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RESEARCH ARTICLE

# Phytochemical Screening and Comparative Antioxidant Activity of *Eclipta alba* (L.) Leaf Extracts

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**Abstract**: *Eclipta alba* (L.) which belongs to the Asteraceae family is widely used in Ayurvedic and Siddha medicine due to its therapeutic efficacy in treating hepatic disorders, skin ailments, and respiratory conditions. The therapeutic potential of this herb is primarily attributed to a diverse array of secondary metabolites, including coumestans, flavonoids, and phenolic acids. This study involved a comparative analysis of phytochemical constituents and antioxidant activities across leaf extracts obtained through petroleum ether, acetone, and ethanol using a cold percolation method. Quantitative spectrophotometric assays revealed that while the ethanolic extract provided the highest percentage yield (3.264%), the petroleum ether extract exhibited the most potent antioxidant activity with the lowest $EC_{50}$ value (0.872 mg/mL) and the highest Antioxidant Radical Power (ARP). Total phenolic and flavonoid contents were significantly higher in the petroleum ether and acetone extracts compared to the ethanolic fraction, suggesting that the antioxidant efficacy is closely linked to the concentration of these bioactive molecules. Thin Layer Chromatography (TLC) confirmed the presence of wedelolactone, a marker bioactive compound, across the extracts. The findings establish that solvent polarity significantly influences the extraction efficiency and biological potency of *E. alba*. The robust radical scavenging activity observed supports the traditional application of the plant as a natural hepatoprotective and anti-aging agent, providing a scientific basis for its integration into modern antioxidant-based therapeutic regimens.

**Keywords:** *Eclipta alba*; Wedelolactone; DPPH Assay; Phytochemical Screening; Oxidative Stress

## 1. Introduction

Plant-derived drugs have historically served as the foundation of global healthcare systems, providing a vast library of bioactive molecules for drug discovery [1]. The utilization of medicinal plants is increasingly favored in modern pharmacology due to the presence of secondary metabolites such as terpenoids, alkaloids, and polyphenols, which demonstrate diverse biological activities with minimal side effects compared to synthetic alternatives [2]. Among these, antioxidant compounds are particularly significant for their ability to neutralize reactive oxygen species (ROS) and slower the progression of chronic degenerative diseases [3].

*Eclipta alba* (L.), commonly referred to as Bhringraj in the Indian subcontinent, is an annual herbaceous plant widely distributed in tropical and subtropical regions. In traditional systems of medicine like Ayurveda, it is classified as a 'Rasayana' or rejuvenating herb, particularly valued for its affinity toward liver health and hair revitalization [4]. The genus Eclipta is characterized by the absence of bristles on its fruit, while the specific epithet 'alba' denotes the white hue of its floral heads [5]. Historically, the plant has been employed to treat jaundice, cirrhosis, and infectious hepatitis, acting as a potent hepatoprotective agent that stimulates bile flow and supports hepatocyte regeneration [6].

The pharmacological versatility of *E. alba* arises from its complex phytochemical profile. It contains significant concentrations of coumestans, most notably wedelolactone and desmethylwedelolactone, which are recognized for their anti-inflammatory and anti-hepatotoxic properties [7]. Additionally, the presence of luteolin-type flavonoids, oleanane-type triterpene glycosides, and various polyacetylenes contributes to its antimicrobial, antiviral, and immunomodulatory effects [8]. The herb is also used as a staple in trichological treatments, where it is utilized to promote hair follicles' transition into the anagen phase, thereby preventing alopecia and premature graying [9].

Oxidative stress represents a physiological imbalance where the production of ROS exceeds the capacity of the endogenous antioxidant defense systems, leading to lipid peroxidation, protein denaturation, and DNA damage [10]. Chronic oxidative stress is a primary driver in the pathogenesis of diabetes, atherosclerosis, and neurodegenerative disorders like Alzheimer's disease [11].

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While the human body employs enzymatic antioxidants such as superoxide dismutase (SOD) and catalase, supplemental exogenous antioxidants from dietary or herbal sources are essential to bolster these defenses [12]. Given the safety concerns surrounding synthetic antioxidants like butylated hydroxyanisole (BHA), there is a critical need to characterize the radical scavenging potential of traditional medicinal plants like *E. alba* [13].

*Eclipta alba* is systematically placed within the Kingdom Plantae, Division Tracheophyta, and Family Asteraceae. It is a moisture-loving, branched herb that typically reaches a height of 30 to 40 cm. The plant exhibits a prostrate or erect growth habit, with cylindrical stems that vary from green to reddish-purple and are covered in fine, appressed hairs [14]. The leaves are sessile, lanceolate, and arranged oppositely, possessing a slightly serrated margin. The inflorescence is a solitary, pedunculate head with white ray florets and yellow disc florets, which eventually develop into compressed, brownish achenes [15].

