



A Review on Tissue Culture Studies in *Eclipta alba* – An Important Medicinal Plant

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ABSTRACT

Eclipta alba (compositae) is an important medicinal herb found as a weed throughout India. Almost all of its parts are used in traditional system of medicine including seeds, roots and shoots and leaves. The review reveals that a number of phytochemical constituents have been isolated from the plant which possess pharmacological activities like antibacterial, antifungal, diuretic, anti-inflammatory, hepatoprotective, anti-venomous, analgesic, anticancerous and various other important medicinal properties. It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. For the last few decades or so, extensive research work has been done to confirm its biological activities and pharmacology of its extracts. Since the plant has enormous medicinal properties, there is an increasing worldwide attention towards harnessing the plant products. There is growing demand for plant raw material from herbal industry and therefore needs prime attention for its cultivation and conservation. Tissue culture techniques have been developed for in situ conservation of this plant. The article gives an account of updated information on its phytochemical, pharmacological properties and developments in tissue culture studies on *Eclipta alba*.

Keywords: *Eclipta alba*, Bhringraja, wedelolactone, micropropagation, Ayurveda.

INTRODUCTION

Eclipta alba (L.) is an annual herb belonging to the family Asteraceae and is commonly known as false daisy. It is conspicuous in the Indian countryside as weed. The plant is erect or prostrate with prolific branching, hairy stem and rooting at nodes (Fig. 1).



Figure 1: *Eclipta alba* L. (Hassk)

The leaves are opposite, sessile and lanceolate. Indigenous nomenclature for *Eclipta alba* is Bhringraja, (India) and the plant is known to natives of South America, Asia and Africa for its various medicinal and therapeutic usages. Its well known usages are treatment of liver cirrhosis, jaundice, gall bladder problems and hepatitis. Its liquid extract is used for curing memory weakness, stress and anxiety, insomnia, chest infections and short-sightedness. The plant has rich medicinal uses as its stem, leaves, seeds and roots contain vital chemical compounds that have been identified for their medicinal properties. The medicinal properties of *Eclipta alba* are antimytotoxic, analgesic, antibacterial, antihepatotoxic, antihemorrhagic, antihyperglycemic, antioxidant, and

immunomodulator for general goodness of health. There are several compounds, viz., coumestans, alkaloids, thiophenes, flavonoids, polyacetylenes, triterpenes and their glycosides, which are extracted from *Eclipta alba*. These chemical compounds are known for their vital role in aforementioned medical usages. *Eclipta alba* leaves are traditionally used for hair rejuvenation since centuries. Its leaf extract is used as liver tonic, anti-venom and in edema and rheumatic joint pains. The shoot extract is known for vital antimicrobial and antifungal properties. Ayurveda recognizes it as one of the most vital plant for curing Kapha and Vata imbalance. A fully mature plant is bitter, sharp and dry in taste.

There is an increased focus on naturopathy and Ayurveda in recent times, which has lent prime focus upon medicinal plants. Their medicinal importance has gained attraction of firms and commercial exploiters. This has resulted in over-exploitation of these plants or their cultivation to a greater extent that is causing regional agro-ecological imbalances. Therefore, there is a need to propagate such practices that can help meet both increased demand and reduce stress on natural resources, viz., land, water and biotic material. Plant tissue culture technology is the right intervention to optimise the commercial utilisation of *Eclipta alba* that can maintain the biodiversity balance as well. Tissue culture protocols developed for this purpose are deployed for conserving the endangered species, rare therapeutic species and other identified species for specific purpose. The micropropagation/clonal techniques are used for mass scale multiplication from shoot tips and nodal segments. One can achieve large numbers in relatively short period of time and less space that is attractive to harness their medicinal properties commercially. Both, the yield of crop and its extracts,

such as primary and secondary metabolites are economically influenced by market conditions. Hence, a proper understanding of response mechanism of the plant to environmental conditions and genetic control needs be rehearsed for correct estimation and output of the plant and its products. The present review is a pioneering contribution to the comprehensive repository of studies in Tissue culture of *Eclipta alba* (L.) Hassk. along with a brief and updated information on its pharmacological properties.

