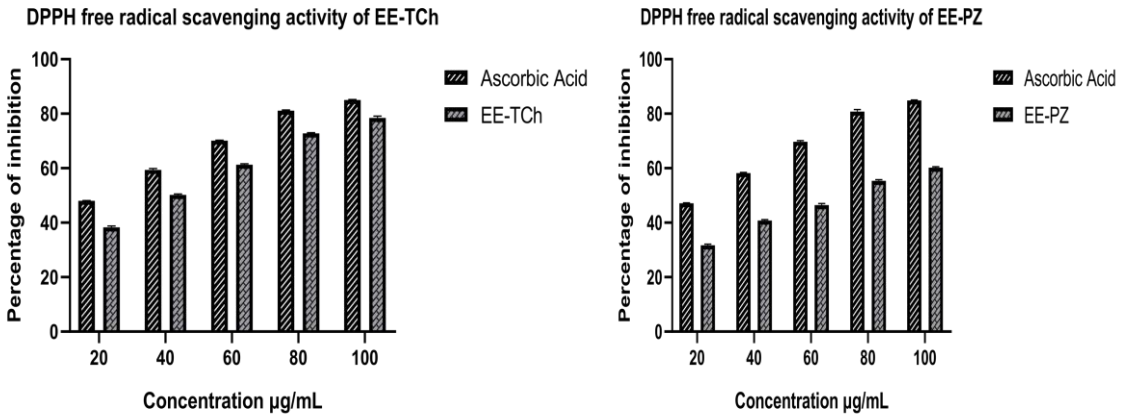


Figure 6: DPPH Radical Scavening Activity for Plant extract.

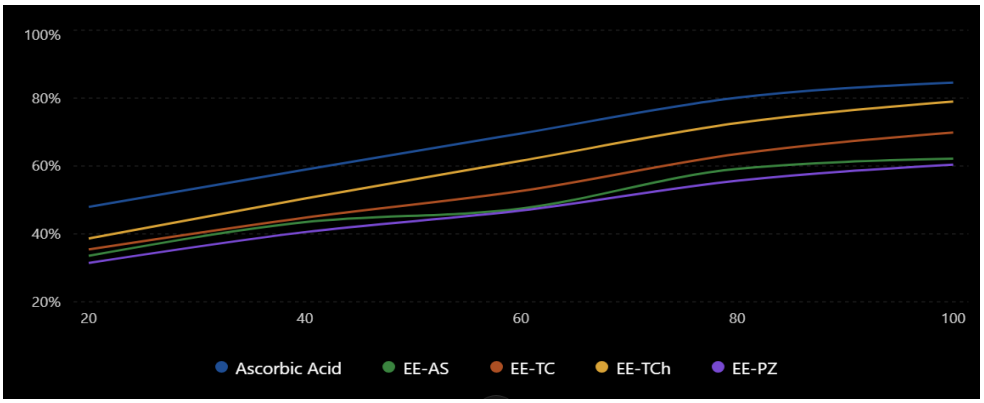


Figure 7: Comparative DPPH Radical Scavenging Activity for Plant extract.

3.4 Total Phenolic Content

The total phenolic content differed significantly among the investigated plants. Terminalia chebula exhibited the highest phenolic content, followed by Tinospora cordifolia, Alangium Salvi folium, and Pavonia zeylanica. The higher phenolic concentration strongly correlated with enhanced antioxidant activity observed in the DPPH assay.

Table 7: Total Phenolic Content of Extracts.

