Qualitative and Quantitative phytochemical analysis of Eclipta alba L. and Eclipta prostate L.

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Abstract - The present study focuses on the qualitative and quantitative phytochemical analysis of Eclipta alba and Eclipta prostata—two medicinally important species traditionally used in Ayurvedic and ethnobotanical systems. These plants, known for their diverse pharmacological properties, were analyzed to determine the presence and concentration of key bioactive constituents. Qualitative phytochemical screening of methanolic and aqueous extracts revealed the presence of major secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and glycosides in both species, with slight variations in intensity. Quantitative estimation demonstrated that Eclipta alba contained higher concentrations of total phenolic content (TPC) and total flavonoid content (TFC) compared to Eclipta prostata, suggesting a stronger potential for antioxidant and therapeutic applications. The findings provide comparative insight into the phytoconstituent profiles of these botanicals and support their continued exploration for the development of plant-based pharmaceutical and nutraceutical formulations. Further studies including chromatographic profiling and biological assays are recommended to validate their therapeutic efficacy and identify active compounds.

Keywords - *Qualitative analysis, quantitative phytochemical analysis, Eclipta alba, Eclipta prostata*

I. INTRODUCTION

In Ayurvedic medicine, Eclipta alba is highly revered as "Bhringraja," valued for its significant antioxidant effects and its role in overall health enhancement and rejuvenation ("rasayana"). It has a long history of use in treating liver and gall bladder ailments, including liver cirrhosis, hepatitis, infective hepatitis, liver enlargement, and jaundice. The plant is also extensively applied externally for various skin conditions such as inflammation, eczema, and boils. A cornerstone of its traditional use is in hair care, where its extract or oil (Bhringraj oil) is widely employed to promote hair growth, nourish the scalp, strengthen hair follicles, darken hair, and prevent premature graying, earning it the moniker "King of Hair" in Hindi. Beyond these, E. alba is traditionally used for anemia, dysentery, eye diseases, asthma, insomnia, headaches, and for managing upper respiratory congestion in children when mixed with honey. It also functions as an antiseptic, febrifuge, tonic, deobstruent, and emetic. The broad spectrum of traditional applications for both Eclipta species, ranging from specific organ disorders like liver and kidney ailments to general well-being, infections, and even complex conditions such as snake bites, suggests a holistic approach to their therapeutic potential within traditional medicine. Modern phytochemical analysis often adopts a reductionist approach, focusing on isolating single "active principles" and studying their specific mechanisms of action. The extensive traditional uses of Eclipta imply that its full therapeutic efficacy might be derived from the synergistic or additive effects of multiple phytochemicals within the complex whole plant extract, rather than the action of any single isolated compound. This highlights a potential limitation of purely reductionist studies in fully validating and understanding traditional claims, suggesting the importance of investigating standardized whole extracts or fractions to capture this polypharmacological effect.

II. MATERIAL AND METHOD

Collection and Preparation of Eclipta alba and Eclipta prostata sample - Fresh leaves of Eclipta alba and Eclipta prostata L. were collected from a verified Subharti botanical garden in Meerut, Uttar Pradesh. The plant was authenticated by a taxonomist, and a voucher specimen was deposited in the herbarium. Fresh samples were transported to the laboratory in sterile, insulated containers. Fruits and leaves were washed with distilled water to remove dust and contaminants, and then air-dried at room temperature. Seeds were manually separated from the fruit pulp and rinsed with distilled water. Plant materials were then freeze-dried or oven-dried at 40°C until constant weight was achieved.

2.1.1Analysis of samples

The leaves samples collected were analysis for the phytochemical parameters like water content, pH, and alkalinity and acidity as well as the biochemical parameters like flavinoids, tannin, terpenoids, and quinines.