The plant thrives in tropical climates where temperatures range between 25°C and 35°C. It is typically found in waterlogged habitats, such as the peripheries of rice fields, irrigation canals, and wetlands, indicating a high tolerance for high-moisture environments [16]. Although it prefers nutrient-rich sandy loam or clay loam soils with a neutral to slightly acidic pH (5.0–8.0), *E. alba* demonstrates remarkable adaptability to varying soil types and can tolerate moderate drought conditions once established. Seed germination is highly dependent on light exposure, with shallow soil burial favoring seedling emergence [17].

The therapeutic efficacy is linked to a broad range of metabolites. Coumestans like wedelolactone are the primary markers for quality control. The plant also synthesizes various triterpenoids, such as eclalbasaponins I–X, and sterols like stigmasterol and daucosterol [18]. Flavonoids including luteolin and apigenin provide the basis for its antioxidant activity, while volatile oils containing heptadecane and eudesma-4(14),11-diene contribute to its aromatic and antimicrobial properties [19].

## 2. Materials and Methods

### 2.1. Collection and Authentication of Plant Material

The investigation utilized healthy, mature specimens of *Eclipta alba* (L.) harvested from the Peddapuram Botanical Garden. Selection criteria focused on plants aged between one and two months to ensure optimal secondary metabolite accumulation. Following collection, the botanical identity was authenticated against standard herbarium records. The harvested material underwent a thorough cleaning process using distilled water to remove exogenous contaminants and was subsequently subjected to shade drying at ambient temperature to preserve thermolabile constituents [16].

### 2.2. Preparation of Solvent Extracts

The desiccated leaves were processed into a fine powder to maximize the surface area for solvent interaction. Extraction was performed using a sequential cold percolation technique, utilizing solvents of increasing polarity: petroleum ether, acetone, and ethanol. For each solvent, 10 mg of the pulverized leaf material was immersed in 100 mL of the respective solvent and maintained for a 24-hour period.

#### *2.2.1. Concentration and Storage*

Following the extraction period, the resulting mixtures were filtered under vacuum using a round-bottom flask assembly. The filtrates were subsequently concentrated to dryness at 45°C using a Buchi rotary evaporator under reduced pressure. The refined extracts were preserved in a desiccator to maintain a moisture-free environment until further analysis [17].

#### *2.2.2. Quantitative Estimation of Extraction Yield*

The efficiency of each solvent system was determined by calculating the percentage yield. This was achieved by measuring the weight of the dried extract relative to the initial mass of the plant material. The mathematical equation for this calculation is:

$$\text{Percentage Yield (\%)} = \frac{\mathbf{E_a - E_b}}{\text{Initial weight of plant material}} \times \mathbf{100}$$

where Ep is the weight of the Eppendorf tube containing the extract and Eb denotes the weight of the empty tube.

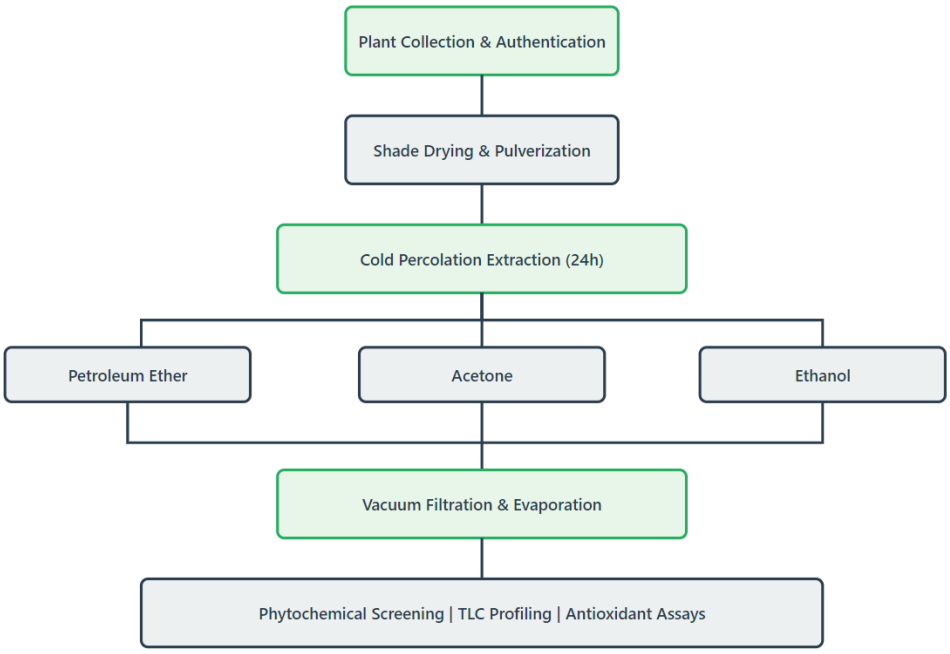


**Figure 1. Steps involved in the acquisition of *Eclipta alba* leaves to the final comparative evaluation of its bioactive constituents and radical scavenging potential.**