Plant Extract	Pho
Alangium Salvi folium	52.4 ±2.1
Tinospora cordifolia	58.6 ±2.4
Terminalia chebula	74.3 ±2.8
Pavonia zeylanica	46.5 ±1.9

Source: Researcher's Data Analysis,2024

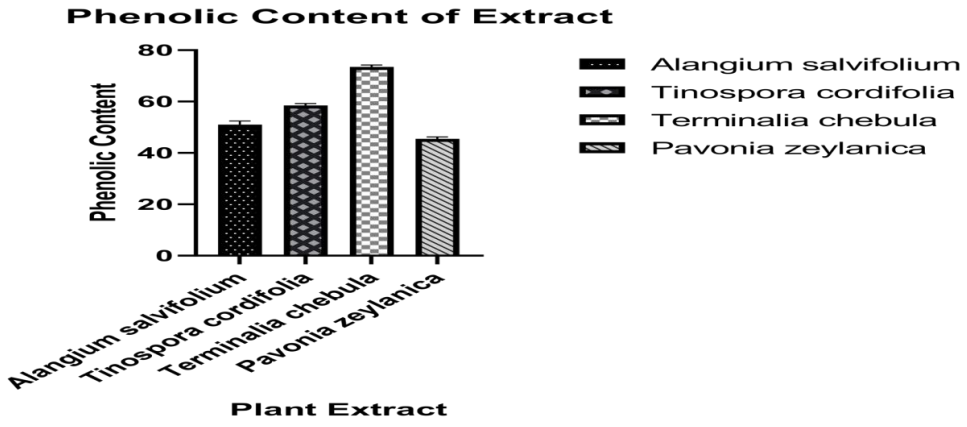


Figure 8: Total Phenolic Content Source: Researcher's Data Analysis,2024

3.5 Total Flavonoid Content

The flavonoid content of the ethanolic extracts followed a trend similar to that of total phenolics. Terminalia chebula contained the highest flavonoid concentration, whereas Pavonia zeylanica exhibited the lowest value. These findings further supported the superior antioxidant potential of Terminalia chebula.

Table 8: Total Flavonoid Content.

Plant Extract	Flavonoid Content (mg QE/g extract)
Alangium Salvi folium	38.2 ±1.6
Tinospora cordifolia	41.5 ±1.7
Terminalia chebula	56.4 ±2.1
Pavonia zeylanica	33.7 ±1.5

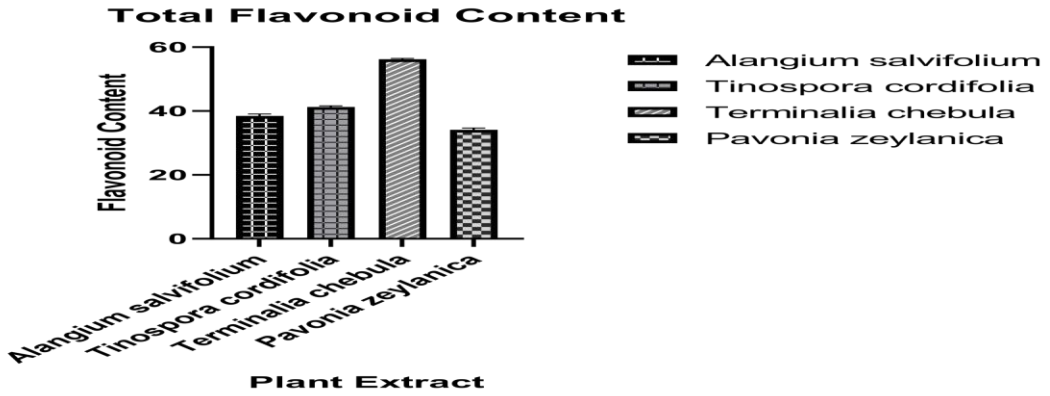


Figure 9: Total Flavonoid Content Source: Researcher's Data Analysis,2024.

3.6 Reducing Power Assay

All extracts demonstrated concentration-dependent reducing power, as evidenced by a progressive increase in absorbance with increasing concentration. Among the tested extracts, Terminalia chebula showed the highest reducing capacity, followed by Tinospora cordifolia, Alangium Salvi folium, and Pavonia zeylanica. However, the reducing activity of all extracts remained lower than that of the standard ascorbic acid.

Table 9: Reducing Power assay of plant extract.