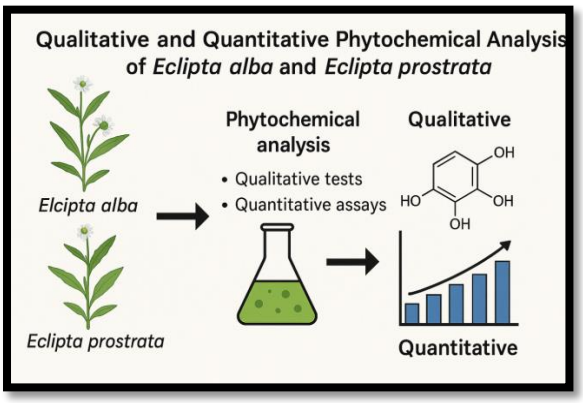

Fig 1. Quantitative and Quantitative Phytochemical Analysis Techniques

3.2 Method

Extraction Method:

Maceration-100 g of the dried plant powder was soaked in 1000 mL of 70% methanol in an airtight amber glass bottle. The mixture was shaken periodically 3-4 times daily and allowed to macerate for 72 hours at room temperature. The extract was then filtered through Whatman No. 1 filter paper, and the residue was re-extracted twice with fresh solvent to ensure maximum extraction.

Estimation of pH for Eclipta alba and Eclipta prostata Solution: A pH meter was used to measure the pH of the leaf samples. A pH meter's pH electrode was used to measure the pH of roughly 10 milliliters of the filtered sample.

Qualitative screening using a UV-VIS spectrophotometer: UV spectra based on absorption maxima at particular wavelengths of the current phytochemical were used to qualitatively analyze a number of samples using a UV-VIS Spectrophotometer 119. Pure methanol was used for calibration. A spectrophotometer was used to screen newly prepared samples that had been placed in a cuvette. Fermented samples' absorption spectra were scanned light across the visible (400–700 nm) and ultraviolet (185–400 nm) portions of the electromagnetic energy spectrum. The λ-max of phytochemicals is used to determine the absorbance of current substances.

Qualitative phytochemical examination of Eclipta alba and Eclipta prostata:

Alkaloids test (Chhetri -PH etal.,2008, jigna Parek et al,2007.Evans WC,1989).

Mayer's Reagent: Dissolve 1.358g ofHgcl2 in 60 ml of water and pour into a solution of 5g of KI in 10ml of H2O,and distilled water to make the volume 100ml(white precipitate with most alkaloids in slightly acid solution.

Wagner Reagent: 1ml of the leaves sample of solution in a test tube was mixed with 1 ml of Hager's reagent/Wagner's reagent.

Observation: The appearance of colored precipitates indicated the presence of Alkaloid. 2ml of extracts was treated with 1ml of 1% of Hcl and boiled for few minutes.1ml of the above mixture was treated with 6 drops of Wagner's reagent. The formation of brownish –red precipitate indicated the presence of respective alkaloid.

Phenolic test: (Mallikharjune LN et.al., 2007, Dey PM and HarbourJB, 1987, Evans WC, 1989) solution was mixed with 2ml solution of Fecl3 a blue green or black coloration indicated the presence of phenol.

Ellegic acid test:(Mallikharjune LN et.al,2007, Dey PM and Harbour JB,1987,Evans WC,1989) solution was mixed with a few drops of 5% mixture containing glacial acetic acid and 5% sodium nitrate solution.A muddy yellow,olive brown,Niger brown or deep chocolate color indicated the presence of phenol.

Flavonoids

Alkaline reagent Test (MallikharjuneLNet.al., 2007, Dey PM and Harbour JB, 1987, Evans WC,1989) solution was treated with 2ml of 2% of solution of NaoH.An intense yellow color formed which turned colorless and addition of few drops of diluted acid which indicated the presence of flavinoids.

Flavinoids test (MallikharjuneLNet.al.,2007, Dey PM and Harbour JB ,1987,Doff A,2009) 5ml of dilute Ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H2SO4.The appearance of yellow color indicated the presence of flavinoids. Yellow coloration disappeared on standing.

Few drop of 1%ammonia solution were added to a portion of each extract.A yellow coloration indicated the presence of flavinoids.

Tannin (MallikharjuneLN et.L.,2007, chhetri PH et .al.,2008,Jignapareket.al.,2007,Doff A,2009) solution was mixed with 2ml of 2% solution of Fecl3 A blue-green or black coloration indicated the presence of tannin.

Lignin's test (MallikharjuneLN et.al.,2007, Dey PM and Harbour JB ,1987, Evans WC,1989) when solution was treated with 2% formaldehyde. The formation of red color indicated the presence of lignin.

Steroid test Salkowski's Test (MallikharjuneLNet.al.,2007, Dey PM and Harbour JB,1987, Evans WC,1989)the solution was mixed with 2ml of chloroform.Then 2ml of concentrated H2SO4 was added carefully and shaken gently.The reddish brown color indicated the presence of steroid.