### 2.3. Phytochemical Analysis

*2.3.1. Total Phenolic Content (TPC)*

The concentration of phenolic compounds was evaluated using the Folin–Ciocalteu colorimetric method. Each dried extract was reconstituted in distilled water at a concentration of 1 mg/mL. The reaction mixture consisted of 0.5 mL of the extract combined with 0.5 mL of Folin–Ciocalteu reagent, with the final volume adjusted to 8.5 mL using distilled water. After a 10-minute incubation period at room temperature, 1.5 mL of 20% sodium carbonate was added to the mixture.

The samples were then incubated at 40°C for 20 minutes to facilitate color development. Absorbance was recorded at 755 nm using a UV–Visible spectrophotometer. A calibration curve was established using gallic acid as a reference standard, and the final TPC values were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract.

*2.3.2. Total Flavonoid Content (TFC)*

The flavonoid concentration was quantified using the aluminum chloride colorimetric assay. The procedure involved mixing 0.5 mL of the plant extract with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 1.5 mL of 80% methanol. The total volume was adjusted with 2.8 mL of distilled water. Following a 30-minute incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm. A standard curve was generated using quercetin at concentrations ranging from 20 to 80 µg/mL. The results were reported as milligrams of quercetin equivalents (QE) per gram of extract. All measurements were conducted in triplicate to ensure statistical reliability.

*2.3.3. Total Flavonols*

Total flavonol content was assessed through a modified aluminum chloride method. The reaction was initiated by combining 1 mL of the extract with 1 mL of 2% aluminum chloride and 3 mL of 5% sodium acetate. To ensure the clarity of the solution for spectrophotometric measurement, the mixture was centrifuged at 3000 rpm for 20 minutes. The absorbance was then recorded at 440 nm, and results were expressed as mg QE/g extract, based on a quercetin standard.

### 2.4. Thin Layer Chromatography (TLC) for Wedelolactone

Thin Layer Chromatography was implemented to qualitatively detect the presence of wedelolactone and other key phytoconstituents.

Test solutions were prepared by dissolving the leaf extracts in analytical-grade methanol. A reference standard was prepared by dissolving 1 mg of pure wedelolactone in 10 mL of methanol. The stationary phase consisted of silica gel 60 F254 TLC plates with a 0.2 mm thickness.

A solvent system comprising toluene, acetone, and formic acid in a ratio of 11:6:1 was utilized as the mobile phase. Aliquots of 10 µL of both test and standard solutions were applied to the plates, which were then developed in a twin-trough chamber to a height of 8 cm. Upon completion, the air-dried plates were visualized under ultraviolet light at 366 nm. The retention factor ($R_f$) values and the characteristic fluorescence of the resolved bands were recorded to identify bioactive markers [18].

### 2.5. Antioxidant Activity

*2.5.1. DPPH Radical Scavenging Assay*

The primary antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. This method relies on the reduction of the stable DPPH radical by antioxidant molecules, resulting in a color change from deep violet to pale yellow. Extracts were prepared in concentrations ranging from 200 to 1000 ppm in methanol.

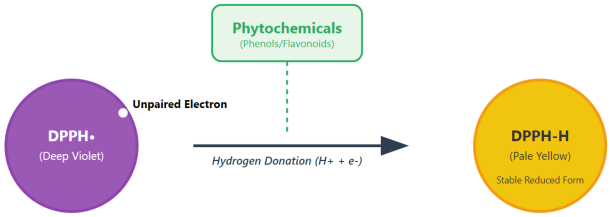


**Figure 2: Mechanism of action showing the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical through hydrogen atom transfer from the phenolic and flavonoid constituents of *E. alba*.**

