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A review on physicochemical & pharmacological activity of *Eclipta alba*

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Abstract

Drugs of natural origin play a significant role in the public health care system of any nation. Indian Materia Medica includes about 2000 drugs of natural origin of which approximately 400 are mineral and animal origin while the rest are of vegetable origin Ayurveda, Siddha and Unani systems 600-700 herbs for medicinal use. The traditional knowledge with its holistic and systematic approach supported through experimental base can serve as an innovative and powerful discovery of natural 5 α -reductase inhibitor. *Eclipta alba* (Bhringaraja) having important role in the traditional Ayurvedic and Unani systems of holistic health and herbal medicine of the east. The principal constituents of *Eclipta alba* are coumestan derivatives like wedololactone [1.6%], demethylwedololactone, desmethyl-wedelolactone-7 glucoside and other constituents are ecliptal, β -amyirin, luteolin-7-O-glucoside, hentriacontanol, heptacosanol, stigmaterol. All the parts of *Eclipta alba* and chemical constituents are used as anticancer, antileprotic, analgesic, antioxidant, antimyotoxic, antihemorrhagic, antihepatotoxic, antiviral, antibacterial, spasmogenic, hypotensive, hepatoprotective ovidal, promoter for blackening and growth of hair. Therefore this plant plays a momentous role in medicinal field and it has promising cosmetic as well as therapeutic application & hence its extraction is essential. As per report the alcoholic extract of *E. alba* shows no signs of toxicity in rats and mice and the minimum lethal dose was found to be greater than 2.0g/kg when given orally and intra-peritonally in mice. This article highlights chief constituents, extraction procedure, phytochemistry, Bio activity, pharmacological activities, phytochemical screening & toxicity studies of *Eclipta alba*.

Keywords: Ayurvedic system, *Eclipta alba*, Hepatoprotective, Phytochemistry, Synthetic drugs

1. Introduction

E. alba, also known as Bhringraj, is a small branched perennial herbaceous plant along with a history of traditional medicinal uses in various countries especially in tropical and subtropical regions of the world. It belongs to the family of Asteraceae. Throughout India, it commonly grows as a natural weed, in Himalayas arises to 1800 m, commonly found in regions of upper northern plains, in grazing lands, Chota Nagpur roadsides and in territories of Orissa and Bihar, Punjab, Western India, South India. It is perpendicular or prone, many are branched, perennial, almost hairy, rooting at the buds, opposite leaves, stalk less and simple leaves. The plant has a bitter, hot, sharp, dry taste and is used in Ayurveda [a primary health care system of India], for the treatment of vitiated conditions of *kapha* and *vata*. Indian Materia Medica includes about 2000 drugs of natural origin of which approximately 400 are mineral and animal origin while the rest are of vegetable origin Ayurveda, Siddha and Unani systems 600-700 herbs for medicinal use [1]. The World Health Organization (1980) has also recommended the evaluation of the effectiveness of plants in conditions where there is lack of safe synthetic drugs [2]. Traditionally, it is extensively used against jaundice, in treatment for night blindness, headache and diseases pertaining to hair and its growth. It is also considered as a rejuvenator³. It is commonly found in India, China, Taiwan, Philippines, Japan and Indonesia. Leaves of this plant are 2.5-7.5 cm long. On a long stalk, it has small white daisy like flowers and short, prostrate or circular, brown stem. It has been reported that *E. alba* grows in India, Bengal, Sri Lanka, Myanmar, Malaysia, Japan, China, Korea, Hong Kong and Pakistan (Mahmood *et al.*) [4]. As per Ayurvedic Pharmacopoeia of India this plant is considered as hepatoprotective. The full taxonomic hierarchy is given below

Scientific Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Eclipta L.
Species: Eclipta prostrata

Vernacular Names

English: Bhringaraj
Sanskrit: Bhringaraj
Hindi: Bhangara, Bhangaraiya
Bengali: Kesuriya, Kesari
Tamil: Karisalai
Marathi: Maka
Telugu: Guntagalagara

This plant has several therapeutic uses like Bhringaraj oil is a famous hair tonic for maintaining dark hair and reversing baldness. It is often translated as 'king of the hair'. It is decocted in coconut oil and as this is a 'Cooling' oil it is used externally for 'hot' and inflammatory head problems such as headaches, sinusitis and ear infections. The herb is also beneficial for heat problems (Caldecott 2006) [5]. *Eclipta alba* is also used as medicine alterative, anti-inflammatory, haemostatic, antipyretic, vulnerary, tonic, hepatoprotective. In Ayurvedic medicine the leaf extract is considered as a powerful liver tonic, rejuvenative besides it has also some traditional uses like athlete foot, eczema, dermatitis, on the scalp to address hair loss & the leaves has been used in the treatment of scorpion stings. Ayurvedic action of *Eclipta alba* is given in the table below-