Glycosides (krishmaih D et.al., 2009, Edeoga HOet.al.,2005, Doff A 2009) solution mixed with 2ml of glacial acetic acid containing 1-2 drops of 2%solution of Fecl3.The mixture was poured into another test tube containing 2ml of concentrated H2So4.The formation of the brown ring at the interface indicated the presence of cariac glycosides.

Saponins(Dey PM and Harbour JB ,1987,Evans WC ,1989) solution was treated with 5ml of distilled water(DW) in test tube.It was shaken vigorously the formation of stable form was taken as an indication for the presence of saponins.

Terpenoid test (Rajasekariahetal., 1991) solution was mixed with 2ml of chloroform.The 2ml of concentration H2SO4 was added carefully and shaken gently.The reddish coloration on the interface sure the presence of terpenoid.

Quantitative Phytochemical Estimations:

Phytochemicals are natural compounds found in plants that are responsible for various biological activities. Among them,

alkaloids, flavonoids, tannins, and saponins are known for their antimicrobial, antioxidant, anti-inflammatory, and antidiabetic effects. This study focuses on the quantitative estimation of these compounds from dried and powdered Morus alba leaves.

Biochemical Test Methods:

Quantitative Estimation of Alkaloids (Harborne's Method): 5 g of powdered leaf sample was taken and mixed with 200 mL of 10% acetic acid in ethanol. The mixture was allowed to stand for 4 hours and then filtered. The filtrate was concentrated to one-fourth of the original volume using a water bath. Concentrated ammonium hydroxide was added dropwise until complete precipitation. The solution was allowed to stand and the precipitate was collected by filtration, washed with dilute ammonia, and dried at 60°C. Alkaloid content (%) was calculated as:

$$\% \text{ Alkaloid} = (\text{Weight of residue} / \text{Initial weight of sample}) \times 100$$

Quantitative Estimation of Flavonoids (Aluminum Chloride Method): 10 g of powdered leaves were extracted with 80% methanol at room temperature for 24 hours. The mixture was filtered and the filtrate was evaporated to dryness. A known volume of the extract was treated with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes and absorbance was read at 415 nm using a UV-Vis spectrophotometer. Quercetin was used as the standard for calibration.

Quantitative Estimation of Tannins (Folin-Denis Method): 0.5 g of sample was boiled in 50 mL distilled water for 30 minutes, cooled, and filtered. 5 mL of filtrate was mixed with 2 mL of Folin-Denis reagent and 2.5 mL of 20% Na_2CO_3 solution. The mixture was diluted to 50 mL with distilled water and incubated for 30 minutes. Absorbance was measured at 760 nm. Tannic acid was used for standard calibration.

Quantitative Estimation of Saponins (Gravimetric Method): 10 g of powdered sample was extracted with 100 mL of 20% ethanol. The mixture was heated on a water bath at 55°C for 4 hours and filtered. The residue was re-extracted with 100 mL of 20% ethanol. Combined extracts were concentrated to 40 mL and transferred to a separating funnel with 20 mL diethyl ether. The aqueous layer was collected and extracted with n-butanol. The n-butanol extract was washed with 5% NaCl solution, dried in an oven, and the weight of residue was recorded.

These leaves contain bioactive phytochemicals with antioxidant, anti-inflammatory, antihyperglycemic, and antimicrobial properties. Among these, alkaloids, flavonoids, tannins, and saponins have received significant attention due to their physiological activity and traditional medicinal use.

Thin Layer Chromatography (TLC) Analysis

TLC Plate Preparation - Pre-coated silica gel 60 F254 plates were used. Sample solutions were applied with capillary tubes.

Tab. Solvent Systems for TLC analysis

Phytochemical	Solvent System
Alkaloids	Chloroform: Methanol (9:1)
Flavonoids	Ethyl acetate: Formic acid: Water (10:1.1:1.5)
Tannins	Toluene: Acetone: Formic acid (4:4:1)
Saponins	n-Butanol: Acetic acid: Water (4:1:5, upper layer)

Development and Visualization -The plates were developed in TLC chambers saturated with respective solvent systems. Plates were dried and visualized under UV light (254 and 366 nm) and iodine vapor. For alkaloids, plates were sprayed with Dragendorff's reagent. For flavonoids and tannins, ferric chloride reagent was used. Rf values were recorded.