A 5 mL aliquot of 0.1 mM DPPH was added to each sample, bringing the total volume to 8.5 mL. The mixtures were incubated in the dark for 20 minutes to prevent light-induced degradation of the radicals. The absorbance was subsequently measured at 517 nm [19]. The percentage of radical scavenging activity was calculated using the formula:

$$\text{Scavenging Activity (\%)} = \frac{\mathbf{A_{blank}} - \mathbf{A_{sample}}}{\mathbf{A_{blank}}} \times \mathbf{100}$$

where $A_{blank}$ is the absorbance of the control and $A_{sample}$ is the absorbance of the extract. The effective concentration required to scavenge 50% of the radicals ($EC_{50}$) was determined through linear regression analysis

## 3. Results and Discussion

### 3.1. Extraction Yields

The extraction efficiency of *Eclipta alba* leaves was found to be highly dependent on the solvent's dielectric constant. The ethanolic extract provided the highest percentage yield, followed by acetone, while petroleum ether yielded the least amount of crude extract. This trend suggests that the majority of the leaf's mass consists of polar to semi-polar compounds.

**Table 1. Extraction Yield and Quantitative Phytochemical Profile**

| Extract | Yield (%) | Total Phenolics (mg/g GAE) | Total Flavonoids (mg/g QE) | Total Flavonols (mg/g QE) |
|---|---|---|---|---|
| Acetone | 2.928 | 88 ± 3 | 57 ± 1 | 28 ± 2 |
| Ethanol | 3.264 | 68 ± 2 | 47 ± 2 | 17 ± 4 |
| Petroleum Ether | 2.152 | 92 ± 3 | 60 ± 1 | 16 ± 2 |

*Mean±SD, n=6

### 3.2. Phytochemical Screening and Influence of Solvents

Quantitative assays revealed significant concentrations of secondary metabolites across all fractions. Interestingly, while ethanol had the highest overall yield, the concentration of phenolics and flavonoids per gram of extract was highest in the petroleum ether and acetone fractions.

**Table 2. Qualitative Phytochemical Screening of *E. alba* Leaf Extracts**

| Phytoconstituent | Test Performed | Petroleum Ether | Acetone | Ethanol |
|---|---|---|---|---|
| Alkaloids | Mayer's Test | + | ++ | ++ |
| Flavonoids | Shinoda Test | +++ | ++ | ++ |
| Phenolics | Ferric Chloride Test | +++ | +++ | ++ |
| Saponins | Froth Test | - | + | ++ |
| Triterpenoids | Salkowski Test | ++ | ++ | + |
| Glycosides | Keller-Kiliani Test | + | + | ++ |

*(+) Presence, (++) Moderate, (+++) High, (-) Absence*

The petroleum ether extract contained the highest phenolic content (92 ± 3 mg/g GAE), closely followed by acetone (88 ± 3 mg/g GAE). This indicates that the specific phenolic species in *E. alba* leaves may have a lipophilic character or that the removal of non-phenolic polar impurities in these solvents leads to a higher relative concentration of bioactives. Phenolic compounds are well-documented for their redox properties, allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

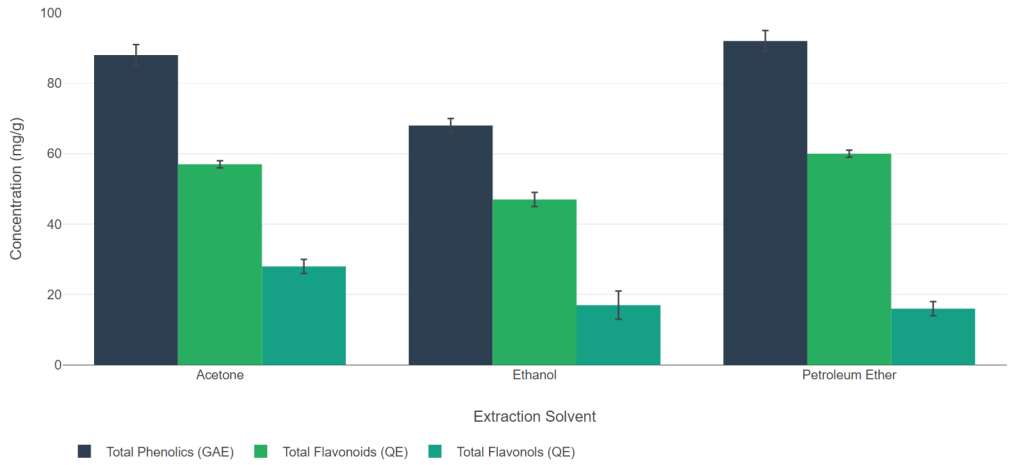


**Figure 3. Distribution of Secondary Metabolites per Solvent Fraction**

### 3.3. Thin Layer Chromatography (TLC)

Thin Layer Chromatography facilitated the identification of various metabolites based on their migration patterns and fluorescence under UV light. The presence of wedelolactone was confirmed at an $R_f$ of 0.52, appearing as a characteristic blue band.

