

Preliminary secondary metabolites detection of aqueous extracts obtained from different tissues of *in-vitro* originated *Eclipta alba*

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ABSTRACT

Eclipta alba is well known herbal plant for its vast range of pharmaceutically important properties. Some of its major activities are antimytotoxic, antimicrobial, antihepatotoxic, antioxidant, antihyperglycemic, rejuveniser and antivenom action. The present study reveals the presence of secondary metabolites responsible for imparting pharmacological properties in different parts like apical tissue, meristem cells, whole plant, Callus, Seedlings, Mature seeds, immature seeds and dried seeds obtained via plant tissue culture technique. The results shows the absence of Alkaloids in tissue cultures plants which has been normally reported to be present in huge amounts in naturally occurring plants in aqueous extracts but other metabolites viz., Flavonoids, Tannins and phenolic groups reported earlier to be occurring in *Eclipta alba* and its parts were found to be present in aqueous extracts of tissue cultured plant.

Keywords: *Eclipta alba*, Phytochemicals, plant tissue culture, quantification

Introduction

Eclipta alba, commonly known as bhringraaj is very well known herbal plant in India especially due to its use as hair tonic. The vast range of secondary metabolites showing antimytotoxic, antimicrobial, antihepatotoxic, antioxidant, antihyperglycemic, rejuveniser and antivenom action etc. has been investigated and reported in *Eclipta alba*. The bioactive compounds responsible for these activities are alkaloids, flavonoids, Phenolic compounds, Tannins, Saponin etc.¹ The world is moving towards use of herbal remedies for treatment due to less or negligible side effects associated with these drugs. The demand and commercialization of herbal drugs create over exploitation of already existing plants and also huge attraction of farmers to cultivate them on large scale. This is surely creating an agro-ecological imbalance². Therefore the need of the day is to develop some protocols so that these plants can be generated in laboratories in large amount to fulfil the need and also maintain the balance in environment. As it is evident that after *in vitro* culture some variations are induced in plants and their metabolites so a study was made to interpret those variations so that the importance and implementation of tissue cultured *Eclipta alba* can be recognized more clearly.

Material and Method

The plants were raised by tissue culture technique. The air dried tissues were pulverized to fine particles and subjected to hot extraction using soxhlet apparatus. 100 gram of each tissue powder was extracted with distilled water at 80°C. The extracts were collected and concentrated using water bath to obtain crude extracts and remove the solvent. The the extracts were used to determine the presence of useful secondary metabolites.

Phytochemical Testing: Chemical tests were carried out for different extract to detect the presence of bioactive components like **Alkaloids, Carbohydrates, reducing sugars, Flavonoids, Glycosides, Tannin and Phenolic compounds, Saponin, Triterpenoids and Steroids** in them by using standard methods of Kokate *et al.*³.

Results

The result clearly depicts the absence of Alkaloids in aqueous extracts of *in-vitro* generated tissues of *Eclipta alba* while it was present in the Mature and Immature seeds of natural origin. The carbohydrates were found to be present in mature seeds (Natural) and Callus (*in-vitro*). Reducing sugars, Glycosides, Triterpenoids and Steroids were absent in all the tissues. The flavonoids were present in immature seeds, apicals tissues, maristamatic tissues and whole plants. Tannins and Phenolic compounds were absent in mature seed, dried seeds, seedling plants whereas these metabolites were found to be present in immature seed, Apical tissues, maristamatic cells, whole plant and Callus. Saponin was present in dried seeds, apical meristem and seedling plants.

The results clearly shows the absence of Alkaloids in tissue cultures plants which has been normally reported to be present in huge amounts in naturally occurring plants in aqueous extracts but other

metabolites viz., Flavonoids, Tannins and phenolic groups reported earlier to be occurring in *Eclipta alba* and its parts were found to be present in tissue cultured plants.⁴ *Eclipta alba* is highly important plant for its huge medicinal importance and is in great demand in herbal drug industry and among the people trusting its miraculous ayurvedic effects without any observable side effects. Recently large interests in natural medicines that are obtained from plant parts or plant extracts have been created worldwide. On the order of 40% or more of the pharmaceuticals currently used in Western countries are derived or at least partially derived from natural sources.⁵

Although the results are only based on the aqueous extracts so further analysis is required for clarifying the changes in secondary metabolite profile of tissue cultured *Eclipta alba* so that its proper and real value can be predicted for production and commercialization of tissue cultured plants to maintain the ecological balance and harmony being disturbed due to overexploitation of naturally occurring *Eclipta elba*.

S. No	Test	Results							
		S1	S2	S3	P1	P2	P3	P4	P5
Alkaloid									
1	Mayer's Test	+ ve	+ ve	- ve	- ve	- ve	- ve	- ve	-ve
2	Wagner's Test	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Hager's Test	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrate									
1	Molish's Test	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
2	Barfoed's Test	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Reducing Sugars									
1	Fehling's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Benedict's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoids									
1	Alkaline reagent Test	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
2	Shinoda Test	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
3	Lead Acetate Test	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Glycoside									
1	Borntrager's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Legal's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Killer-Killiani Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Tannin and Phenolic Compounds									
1	Ferric Chloride Test	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
2	Lead Acetate Test	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
3	Dilute Iodine Test	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve

Saponins									
1	Foam Test	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
Triterpenoids and steroids									
1	Salwonski Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Libberman & Burchard's Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 1: The results showing of various tests performed for detection of secondary metabolites in different crude extracts isolated from *in- Vitro* cultured plant and plant parts.

Secondary Metabolite	Result							
	Mature seeds (S1)	Immature Seeds (S2)	Dried Seeds (S3)	Apical Tissues (P1)	Meristamatic cells (P2)	Whole Plant (P3)	Callus (P4)	Seedling Plants (P5)
Alkaloid	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Carbohydrate	Present	Absent	Absent	Absent	Absent	Absent	Present	Absent
Reducing Sugar	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Flavonoids	Absent	Present	Absent	Present	Present	Present	Absent	Absent
Glycoside	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Tannins and Phenolic compounds	Absent	Present	Absent	Present	Present	Present	Present	Absent
Saponins	Absent	Absent	Present	Present	Absent	Absent	Absent	Present
Triterpenoids and Steroids	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2: The results summarizing the presence /absence of various metabolites in different crude extracts mined from *in- Vitro* cultured plant and plant parts.

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