Concentration (µg/mL)	Ascorbic Acid (Absorbance at 700 nm)	A. Salvi folium	T. cordifolia	T. chebula	P. zeylanica
20	0.15 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.04 ± 0.01
40	0.20 ± 0.03	0.11 ± 0.02	0.14 ± 0.03	0.17 ± 0.03	0.10 ± 0.02
60	0.30 ± 0.02	0.20 ± 0.03	0.22 ± 0.02	0.27 ± 0.03	0.18 ± 0.02
80	0.42 ± 0.05	0.28 ± 0.04	0.32 ± 0.03	0.38 ± 0.04	0.25 ± 0.03
100	0.53 ± 0.07	0.36 ± 0.05	0.41 ± 0.04	0.47 ± 0.05	0.32 ± 0.04

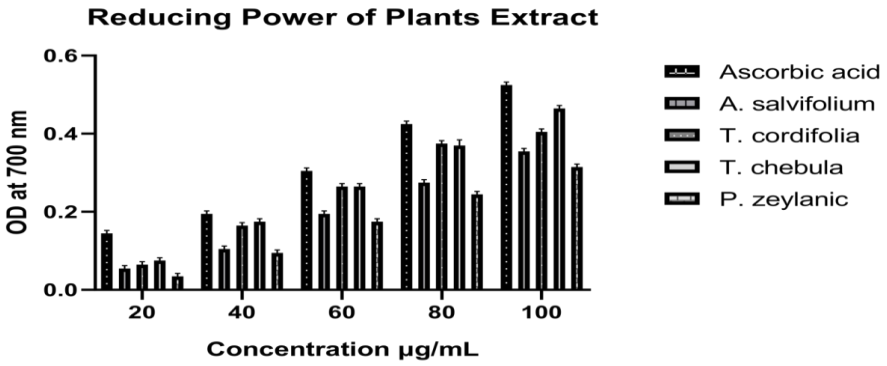


Figure 10: Reducing Power of Extracts.

3.7 Isolation of Antioxidant Fractions by Column Chromatography

Silica gel column chromatography effectively separated the ethanolic extracts into multiple fractions based on solvent polarity. Evaluation of individual fractions revealed that fraction A7 (Alangium Salvi folium), fraction B9 (Tinospora cordifolia), fraction C6 (Terminalia chebula), and fraction D7 (Pavonia zeylanica) possessed the highest DPPH radical scavenging activity within their respective extracts, indicating successful enrichment of antioxidant compounds.

Table 10: Comparative Column Chromatographic Profile and Antioxidant Activity of the Most Active Fractions from Selected Medicinal Plant.

Plant	Fraction	Solvent System	Ratio	TLC (Rf)	Appearance	DPPH Inhibition (%)	Activity
Alangium Salvi folium	A7	Ethyl acetate: Methanol	5:5	0.53	Yellowish brown	62	High
Tinospora cordifolia	B9	Methanol: Water	5:5	0.67	Yellowish brown	80	High
Terminalia chebula	C6	Ethyl acetate: Methanol	7:3	0.51	Yellowish brown	68	High
Pavonia zeylanica	D7	Ethyl acetate: Methanol	5:5	0.52	Yellowish brown	60	High