Pharmacological activities

The plant has high therapeutic and medicinal value due to its chemical composition. The whole plant contains Nicotine¹, Ecliptine & Coumarin², Wedelolactone^{3,4}, Dimethylwedelolactone-7-glucoside^{5,6}, Stigmasterol^{7,8}, Ecliptal⁹, α -Formyl terthienyl¹⁰, Triterpine glycosides and Eclalbasaponins I-VI¹¹ VII-X¹² and XI and XII¹³. Roots contain Thiophenes¹⁴, Heptacosanol¹⁵, Ecliptal^{14,16,9,17}, Stigmasterol¹⁸. Wedelolactone, an active principle of the plant is used for the treatment of hepatitis, cirrhosis. A simple HPTLC method has been developed for quantification of wedelolactone and is considered to be beneficial for herbal formulations.¹⁹ Identification and quantification of Wedelolactone has been carried out using the simple and sensitive reverse phase high performance liquid chromatography-photo diode array (HPLC-PDA) method.²⁰ A few important phyto-chemicals have been obtained from various parts of *Eclipta alba* - Stigmasterol, α -terthienylmethanol, Wedelolactone [1.6%], Desmethylwedelolactone, Desmethyl-wedelolactone-7-glucoside from leaves; Hentriacontanol, Heptacosanol & Stigmasterol, Ecliptal from roots; β -amyryn & Luteolin-7-O-glucoside, Apigenin, Cinnaroside, Sulphur compounds from aerial parts; Wedelolactone from Stems; Sterols from seeds; Large amounts of resin, Ecliptine, Reducing sugar, Nicotine, Stigmasterol, Triterpene saponin, Eclalbatin together with α -amyryn, Ursolic acid, Oleanolic acid from whole plant (Fig. 2).²¹

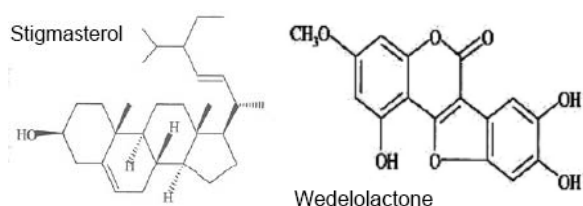


Figure 2: Active compounds

Hepatoprotective activity

Extensive research corroborates the hepatoprotective activity of *Eclipta alba*.²²⁻²⁹ Aqueous leaf extract of *Eclipta alba* has been reported to possess the restorative potential so as to suppress the hepatotoxicity in the male albino rats.³⁰

Hair growth activity

The plant is traditionally used in treatment of premature greying of hair. It is reported to improve hair growth and colour.³¹⁻³⁵

Antimicrobial properties

Various scientists have studied the anti-microbial potential of *Eclipta alba*. Antifungal activity of the plant has been confirmed by various workers.³⁶⁻³⁸ *Eclipta alba* has been found to possess antiviral^{39,40}, antibacterial activity⁴¹⁻⁴⁵. Antimicrobial potentials of methanol, petroleum ether, dichloromethane, ethylacetate, butanol and water extracted samples from the aerial parts of *Eclipta alba* have been evaluated against nine microbial species and confirmed the antimicrobial potential by Disc diffusion method.⁴⁶ Antimicrobial activity has been compared for methanolic extracts of leaves and stems of *Eclipta alba* and natural oils and it was found that natural oils were more effective as compared to the methanolic extracts of *Eclipta alba*.⁴⁷ The phytochemical profile and antibacterial activities of extracts from *Eclipta alba* have been evaluated where it has been confirmed that the latter can be used for therapeutic purposes.⁴⁸ The studies on phytochemical content of the plant, such as, alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, reducing sugars, anthraquinones, and cardiacglycoside, physiochemicals, and its anti-fungal activity has been conducted.⁴⁹ The plant showed very promising results against the fungal strains, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium solani* and *Aspergillus flavus*. A first large-scale field testing of *Eclipta alba* has been reported for seed treatment of sorghum and its direct comparison with a thiram-based pesticide and seed priming has been done.⁵⁰

Anti inflammatory activity

Eclipta alba is known to be effective against chronic inflammation.^{51,52} The bronchodilatory and antidermatitis (inflammation) property of the plant has also been studied.⁵³⁻⁵⁵ The wedelolactone derived from *Eclipta alba* has found to be responsible for anti-inflammatory property.⁵⁶

Anti-venomous activity

Anti-venom potential of *Eclipta alba* has been confirmed by various researchers.⁵⁷⁻⁶⁰

Miscellaneous activity

Preliminary studies revealed the anti-hemorrhagic activity^{61,62}, anti-aggressive activity^{63,64}, anti-oxidant activity⁶⁵⁻⁶⁸, anti-cancer activity⁶⁹⁻⁷¹ and anti-malarial activity⁷²⁻⁷⁵. It has been found that combined ethanolic biherbal extract from *Eclipta alba* and *Piper longum* has more potent antioxidant potential action than its individual preparation of *Eclipta alba* extract or *Piper longum* extract.⁷⁶ The biological properties of different extracts of whole plant of *Eclipta alba* have been evaluated in terms of its antimicrobial, antioxidant, cytotoxic activities and a phytochemical analysis was performed to find out the active compounds responsible for these activities.⁷⁷ It was found that compounds like Naphthoquinone, Hydrazine carboxamide of biological significance causing growth arrest and apoptosis in lung cancer lines. The study has been conducted on larvicidal

and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba* against dengue vector, *Aedes aegypti* and it was concluded that the crude extract of *E. alba* had an excellent potential for controlling *Ae. aegypti* mosquito.⁷⁸

