Antioxidant and Antitumor Activities of Leaf Extract of Eclipta Alba

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Abstract – Medicinal plants are nature’s hidden and unexplored treasures (nature’s pharmacy) for humanity since times immemorial. The plant Eclipta Alba has many medicinal value used in the traditional Ayurvedic and Unani System. Eclipta Alba (L.) commonly known as bhringraj as well as false daisy, a species of plant in the family Asteraceae. This herb contains many bioactive components such as coumestans i.e. wedelolactone and dimethyl wedelolactone, flavonoids, steroids, etc. We have examined it antioxidant activity through DPPH & reducing power assay and we got in it total phenolic content, flavonoids and sterol as good antioxidant agents. The antioxidant activity was assessed through DPPH and reducing power assay, which was explained in terms of effective concentration EC50/IC50.

Keyword: Eclipta Alba, Flavonoids, Antitumor, Antioxidants and Wedelolactone.

Introduction
The use of synthetic drugs causes many side effects as well as resistance in pathogenic microbes. Thus, there is a need to focus on developing herbal drugs. Among them, a very well-known plant is E. Alba (L.) Hassk. It belongs to the family Asteraceae and is native of India and its neighbouring countries. Past ethnomedicinal literature revealed that plant and plant parts are highly of medicinal value but still these plants only used in limited areas like respiratory tract disorders (including asthma), gastrointestinal disorders, fever, hair loss. Many types of research were done and found E. Alba was effective against many diseases, but still, there is little dark area which remains to be enlightened to the world1. According to Saint Vallalaar, Eclipta is number one herb in hierarchy and has been reported to possess antimicrobial, anti-cancer and antioxidant properties and promote hair follicle growth (Soni and Soni, 2017). Active ingredients of Eclipta Alba are as follows coumestans i.e wedeloactone, alkaloids i.e, Ecliptalbine, flavonoids, glycosides, polycytylenes and triterpenoids. The leaves contain stigma-sterol, α-terthienyl methanol, wedelolactone, dimethylwedelolactone and dimethyl wedelolactone-7-glucoside2.
Emerging research evidence has suggested that the medicinal plants containing a wide variety of natural antioxidants, such as phenolic acids, sterols, flavonoids are of great value in preventing the onset and/or progression of many human diseases (Halliwell et al. 1992).

Material and Methods

Ethics Statement: All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee (IAEC) was obtained as per the prescribed guidelines.

Plant Material: *Eclipta alba* was collected and the sample was verified by Dr. K. R. Arya, Principal Scientist, Botany Division, CSIR-Central Drug Research Institute Lucknow (UP), India.

Preparation of *Eclipta Alba* extract

*Eclipta Alba* was dried in an oven at 40 °C for 5 days and then blended in an electric blender. The powder was kept within 80% alcohol and left at room temperature for overnight soaking. The crude content was filtered through 125 mm Whatman qualitative filter paper under sterile condition. This procedure was repeated 5 times and then solvent (alcoholic extract of *Eclipta Alba*), thus collected, were evaporated to dryness under reduced pressure using a rotary evaporator below 50 °C. The residue was further subjected to dryness by incubating them for 8 days at 37°C. The extract was stored at 4 °C until use. The yield of the extract was 12.5% (w/w).

Phytochemistry: *Eclipta Alba* (L.) contains wide range of active principles which include coumestans, alkaloids, flavonoids, glycosides, and triterpenoids. The leaves contain stigmasterol, β-terthienyl- methanol, wedelolactone, dimethylwede-lolactone and dimethyl wedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene substituted thiophene. The aerial part contains phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β-glucose side of phytosterol, a glucoside of a triterpenic acid and wedelolactone. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid occur in this plant.

Sterols and Flavonoids: Sterols seen in *E. Alba* are phytosterol, β-glucoside of phytosterol, daucosterol and stigmasterol-3-oglucoside in the entire plant body. Flavonoids like apigenin, luteolin and luteolin-7-glucoside.