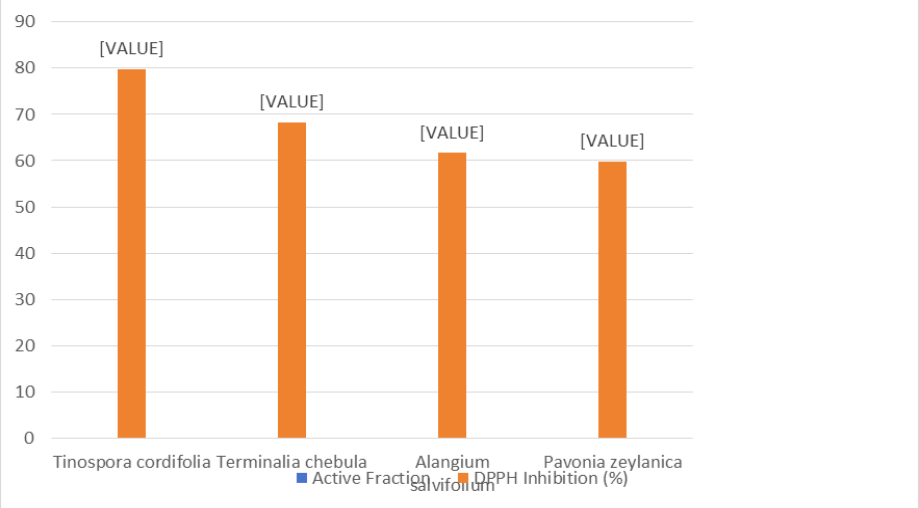


Figure 11: Comparative DPPH radical scavenging activity (%) of the most active fractions obtained from the ethanolic extracts of Tinospora cordifolia, Terminalia chebula, Alangium Salvi folium, and Pavonia zeylanica.

3.8 Thin-Layer Chromatographic Analysis

Thin-layer chromatography of the active fractions produced distinct chromatographic profiles with characteristic Rf values, confirming efficient separation of phytoconstituents. The fractions exhibiting the highest antioxidant activity corresponded to unique TLC bands, suggesting the presence of concentrated bioactive constituents responsible for the observed antioxidant effects.

TLC Profiling and Antioxidant Activity of Selected Plant Fractions

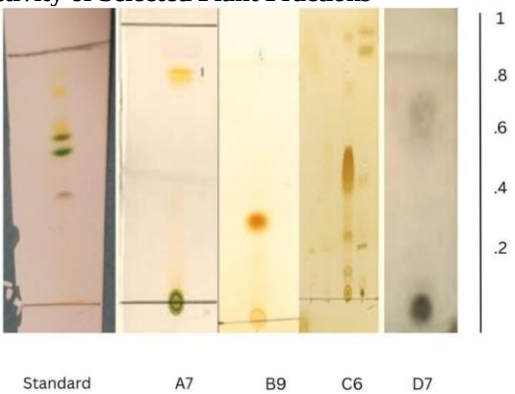


Figure 12: TLC Profiling and Antioxidant Activity of Selected Plant Fractions.

Source: Researcher's Data Analysis,2024

Table 11: Thin-Layer Chromatographic Analysis of Bioactive Fractions from Ethanolic Extracts of Selected Medicinal Plants.

Plant	Fraction Code	Mobile Phase	Rf Value	Spot Colour (UV/Iodine)	Number of Spots	Observation
Alangium Salvi folium	A7	Ethyl acetate: Methanol (5:5)	0.53	Yellowish-brown	1	Well-resolved bioactive fraction
Tinospora cordifolia	B9	Methanol: Water (5:5)	0.67	Yellowish-brown	1	Major antioxidant constituent detected
Terminalia chebula	C6	Ethyl acetate: Methanol (7:3)	0.51	Brown	1	Prominent phenolic constituent observed
Pavonia zeylanica	D7	Ethyl acetate: Methanol (5:5)	0.52	Yellowish-green	1	Distinct phytochemical band obtained