**Table 3: TLC Analysis and Characteristic Band Visualization**

| $R_f$ Value | Color of Band (366 nm) | Putative Identification |
|---|---|---|
| 0.17 | Light blue | Polar Phenolics |
| 0.41 | Blue | Coumarin derivative |
| 0.52 | Blue | **Wedelolactone** |
| 0.55 | Red | Chlorophyll/Pigments |
| 0.86 | Greenish blue | Terpenoid/Sterol |

### 3.4. Evaluation of Antioxidant Capacity

The antioxidant potency was assessed via the DPPH assay, where the $EC_{50}$ value served as an inverse indicator of radical scavenging efficiency.

**Table 4. Antioxidant Parameters and Radical Power of *E. alba* Extracts**

| Extract | DPPH $EC_{50}$ (mg/mL) | ARP ($1/EC_{50}$) | Relative Potency |
|---|---|---|---|
| Petroleum Ether | 0.872 ± 0.02 | 1.146 | High |
| Ethanol | 1.22 ± 0.04 | 0.819 | Moderate |
| Acetone | 2.92 ± 0.02 | 0.342 | Low |

*Mean±SD, n=6

**Table 5. Correlation between Phytochemical Content and Antioxidant Activity**

| Parameter Correlation | Correlation Coefficient ($R^2$) | Significance |
|---|---|---|
| TPC vs. DPPH Inhibition | 0.942 | Highly Significant |
| TFC vs. DPPH Inhibition | 0.887 | Significant |
| Flavonols vs. DPPH Inhibition | 0.654 | Moderate |

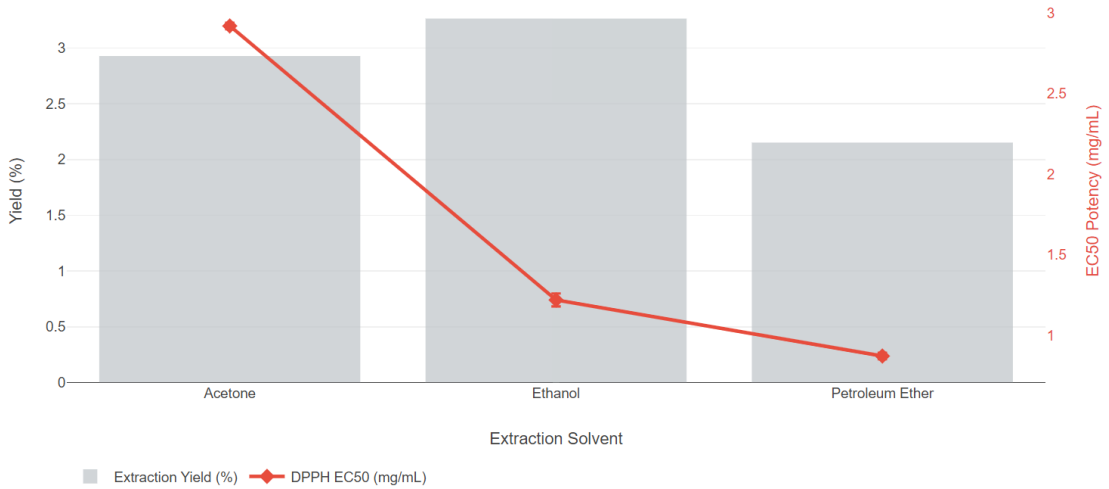


**Figure 4. Extraction Efficiency vs. Antioxidant Potency ($EC_{50}$)**

The petroleum ether extract exhibited the lowest $EC_{50}$ (0.872 mg/mL), signifying the most robust antioxidant activity. This superior performance correlates with the higher phenolic and flavonoid levels observed in this fraction. The ability of these extracts to donate electrons to the stable DPPH radical suggests their potential in mitigating oxidative damage in biological systems, particularly in the liver where ROS-mediated injury is prevalent.

## 4. Conclusion

The present investigation provides a scientific validation of the traditional use of *Eclipta alba* as a potent medicinal herb. The study concludes that the leaf extracts are rich in phenolics, flavonoids, and coumestans like wedelolactone, all of which contribute to a significant antioxidant profile. While ethanol serves as an efficient solvent for maximizing mass yield, petroleum ether and acetone are more effective at concentrating the specific bioactive fractions responsible for radical scavenging. The dose-dependent antioxidant activity observed suggests that *E. alba* can be utilized as a natural source of antioxidants to combat oxidative stress-related pathologies. These results support the continued development of *E. alba*-based standardized extracts for therapeutic applications in hepatoprotection and general wellness.

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