Ayurvedic action of Eclipta alba

Rasayana Rejuvenative
Medhya Promotes the intellect
Kesya Benefits hair
Dantya Benefits the teeth
Tvacya Benefits itching
Kusthahaghna Destroys skin diseases
Raktasodhana Purifies the blood
Raktastambhana Stops bleeding
Visaghna Destroys poisons in the body
Caksusya Benefits the eyes
Krmighna Destroys worms
Pandughna Reduces anaemia

2. Ethnopharmacological Relevance

Eclipta alba (L.) has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases [6].

The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus [7]. The whole plant of *Eclipta alba* works as a best medicine for hair growth. The presence of β -sitosterol in *Eclipta alba* help to rebuild hair in androgenic alopecia (Roy *et al.*, 2008) [8]. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly used to enhance memory and learning [9]. The plant has a reputation as an anti-ageing agent in Ayurveda [10]. It is used as a general tonic for debility. Externally it is used for inflammation, 10, 11, minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding [11]. The water extract of *Eclipta prostrata* (whole plant) exhibited the most potent inhibitory activity against HIV-1 integrase (HIV-1 IN) [12]. Considering the ethno-medicinal significance of the plant, it is of interest to review the ethno-pharmacological reports on the plant & selective phytoconstituents through data base searches.

3. Extraction Procedure of Eclipta alba

Eclipta alba whole plant was cut into small pieces by knife. 250 g of dried small pieces *Eclipta alba* (whole plant) was taken in two separate 2000 ml conical flask and added 1000 ml of methanol and 1000 ml of petroleum ether. It was kept for 72 hrs in air tight condition at 25 to 30 °C temperature. After that, it was filtrated by normal filter paper. Filtrate was kept in a 1000 ml beaker. After filtration; the filtrate was concentrated by rotary evaporator at 40 to 45°C temperature and other ambient condition. The percentage yield of extraction was 1.16% w/w. The extract was stored in glass vials in air tight condition at room temperature with proper label.

4. Phytochemistry

The plant *Eclipta alba* contains the triterpenoid saponins eclalbasaponin I, eclalbasaponin II, eclalbasaponins III–VI, XI and XII, eclipta saponin C and D, eclalbatin, the flavonoids apigenin and luteolin-7-glucoside, as well as the coumestans, wedelolactone (0.5-0.55%), demethylwedelolactone [13], isodemethylwedelolactone and strychnolactone. Alkaloids include 25- β -hydroxyverazine and ecliptalbine, as well as small amounts of nicotine (0.078%) in the aerial portions. Other constituents are α -formylterthienyl, α -terthienyl, polyacetylenicthiophenes, dithienylacetyl esters I, II, and III, β -sitosterol, stigmasterol, daucosterol, stigmasterol-3-O-glucoside, nonacosanol, stearic acid, lacceroic acid, 3, 4-dihydroxy benzoic acid, α -amyrin, ursolic acid and oleanolic acid (Caldecott 2006) [14]. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant [15]. According to Zhang and Chen, several saponins in the plant i.e. eclalbosaponins have been characterized [16].

Table 1: Parts containing chemical constituents of *Eclipta alba*

Sl. No	Parts	Chemical Constituents
1	Leaves	Wedelolactone [1.6%], Desmethylwedelolactone, Desmethylwedelolactone 7-glucoside, stigmasterol
2	Roots	Hentriacontanol, Heptacosanol & Stigmasterol, Ecliptal, Eclalbatin.
3	Aerial parts	β -amyrin & Luteolin-7-O-glucoside, Apigenin, Cinnaroside, Sulphur compounds, Eclalbasaponins I-VI
4	Stems	Wedelolactone [17-19], wedelic acid, L-terthienyl methanol, luteolin [18].
5	Seeds	Sterols [17-19], Ecliptalbine (alkaloid)
6	Whole plant	Resin, Ecliptine, Reducing sugar, Nicotine, Stigmasterol, Triterpene saponin, Eclalbatin, Ursolic acid, Oleanolic acid