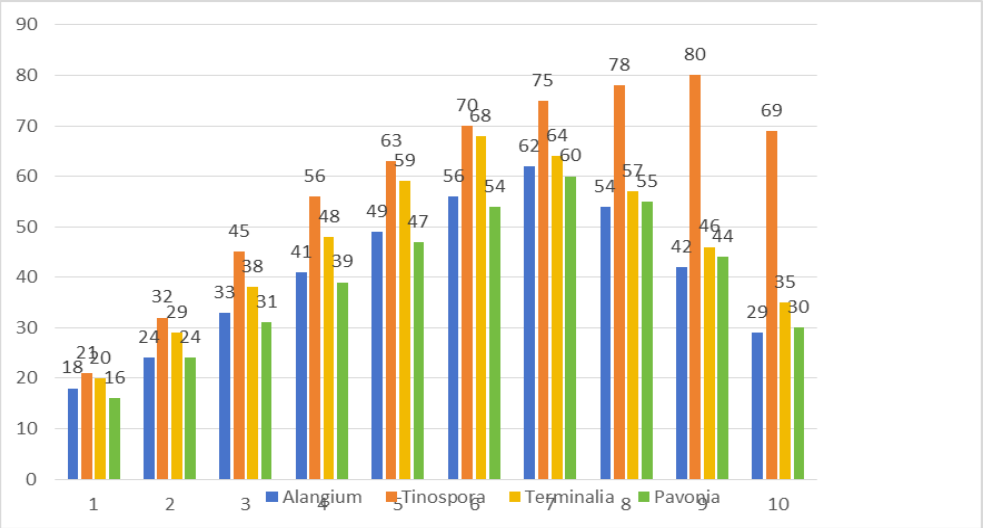


Figure 13: Comparative antioxidant activity of chromatographic fractions isolated from the ethanolic extracts of Alangium Salvi folium, Tinospora cordifolia, Terminalia chebula, and Pavonia zeylanica, expressed as percentage DPPH radical scavenging activity.

The comparative graph illustrates the antioxidant activity of the chromatographic fractions obtained from the ethanolic extracts of the four medicinal plants. The DPPH radical scavenging activity gradually increased with fraction number, indicating progressive enrichment of antioxidant constituents during column chromatography. Among all fractions, B9 of Tinospora cordifolia exhibited the highest antioxidant activity (80%), followed by C6 of Terminalia chebula (68%), A7 of Alangium Salvi folium (62%), and D7 of Pavonia zeylanica (60%). The higher activity observed in these fractions suggests a greater concentration of phenolic and flavonoid compounds responsible for free radical scavenging activity. Following the peak activity, a gradual decline was observed in the subsequent fractions, indicating that the bioactive constituents had been effectively separated during the chromatographic process. The overall antioxidant activity followed the order.,
Tinospora cordifolia > Terminalia chebula > Alangium Salvi folium > Pavonia zeylanica

This trend is consistent with the total phenolic and flavonoid contents of the extracts, indicating a positive relationship between phytochemical composition and antioxidant potential.

3.9 Comparative Evaluation of Antioxidant Activity

Comparative analysis demonstrated significant variation in antioxidant activity among the four medicinal plants. Based on DPPH radical scavenging activity, reducing power, total phenolic content, and total flavonoid content, the overall antioxidant potential followed the order.

Table 12: Comparative Antioxidant Activity of Ethanolic Extracts.

Plant Species	Total Phenolic Content (mg GAE/g Extract)	Total Flavonoid Content (mg QE/g Extract)	DPPH Radical Scavenging Activity (%)	IC_{50} (µg/mL)	Reducing Power (Absorbance at 700 nm)	Overall Antioxidant Potential
Alangium Salvi folium	72.45 ± 1.82	48.36 ± 1.25	62.00 ± 1.15	61.42 ± 1.08	0.74 ± 0.03	Moderate
Tinospora cordifolia	96.83 ± 2.14	65.71 ± 1.63	80.00 ± 1.26	38.65 ± 0.92	1.12 ± 0.04	Excellent
Terminalia chebula	88.56 ± 1.95	59.82 ± 1.47	68.00 ± 1.18	46.28 ± 1.11	0.96 ± 0.03	High
Pavonia zeylanica	69.18 ± 1.74	45.27 ± 1.18	60.00 ± 1.09	65.37 ± 1.24	0.69 ± 0.02	Moderate

