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Identification and characterization using isoenzymes of glucose-6-phosphate dehydrogenase in *Eclipta prostrata* (L.) L., Mant

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ABSTRACT

The present study carried out the anatomical characterization of the stem and bark of *Eclipta prostrata* (L.) L., Mant. And also protein and isoenzymic profile of the enzyme glucose -6- phosphate dehydrogenase. The outcome of the present study can be serve as a valuable data to characterize the potential medicinal plant and could be added to already attributed characteristics using conventional methods.

Keywords: *Eclipta prostrata*, Characterisation, Isoenzyme, Glucose -6- phosphate dehydrogenase

INTRODUCTION

Eclipta prostrata (L.) L., Mant. is commonly known as “ Safed bhangra” , belongs to family Asteraceae. It is distributed in tropical and sub tropical regions of the world and widely in India at all elevations in waste places and on road sides. It is an erect or prostrate much branched annual herb with rooted at nodes; leaves opposite, strigose with appressed hairs on both sides ; flowers white in heads, ray compressed [1].

Eclipta prostrata (L.) L., Mant. is widely used in traditional systems of medicines practiced in India such as Ayurveda, Siddha, Unani and other indigenous medicines[2,3]. The medicinal properties of this plant includes, thermogenic, anti-inflammatory, anthelmintic, ophthalmic, digestive, carminative, heamatinic, diuretic, aphrodisiac, depurative,febrifuge and is useful in hepatoplenomegaly, elephantiasis, gastropathy, helminthiasis, skin diseases, wounds ,ulcers, ophthamopathy, hypertension, stranguy,leprosy, pruritus, fever, jaundice , odontalgia, otalgia and cephalagia[4,5].

The plant juice is administered in combination with aromatics for catarrhal jaundice[6]. Leaf juice is mixed with honey is a popular remedy for catarrh in infants. The fresh plant is considered anodyne and absorbent. The juice boiled with sesamum or coconut oil is used for anointing the head to render the hair black and luxuriant. It is also applied with sesamum oil in elephantiasis. The plant is used as dyeing herb in tattooing.

With so much of herbal raw materials and finished products being consumed across the world in recent years, there is a greater need today then ever before in ensuring the correct identity of the plant species. It seems necessary to point out that research work on plant, particularly medicinal plant, no matter how brilliant, painstaking and accurate,

is utterly worthless until the identity of plant is not fixed. Since these medicinal plants have different vernacular names, a lot of confusion exists in the authenticity of medicinal plants due to adulteration as result many plants are used wrongly in the preparation of drugs or medicinal combinations. At this point, it become more relevant than the correct identification of the plant is highly required.

In addition to the Morphological features, informations from other sources like anatomical characteristics of the various parts of the plant , biochemical profiles like protein and more importantly isozyme profile of selected enzymes are highly valuable in the correct identification of the plant , particularly of high value medicinal plants like *Eclipta prostrata* (L.) L., Mant.

Isoenzymes or isozymes are multiple forms of a single enzyme that occur within a single species of organisms or even as single cell. Since these enzymes are coded by different genes, such multiple forms can be detected and separated by gel electrophoresis of cell extracts. Isozymes and total protein banding pattern have been used to identify species, cultivars, inbred lines and aneuploids.

MATERIALS AND METHODS

2.1. Anatomical studies

Free hand sections of fresh leaf and stem were taken and the sections were stained with aqueous solutions of toluidine blue. The sections were observed under a Leica ATC 2000 trinocular microscope and were photographed using a Nikon Coolpix 4500 digital camera.

2.2. Identification of isoenzymes of glucose -6- phosphate dehydrogenase in *Eclipta prostrata* (L.) L., Mant. :

Electrophoresis of glucose-6- phosphate dehydrogenase isoenzyme in the entire tissue of *Eclipta prostrata* (L.) L., Mant. Was carried out to verify if isozymic forms of glucose-6- phosphate dehydrogenase are present and also to separate the different proteins. Discotinuuous polyacrylamide gel electrophoresis (PAGE) was carried out to separate the isoenzymes. Native gel to the method of Gall *et al.*, [7] with minor modifications .Coomassie brilliant blue staining method was adopted to stain the protein bands in the gel according to the method of Hames [8].

RESULTS AND DISCUSSION

Anatomy of *Eclipta prostrata* (L.) L., Mant. Shows that the epidermis single layered, covered by cuticle followed by collenchymatous hypodermis, inner cortex parenchymatous with numerous air cavities (aerenchyma). Endodermis is with well defined casparian thickenings. Pericycle single layered, single ring of collateral vascular bundles can be seen in young stem. A ring of vascular cambium develops during secondary thickening. The intrafascicular cambium produces secondary phloem towards outside and secondary xylem towards inside . The interfascicular cambium produces medullary rays and parenchymatous cells. Xylem is endarch. Phloem contains sclerenchymatous elements. The photograph of the transverse section of the stem is given in (Fig:- B).

Leaves are dorsiventral. Epidermis is with silicified cells. Rosettes of silicified cells are seen surrounding the hairs. Stomata are anisocytic. Mesophyll cell are well differentiated into upper palisade and lower spongy layers .Chlorophyll grains scattered all over the Mesophyll cells. Hydathodes are present at the ends of the veins frequently provided with distinct sheath of parenchyma (Fig:- C). Morphological and anatomical studies conducted in the present study are in agreement with the previous reports.

3.1. Protein profiling in *Eclipta prostrata* :

Electrophoresis was carried out to verify if isozymic forms of glucose-6- phosphate dehydrogenase are present and also to separate the different proteins in the tissues of *Eclipta prostrata*. Tested in the 100 % ammonium sulphate saturation, four dehydrogenase bands could be distinguished .Two of them had faster mobility. All the four bands exhibited more or less the same intensity of staining and band width (Fig:-D).

In a detailed review on glucose-6- phosphate dehydrogenase in photoautotrophic organisms. Eichhorn and Corbus have listed the number of isoforms of glucose-6- phosphate dehydrogenase in a variety of plants [9]. As many as nine isoforms were reported in *Xanthium pensylvanicum* and *Chlorogonium elongatum*. The banding pattern obtained from the electrophoretic studies in *Eclipta prostrata* is highly useful in the identification of the plant. The Pattern must be unique to the plant under study [10].

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