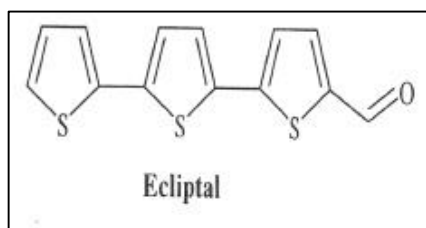


Fig 1: Ecliptal

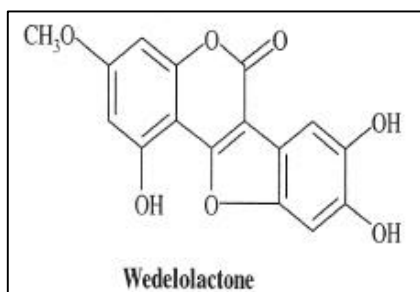


Fig 2: Wedelolactone

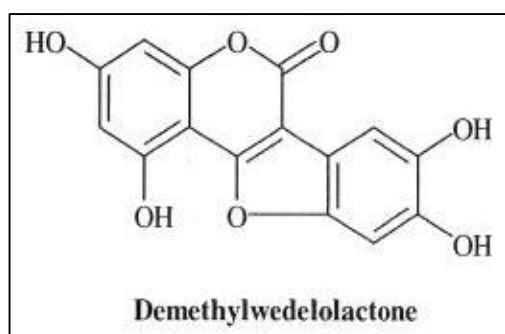


Fig 3: Demethylwedelolactone

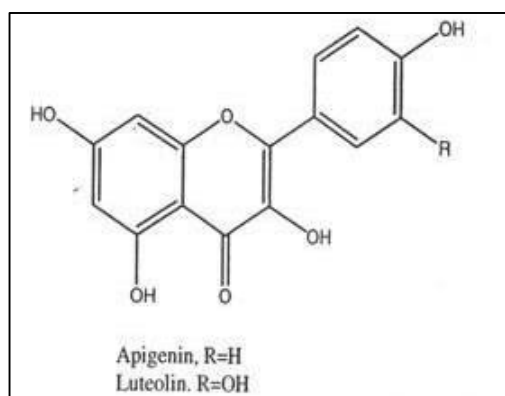


Fig 4: Apigenin, Luteolin

5. Bioactivity

Eclipta alba is a plant used in folk & traditional medicine for cirrhosis and infectious diseases. It is believed to prevent aging and rejuvenate hair, teeth, bone, memory, sight and hearing. The plant was known to possess significant antifungal and insecticidal properties. The biological properties of the plant are treated under two subheadings: (1) pharmacological properties (2) insecticidal properties and other biological properties.

5.1. Pharmacological Properties

5.1.1. Hepatoprotective Activity

There have been an extensive studies carried out to substantiate the hepatoprotective activity of *Eclipta alba*.

Alcoholic extract of the plant is known to show protective effect on experimental liver damage in rats and mice [20]. The plant has been reported to exhibit hepatoprotective action on subcellular levels of functional markers [21], in inflammation and liver injury [22]. The ethanol / H₂O [1:1] extract of *Eclipta alba* significantly counteracted CCl₄ induced inhibition of the hepatic microsomal drug metabolizing enzyme amidopyrine-N-demethylase and membrane bound glucose 6- phosphatase. The loss of hepatic lysosomal acid phosphatase and alkaline phosphate was significantly restored by the extract. The methanolic extract of leaves and the chloroform extract of roots of *Eclipta alba* showed significant activities and respectively causing 72.8% & 47.96% reduction of lysosomal enzyme. The triterpenoid eclabasaponin fraction from methanolic extract of leaves produced significant (78.78%) and the alkaloidal fraction (60.65%) reduction of carbon tetrachloride induced increase in lysosomal enzyme in blood. Coumestan fraction and triterpenoidal saponin fraction from the chloroform extract of roots produced very significant (75.6%) and (52.41%) respectively reduction of carbon tetra chloride induced increase in lysosomal enzyme levels in blood. The plant is reported to exhibit protective effect on carbon tetrachloride induced acute liver damage, by reducing centrilobular necrosis, hydro pic degeneration and fatty change of the hepatic parenchymal cells [23]. The ethyl acetate fraction showed improved and effective protection in doses of 20, 40 and 80 mg/kg in rats [24]. Wagner *et al.* [1986] confirmed that the coumestan constituents of the plant wedelolactone and demethylwedelolactone are responsible for the potent anti-hepatotoxic activities in carbon tetrachloride, galactosamine and phalloidin induced liver damage in rats [25]. Wedelolactone has been reported to be a potent and selective 5-lipoxygenase inhibitor with an IC₅₀ of 2.5 μm and it dosages so by an oxygen radical scavenging mechanism [26].