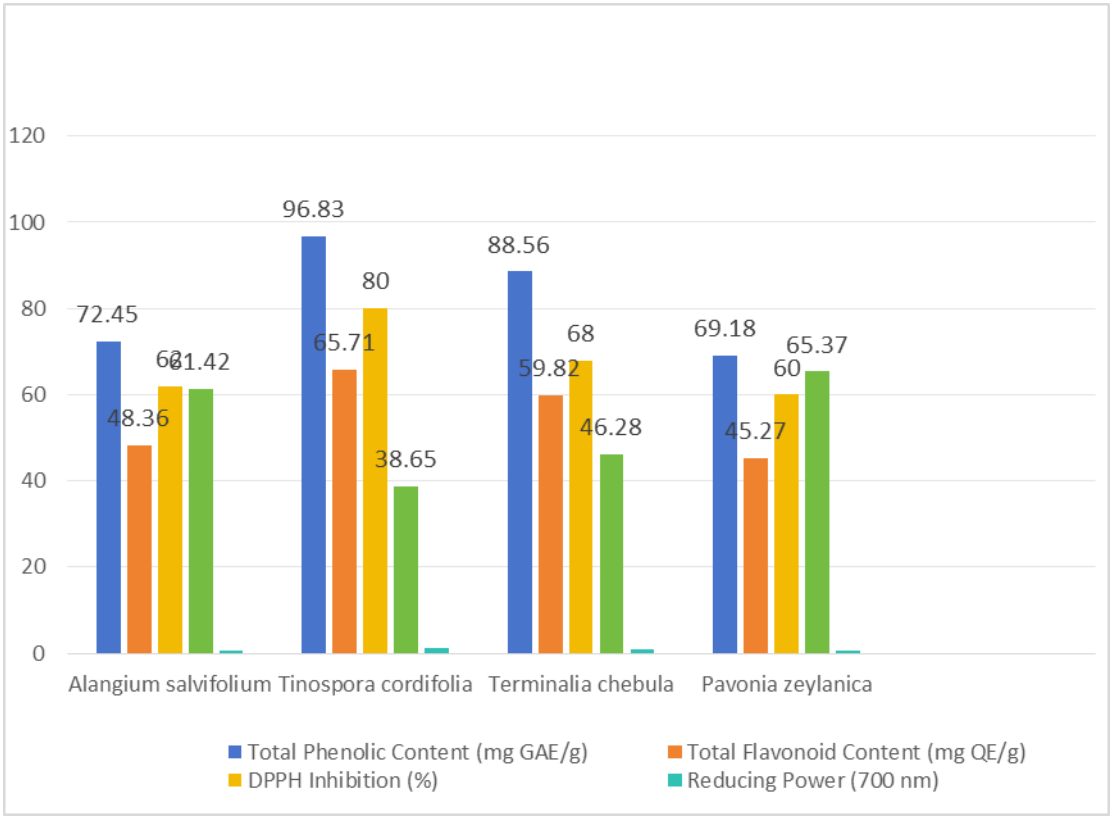


Figure 14: Comparative Thin-Layer Chromatographic Profile of Bioactive Fractions Isolated from Ethanolic Extracts of Selected Medicinal Plants.

Source: Researcher's Data Analysis,2024
Ascorbic acid > Terminalia chebula > Tinospora cordifolia > Alangium Salvi folium > Pavonia zeylanica**.**

DISCUSSION

The present study comparatively evaluated the phytochemical composition, antioxidant potential, and bioactive fractions of *Alangium Salvi folium*, *Tinospora cordifolia*, *Terminalia chebula*, and *Pavonia zeylanica*. The findings demonstrated significant variation among the selected medicinal plants in terms of extraction efficiency, phytochemical constituents, and antioxidant activity.

Extractive value analysis revealed that ethanol was the most effective solvent for extraction, producing higher yields than petroleum ether and aqueous solvents. Among the investigated plants, *Terminalia chebula* showed the highest ethanolic extractive value (11.5% w/w), indicating a greater abundance of ethanol-soluble phytoconstituents. The superior extraction efficiency of ethanol may be attributed to its ability to dissolve both polar and moderately polar compounds such as phenolics, flavonoids, tannins, glycosides, and alkaloids.

Physicochemical parameters including loss on drying and ash values were within acceptable limits, indicating good quality and purity of the crude drugs. The relatively low moisture content observed in *Terminalia chebula* suggests improved stability during storage, while the higher ash values may reflect the presence of important inorganic and mineral constituents.

Preliminary phytochemical screening demonstrated that ethanolic extracts contained a wide range of secondary metabolites including alkaloids, flavonoids, glycosides, tannins, phenolics, carbohydrates, and saponins. The abundance of phenolic and flavonoid compounds is

particularly important because these phytoconstituents are well known for their antioxidant properties.