An evaluation for the possible cerebroprotective and antioxidant effects of hydroalcoholic extract of *Eclipta alba* has been done against global cerebral ischemia in the rat.⁷⁹ The results of their study showed that *Eclipta alba* pretreatment ameliorates cerebral ischemia/reperfusion injury and enhances the antioxidant defense mechanism in rats exhibiting cerebroprotective property. Evaluation of hydroalcoholic and aqueous extracts of the plant *Eclipta alba* was carried out for sedative, muscle-relaxant, anxiolytic, nootropic and anti-stress activities. Their results confirm the Neuropharmacological activity of the plant as a Nootropic.⁸⁰ The antistress ability of dietary *Eclipta alba* (L.) ethanol extract has been studied against acid stress in *Labeo calbasu* fingerlings in terms of stress hormones.⁸¹ Their Results indicate that the plant ethanol extract possesses good antistress activity and that dietary supplementation of $\geq 0.1\%$ *E. alba* ethanol extract reduces stress in *L. calbasu* fingerlings exposed to acid stress. The anti-hepatitis C virus activity present in *Eclipta alba* extract was evaluated and it was concluded that the plant extract strongly inhibited RNA dependent RNA-polymerase active of HCV replicase in vitro.⁸² Bio assay based fractionation of the extracts identified three compounds viz., wedelolactone, luteoin and apigenin responsible for the inhibitory activity.

The antianaphylactic activity of alcoholic extract of *Eclipta alba* was explored using rat as the model organism.⁸³ The *Eclipta alba* has been found to possess good antileptic potential due to presence of wedelolactone, luteolin and B-amyrin.⁸⁴ Studies have been done to isolate and characterise the triterpenoids from Methanolic extracts of *Eclipta alba* and the structure of the isolated compound has been investigated as "Lanost-5, 24 dien-3 beta-ol-18, 21 olide-3 beta- yl Tetra decanoate".⁸⁵ The anthelmintic activity of *Eclipta alba* leaf extract has been confirmed using adult earthworm.⁸⁶ A study has also been done to identify the constituents contributing towards its antidiabetic activity and subsequently, eclalbasaponin VI was found to be the most potent.⁸⁷ The anti-hyperlipidemic activity of *Eclipta alba* hassk was studied and identified the plant to be a potential herb for the management of hyperlipidemia and its consequences.⁸⁸

Tissue culture studies

A Perusal of literature revealed that micropropagation of *Eclipta alba* has been done by several researchers. Plant regeneration by organogenesis has been extensively studied in *Eclipta alba* using various explants. *In vitro* micropropagation from nodal segment has been carried out.²⁴ The MS medium⁸⁹ with modification in the vitamin level showed optimal response towards multiplication and growth of shoots with simultaneous rooting. *In Vitro* flowering was also reported in kinetin supplemented MS

basal medium which was reported for the first time.⁹⁰ The micropropagation protocol of elite variety of *Eclipta alba* has been successfully shortened, where multiple shoot formation has been reported from nodal explants on MS medium supplemented with 4.44 micro-molar BAP. Further proliferation of shoots along with rooting was observed on MS with 0.44 μM BAP thereafter followed by successful acclimatisation of plants in field conditions.⁹¹ Establishment of totipotent callus cultures for the plant regeneration has been reported where enhanced production of coumestans such as wedelolactone and Dimethylwedelolactone from regenerated plants was seen as compared to the Natural ones.⁹² An efficient and reproducible method for high frequency axillary shoot proliferation from cotyledonary nodes of *Eclipta alba* has been reported, followed by the establishment of regenerated plants in soil.⁹³ There was no detectable variation seen among the acclimatised plants with regard to morphological growth characteristics and growth features.

Role of basal media, carbon sources and growth regulators was investigated in micropropagation of *Eclipta alba*.⁹³ In their study they revealed that *in vitro* plant production was best achieved on MS medium supplemented with BAP (4.4 μM) +Kinetin (9.2 μM)+ 2iP (2.4 μM) and 3 percent sucrose. A highly efficient and reproducible protocol was reported for mass propagation of *Eclipta alba* through nodal explants on MS media supplemented with BAP 1 mg/l and subsequent rooting was achieved on IBA 1 mg/l.⁹⁴ Further multiplication and maintenance of Shoot cultures was accomplished on MS media containing BAP and GA₃. Our protocol enables easy large scale (at least 400 plants by two successive cultures from a single nodal explant) multiplication of the medicinal plant *Eclipta alba* (Fig. 3).