5.1.2. C. N. S. Activity

Studies indicated that the aqueous extract of *Eclipta alba* and its hydrolyzed fraction at a dose of 300 mg/kg and 300 mg/kg p.o. respectively showed no tropic activity in rats [22-27].

5.1.3. Antimicrobial Activity

Studies revealed the anti- hepatitis B virus properties of *Eclipta alba* [28]. The shoot extract showed antibacterial activity against *Staphylococcus aureus* and *Eclipta Coli* [29].

5.1.4. Anti-inflammatory and Analgesic Activity

The plant has been reported to possess anti-inflammatory and bronchodilator activities, due to the coumarin compounds [30]. Further studies reported confirmed analgesic activity of *Eclipta alba* [31]. Analgesic effect was studied on albino mice using ethanolic and alkaloidal extract of *Eclipta alba*. Standard experimental models such as the tail clip method, the tail flick method and the acetic acid induced writhing response were used which showed both the ethanol extract as well as the total alkaloids produced good analgesic activity in all the different models of analgesia used.

5.1.5. Immunomodulatory Activity

Preliminary studies revealed the immune-modulatory activity of methanolic extract of *Eclipta alba* [32]. Wedelolactone and demethylwedelolactone isolated from *Eclipta alba* exhibited trypsin inhibition in vitro. Both compounds showed potent activity with IC₅₀ values of 2.9 and 3.0 μg/ml, respectively [33].

5.1.6. Hair growth & Alopecia

Eclipta alba is used in hair oil preparations since it promotes hair growth and maintains hair black. 10% w/v of *Eclipta alba* was an chief ingredient in the preparation of herbal formulation for hair growth [34]. Alopecia is a dermatological disorder with psycho social implications on patients with hair loss. *Eclipta alba* is a well-known Ayurvedic herb for hair growth. In the reported work Petroleum ether & ethanolic extracts were incorporated into oleaginous cream (water in oil cream base) and applied topically on shaved denuded skin of albino rats. The time (in days) required for hair growth initiation as well as completion of hair growth cycle was recorded. Minoxidil 2% solution was applied topically and served as positive control for comparison. The result of treatment with 2% and 5% petroleum ether extracts were better than the positive control minoxidil 2% treatment [35]. Roy *et al.* have been reported quantitative analysis of hair growth after treatment with petroleum ether extract [5 %] exhibited greater number of hair follicles in anagenic phase [69 ± 4] which were higher as compared to control [47 ± 13].

5.1.7. Anticancer activity

Methanolic extract of *Eclipta alba* was evaluated for its anticancer activity against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. On day 1, the extract of *Ecliptaalba* at a dose of 250 and 500 mg/kg body weight were administered orally and continued for 9 consecutive days. The anticancer activity was examined by determining the tumour volume, tumour cell count, viable tumour cell count, nonviable tumour cell count, mean survival time and increase in life span in experimental animal models. The extract increased the life span of EAC treated mice and restored the haematological parameters as compared with the EAC bearing mice. Thus, study revealed that the methanolic extract of *Eclipta alba* showed anticancer activity in the tested animal models [36]. Coumestans are also known to act as phytoestrogens. These compounds are present in soya beans and clover. In many countries it is used as diet which acts as chemo- preventive agent in breast and prostate cancer. Dasyscyphin-C (saponins) a newer isolated compound from *Eclipta prostrata* reported to have anticancer-cytotoxic activity [37] under in-vitro conditions in HeLa (Human cervical carcinoma) & vero cell lines at the concentration of 50 µg/ml.

5.2. Insecticidal & Other pharmacological activities

It has been reported that the significance of free carboxylic acid at C-28 position in echinocystic acid derivatives from the methanolic extract *Eclipta prostrata* showed antifibrotic activity [38]. Ethanolic and ethyl acetate fractions of *Eclipta prostrata* were tested for its antibacterial activities against *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* [40]. *Eclipta prostrata* is combined with a non-plant material which is used to bath children suffering from malnutrition for 9 days and used as self-medication by AIDS patients in southern Thailand. Besides *Eclipta alba* is also used with different plants in combinations for treatment of various diseases like; Amalaki, sariva, *triphala* for hair problems from high *pitta*; Manjishtha, kutki, neem, pippali for hepatitis and liver conditions; Jatamansi, brahmi and shankhapushpi for mental disorders from high *vata* and *pitta*; Black pepper for stimulating *rasa* and *rakta dhatu agni* and treating anaemia; Turmeric, neem, licorice for dermatological conditions due to

high *kapha* and *vata*.