Total Phenolic Content: Total phenolic contents in the extracts were determined spectrophotometrically by the Folin Ciocalteau method. Dried extracts were reconstituted in distilled water (1 mg/ml). Folin-Ciocalteau reagent (0.5 ml) was added to the extract solution (0.5 ml), and the total volume was adjusted to 8.5 ml with distilled water.

The tubes were kept at room temperature for 10 min, and thereafter 1.5 ml of sodium carbonate (20%) was added. The tubes were incubated in a water bath at 40 °C for 20 min; the intensity of the blue colour developed was measured by recording the absorbance at 755 nm using a UV-visible spectrophotometer (Varian, CARY-300 Bio). The reagent blank was also prepared using distilled water. For quantification of the total phenolic in the extract, a standard calibration curve was prepared using Gallic acid.

Pharmacological Evaluation

Antimicrobial Effects: Extracts of *Eclipta Alba* is used for relieving infections. It fights against all microorganisms which cause boils, infections and inflammations.

Anticancer Properties: Extraction of *Eclipta Alba* is useful in inhibiting the growth of cancer cells.

Insecticide Properties: The extracts of *Eclipta Alba* are effective as an insecticide and provide an opportunity to chemical pesticides. Pest control can be performed in an eco-friendly way. *Eclipta Alba* juice is used to make hair oil which is powerful in controlling several problems related to hair...
like dandruff, hair falling and hair thinning etc. The juice of the leaves is used to make Kajal which is useful in relieving ailments related to eyes. In Siddha medicine it is used to relieve several diseases. Popular liver tonic with the name Liv.52 contains this herb. A black dye that is obtained from the plant is used for tattooing and hair dyeing.

**Antioxidant Activity**

The antioxidant effects of *E. prostrata* (Syn. *E. Alba*) were evaluated in Charles River Sprague-Dawley rats. The extract at 50 mg/kg and 100 mg/kg dose significantly reduced the oxidative biomarkers such as serum lipid peroxide, serum hydroxyl radical levels. In another study, the in vitro antioxidant activity was evaluated based on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay. An IC$_{50}$ value of extract was determined to be 45.68 µg/mL for the whole plant as compared to the IC$_{50}$ of 3.26 µg/mL of standard ascorbic acid. When evaluated using hydrogen peroxide scavenging assay, the extract showed potent activity with the IC$_{50}$ values of 1.34 µg/mL as compared to ascorbic acid (IC$_{50}$: 1.03 µg/mL). The antioxidants present in the extract of *E. prostrata* showed the reduction of ferricyanide complex (Fe$^{3+}$) to ferrous form (Fe$^{2+}$) in a dose-dependent manner. The highest reducing ability (75.59%) for the whole plant of *E. prostrata* was reported at 250 µg/mL concentration. The IC$_{50}$ value for reducing ability of the extract was 100 µg/mL. The studies are mostly conducted using in vitro methods and detailed mechanism is yet to be established.

**Results and Discussions**

The outcome or result demonstrated that the whole quantity of these alkaloids was considerably different among the solvents, but the ratio pattern of the alkaloid content was established to be helpful in classifying the samples. The total phenol content is maximum in P.ether (92 ± 3) followed by acetone and ethanol 88 ± 3 and 68 ± 2 respectively.

*Eclipta prostrata* (L.) (Syn. *E. Alba*) is widely used as traditional medicine in various countries especially for skin, liver and stomach problems, and for promoting hair growth. Various compounds such as coumestan derivatives, steroidal, phenolic acids and flavonoids were isolated and identified from the extracts. Similarly, many of these activities were performed based on in-vitro methods and mechanisms of action are not explored in detail using animal models. Properly designed clinical studies are necessary to evaluate the safety and efficacy *E. Alba* in future.

**Conclusion**

The leaf of *Eclipta Alba* showed significant antioxidant and antitumor activities in the model tested.

**Disclaimer Statement**

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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