Figure 3: Tissue cultured shoots

An efficient protocol for rapid micropropagation was developed through axillary bud multiplication.⁹⁵ MS medium supplemented with BAP 10 μM was found to be the most effective for shoot regeneration (about 23 shoots per explant). Rooting was best achieved on half strength MS medium augmented with 0.5 μM IBA followed by 90 percent survival of acclimatised plants. Carbohydrates are of prime importance for *in vitro* organogenesis. Carbon requirements depend upon the stage of culture and show differences according to the species.⁹⁶ Role of various carbon sources have been

studied to see their effect on the Regeneration process. Among the three carbon sources tried, sucrose proved to be best for shoot regeneration.⁹³

An efficient micropropagation method from young nodal axils of shoot tip explants has been developed with emphasis to priming *in vitro* plants in view of increasing their hardening ability after transplantation *ex vitro*. Priming with 6.3 µm of chlorocolinchoride (CCC) was found most effective for acclimatisation by increasing number of root and its biomass, elevating chlorophyll level in leaves. Comparison between primed micropropagated and non-treated plants was made and it was seen that primed plants survived by higher frequency (100 %) in soil as compared to non-treated ones (84 percent survival rate).⁹⁷ Multiple shoot induction and plant regeneration on MS medium supplemented with BAP and NAA has been reported.⁹⁸ An improved and efficient *in vitro* regeneration system has been developed through transverse thin cell layer culture. The tTcl nodal explants were used for shoot proliferation because of its lower risk of genetic alteration.⁹⁹ MS media supplemented with 13.2 µm BAP and 4.6 µm Kinetin was found to be best for shoot multiplication. RAPD profile analysis was carried out using RAPD markers to evaluate the genetic fidelity of micropropagated plants and it was seen that they were genetically similar to mother plant.

Somatic embryogenesis is preferred over organogenesis for regeneration in plants as shoot and root development occurs simultaneously so that regeneration procedure is simple and rapid. High frequency somatic embryogenesis and plant regeneration from highly competent nodal explants derived *Eclipta alba* have been studied.¹⁰⁰ In their study they reported callus formation on ME media supplemented with auxins viz. 2,4-D/NAA, that formed somatic embryos when transferred on MS supplemented with NAA in combination with BAP. Establishment of totipotent callus cultures for regeneration of *Eclipta alba* plants may constitute a useful tool for the production of active constituents wedelolactone and dimethyl wedelolactone. Regeneration from totipotent calli of leaf, stem and root, cultured on Murshige and Skoog medium supplemented with various combinations of growth regulators like (IAA, 2,4-D and Kinetin), has been reported.⁹² Estimation of production of coumestins and wedelolactone and dimethyl wedelolactone was done by UV method. A liquid shoot culture system was standardised for rapid proliferation of multiple shoots followed by their subsequent establishment as a system for the production of wedelolactone. The successful proliferation of the shoots in liquid medium, the enhanced production of wedelolactone in cultures, and the leeching of the wedelolactone in the medium enhance the prospects for establishment of an economic bioreactor for the large scale *in vitro* synthesis of the compound.¹⁰¹ *Eclipta alba* is studied not only in India but also worldwide for pharmaceutical purpose. The synthetic seed technology can be used for exchange of germplasm from elite genotypes and axenic plant material across

research laboratories and targeted pharmaceutical industries as they have small bead size and it is relatively easier to deal with these structures.¹⁰²⁻¹⁰⁵ Synthetic seed production and subsequent plant regeneration in *Eclipta alba* using nodal explants and shoot tips and Nodal explants has been reported.^{105,106}

CONCLUSION

The present review concludes that the plant *Eclipta alba* is a wonder plant with multiple forms of medicine in Ayurveda. The increasing demand in pharmaceutical industry warrants its conservation and mass propagation through Plant tissue culture strategy. Morphogenetic studies conducted so far have revealed that Organogenesis is the preferred method of micropropagation over Somatic Embryogenesis. Among various Explants used, Nodal explants have the best morphogenetic potential in terms of Shoot formation. Cytokinin BAP is the most effective Plant Growth Regulator for regeneration. Somatic Embryogenesis has been studied in a very limited way and still has to be worked on in a detailed manner. Clinical and Biochemical analysis need to be further investigated due to the presence of broad range of value added phytochemicals responsible for its medicinal properties.

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