6. Phytochemical screening of *Eclipta alba* Extract

Detection of Phytosterols:

Liebermann-Burchard Test: 10 mg of extract was dissolved in 1 ml of chloroform. 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid, a reddish violet colour developed, indicating the presence of steroids.

Salkowski Test: 1 ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish-blue colour exhibited by chloroform layer and green fluorescence by the acid layer suggested the presence of steroids.

Detection of Triterpenoids

Nollars Test: In the test tube 2 mL of 0.01% anhydrous stannous chloride in thionyl chloride solution and test solution was added. Purple colour formed changed to deep red colour after few minutes indicates the presence of triterpenoids.

Detection of Flavonoids:

Shinoda Test: To the extract magnesium turnings and then conc. hydrochloric acid was added. Red colour was produced.

Detection of Alkaloids

Mayer's Test: 1.2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloid.

Dragendroff's test: 0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orange brown coloured precipitate suggested the presence of alkaloid.

Biuret Test: 1 ml of 40% NaOH mixed with 2 drops of 1% copper sulphate was added to the extract, a violet colour indicated the presence of proteins.

Detection of protein and Amino Acid:

Ninhydrin Test: Extract solution was treated with ninhydrin (Tri-keto hydrindene hydrate) at the pH range of 4-8. Development of purple colour indicated the positive response for amino acids.

Detection of De-oxy Sugars:

Keller Kiliani Test: To 1 g of the sample, 10 ml of 70% ethanol were added and boiled for 2-3 min. it was filtered and to the 5 ml of the filtrate, 5 ml of distilled water and 0.5 ml strong lead acetate solution were added. It was filtered and 5 ml of chloroform were added to the filtrate. Excess chloroform was pipetted off and gentle evaporation of chloroform was done on a porcelain dish. It was cooled and to the residue, 3 ml of glacial acetic acid and 2 drops of 5% ferric chloride were added. The solution was transferred to the surface of 2 ml concentrated sulphuric acid. Reddish brown colour (which changed to bluish green to dark on standing) at the junction confirmed the presence of deoxy sugars in the sample.

Detection of Reducing Sugars:

Fehling's Test: 5 ml of the extract solution, mixed with 5 ml

of Fehling's solution was boiled for 5 minutes. Formation of brick red coloured precipitate demonstrated the positive test for reducing sugars.

Detection of Glycosides:

Borntrager's test: Few ml of dil. sulphuric acid added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink red colour was produced inorganic layer.

Keller Killiani Test: Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulphuric acid. At the junction of liquid reddish brown colour was produced which gradually becomes blue.

Detection of Phenolic compounds and Tannins:

Ferric chloride Test: 5 ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

Potassium dichromate Test: 5 ml of the extract was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish-brown precipitate suggested the presence of tannins.

Detection of Saponins

Foam Test: 1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. Development of stable foam suggested the presence of saponins.

Potassium dichromate test: 1 ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

7. Result

Phytochemical screening of the methanolic extract of *Eclipta alba* showed presence of different type of phyto-constituents as depicted below-

Phytochemical screening of the methanolic extract of *Eclipta alba*

Test Methanolic extract of *Eclipta alba*

Phytosterols	+
Triterpenoids	-
Flavonoids	+
Alkaloids	+
Protein and amino acids	+
Carbohydrates	+
Glycosides	+
Phenolic compounds and Tannins	+
Saponins	+

("+" Indicates positive; "-" indicates negative)

8. Toxicity Studies

In studies conducted by the alcoholic extract of *E.alba* shows no signs of toxicity in rats and mice and the minimum lethal dose was found to be greater than 2.0g/kg when given orally and intra-peritonally in mice ^[40].

9. Conclusion

The traditional knowledge with its holistic and systematic approach supported through experimental base can serve as an innovative and powerful discovery of natural 5 α -reductase

inhibitor. Experimental results revealed that the plant extracts showed potential 5 α -reductase inhibition activity. The plant *Eclipta alba* has promising cosmetic as well as therapeutic application. Based on these findings, we can suggest that these plants extract could potentially be a useful for alopecia. This study provides newer insight for treatment and controlling baldness disorder as well as cosmetic application of the plant. Hair growth gel, cream and lotion are prepared by using plant materials with vitamin B. The formulations are evaluated and results indicated the formulations are good in appearance, homogeneity and easily spreadable and showed significant inhibition of 5 α -reductase enzyme in in-vitro model by comparing with some marketed formulation. Further HPTLC and HPLC method was done for quantification of plant biomarkers used in formulation. The results also showed that 5 α -reductase enzyme inhibition effect of the formulation was better than the effect of marketed hair gel formulation.

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