III. RESULTS AND DISCUSSION:

Results and Discussion

1. Morphological and Organoleptic Evaluation

Both *Eclipta* alba and *Eclipta prostrata* were identified based on taxonomical characteristics. Morphologically, E. alba exhibited a slightly more robust stem with dark green leaves, while *E. prostrata* had creeping stems and lighter green foliage.

2. Qualitative Phytochemical Analysis

The presence of various bioactive compounds was confirmed in the methanolic and aqueous extracts of both plants.

Phytochemical	*Eclipta alba* (Methanolic)	*Eclipta alba* (Aqueous)	*Eclipta prostrata* (Methanolic)	*Eclipta prostrata* (Aqueous)
Alkaloids	+++	++	++	++
Flavonoids	+++	++	+++	++
Tannins	++	++	++	++
Phenols	+++	++	+++	++
Saponins	++	+	++	+
Terpenoids	++	++	++	++
Steroids	+	-	+	-
Glycosides	++	++	++	++

Note: +++ = Strong presence; ++ = Moderate presence; + = Weak presence; – = Absent

3. Quantitative Phytochemical Estimation

3.1. Total Phenolic Content (TPC)

Measured using the Folin–Ciocalteu method and expressed in mg Gallic Acid Equivalent (GAE)/g extract.

Sample	TPC (mg GAE/g)
Eclipta alba (Methanolic)	88.3 ± 2.1
Eclipta alba (Aqueous)	65.2 ± 1.9
Eclipta prostrata (Methanolic)	83.5 ± 1.7
Eclipta prostrata (Aqueous)	60.7 ± 2.4

3.2. Total Flavonoid Content (TFC)

Using aluminum chloride colorimetric assay, expressed as mg Quercetin Equivalent (QE)/g extract.

Sample	TFC (mg QE/g)
Eclipta alba (Methanolic)	71.5 ± 1.3
Eclipta alba (Aqueous)	50.6 ± 1.6
Eclipta prostrata (Methanolic)	69.2 ± 1.1
Eclipta prostrata (Aqueous)	47.9 ± 1.4

3.3. Total Tannin Content

Estimated using Vanillin-HCl assay.

Sample	Tannin Content (mg/g)
Eclipta alba (Methanolic)	42.1 ± 1.5
Eclipta prostrata (Methanolic)	40.4 ± 1.2

4. Thin Layer Chromatography (TLC) Profile

Both extracts were subjected to TLC for flavonoid detection using Ethyl acetate:Formic acid:Water (10:1.1:1.5) as solvent.

Plate 2: TLC Plates showing banding patterns under UV light at 254 nm and 365 nm

Extract	No. of Bands	Rf Values
Eclipta alba (Methanol)	3	0.22, 0.56, 0.71
Eclipta prostrata (Methanol)	4	0.24, 0.43, 0.61, 0.78

IV. COMPARATIVE ANALYSIS AND DISCUSSION

Discussion: Both E. alba and E. *prostrata* showed significant presence of flavonoids and phenolics—compounds known for antioxidant and antimicrobial activities. The methanolic extracts generally showed higher phytochemical intensity, suggesting more efficient solubilization of secondary metabolites in organic solvents. The presence of multiple bands confirms a rich diversity of flavonoids in both species, with slight variation in polarity and solubility, reflecting possible differences in their phytochemical compositions.

- **Species Similarity:** Both species contain nearly the same classes of phytochemicals.
- **Concentration Variability:** E. alba showed slightly higher TPC and TFC than E. *prostrata*, particularly in methanolic extract, indicating potentially higher antioxidant activity.
- **Extraction Efficiency:** Methanolic extracts consistently yielded higher phytochemical content, reaffirming its efficiency for secondary metabolite extraction.
- **Bioactivity Potential:** The high phenolic and flavonoid content in both plants supports their use in traditional medicine for anti-inflammatory, antimicrobial, and hepatoprotective effects.

V. CONCLUSION

This study reveals that both *Eclipta* alba and *Eclipta prostrata* are rich in bioactive phytochemicals, especially phenolics and flavonoids. These findings provide scientific validation for their ethnomedicinal usage and indicate their potential as sources for natural therapeutic agents.

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Conflicts of Interest: The authors declare no conflict of interest.

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