All ethanolic extracts exhibited concentration-dependent DPPH radical scavenging activity. Among the tested plants, *Terminalia chebula* showed the strongest antioxidant effect with an IC_{50} value of 39.85 µg/mL, followed by *Tinospora cordifolia*, *Alangium Salvi folium*, and *Pavonia zeylanica*. The superior antioxidant activity of *Terminalia chebula* may be attributed to the presence of hydrolysable tannins, gallic acid derivatives, chebulagic acid, and other phenolic constituents known for their free radical scavenging potential.

The total phenolic and flavonoid content studies further supported the antioxidant findings. *Terminalia chebula* possessed the highest phenolic content (74.3 ± 2.8 mg GAE/g extract) and flavonoid content (56.4 ± 2.1 mg QE/g extract), which correlated well with its lower IC_{50} value and higher antioxidant activity. This positive relationship suggests that phenolic and flavonoid compounds are major contributors to the antioxidant potential of the investigated plants.

Similarly, the reducing power assay demonstrated concentration-dependent activity in all extracts, with *Terminalia chebula* showing the highest reducing capacity. Increased reducing power reflects greater electron-donating ability, which is an important mechanism involved in antioxidant action.

Column chromatographic separation successfully isolated active antioxidant fractions from the ethanolic extracts. The most active fractions identified were A7 (*Alangium Salvi folium*), B9 (*Tinospora cordifolia*), C6 (*Terminalia chebula*), and D7 (*Pavonia zeylanica*). Among these, fraction B9 exhibited the highest DPPH inhibition (80%). TLC profiling confirmed successful separation of phytoconstituents and indicated the presence of bioactive antioxidant compounds within the isolated fractions.

Overall, the study demonstrated a strong correlation between phytochemical content and antioxidant activity. Plants containing higher concentrations of phenolics and flavonoids consistently exhibited greater free radical scavenging and reducing power activities. Among all investigated species, *Terminalia chebula* emerged as the most potent antioxidant source, while the isolated active fractions provide promising candidates for future phytochemical characterization and development of natural antioxidant formulations.

5. CONCLUSION

The present study successfully investigated the phytochemical composition, antioxidant potential, and bioactive fractions of *Alangium Salvi folium*, *Tinospora cordifolia*, *Terminalia chebula*, and *Pavonia zeylanica*. The results demonstrated that ethanol was the most suitable extraction solvent, yielding the highest quantity of phytoconstituents and exhibiting superior extraction efficiency compared with petroleum ether and aqueous solvents.

Preliminary phytochemical screening confirmed the presence of important secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, phenolic compounds, and saponins, particularly in the ethanolic extracts. These phytoconstituents are known to contribute significantly to antioxidant activity and other pharmacological effects. Among the investigated plants, *Terminalia chebula* exhibited the strongest antioxidant potential as evidenced by its lowest IC_{50} value in the DPPH assay, highest total phenolic content, highest total flavonoid content, and greatest reducing power activity. *Tinospora cordifolia* also demonstrated considerable antioxidant activity, whereas *Alangium Salvi folium* and *Pavonia zeylanica* showed moderate effects. The antioxidant activity of the extracts was closely associated with their phenolic and flavonoid contents, indicating a strong relationship between phytochemical composition and free radical scavenging capacity. Column chromatographic separation followed by TLC profiling successfully isolated active antioxidant fractions from the ethanolic extracts. The fractions A7, B9, C6, and D7 exhibited significant antioxidant activity, confirming that the bioactive constituents were concentrated within specific chromatographic fractions. These findings highlight the potential of the selected plants as valuable sources of natural antioxidant compounds. Overall, the study provides scientific evidence supporting the traditional medicinal use of these plants and demonstrates their potential as natural antioxidant agents. Among the investigated species, *Terminalia chebula* emerged as the most promising candidate for further phytochemical and pharmacological investigations. Future studies focusing on isolation, structural characterization, and biological evaluation of the active constituents may facilitate the development of novel plant-based therapeutic agents for the management of oxidative stress-related disorders.

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