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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND INVITRO STUDY OF HYPOLIPIDEMIC ACTIVITY ON THE STEM OF *ECLIPTA PROSTRATA* LINN

Keerthana V[1], Keerthika S[1], Kingcy Shanoba K[1], Kishore D[1], Johnsy Mary F[2]*, Senthil Kumar M[2]

[1]Annai Veilankanni's College of Pharmacy, Saidapet, Chennai-600 015, India
[2]Associate professor, Annai Veilankanni's College of Pharmacy, Saidapet, Chennai- 600 015, India.

ABSTRACT
The study examines macroscopic and microscopic features of the plant, physiochemical constants, as well as phytochemical compositions. Folklore and Indian traditional medicine recognize *Eclipta Prostrata* as a medicinal plant of great value, but its use is relatively underexplored in the literature. As a result of the powder microscopy, essential structures such as epidermis, trachea, fibers of lignified material, oil glands, and the cork cells could be observed. The ash value, extractive values, and foaming index were determined in order to determine physiochemical constants. After using successive solvent extractions, the ethanol extract yielded 6.02% weight-to-weight after successive solvent extractions. An in-vitro study was conducted to evaluate the inhibitory effects of the ethanolic extract on HMG-CoA reductase and the effects on cholesterol levels. A 46.04 g/ml IC50 value was found for the test sample (HCAS) and a 10.89 g/ml IC50 value was found for the standard drug Atorvastatin. According to these findings, *Eclipta Prostrata* may have a role to play in the management of hyperlipidemia in the future. These standards can distinguish plants from related species based on their pharmacognostical properties. In this study, useful insights are provided into the medicinal properties of *Eclipta Prostrata*, emphasizing the potential hypolipidemic effects of this plant.

Keywords: *Eclipta Prostrata*, Pharmacognostical, Phytochemical, Hypolipidemic, HMG-CoA Reductase.

INTRODUCTION

Eclipta Prostrata Linn. was a medicinal plant belonging to the Asteraceae family that is known as False Daisy or Bhringraj. Plants of this herbaceous species are widespread in tropical and subtropical regions, including in India where it has been used in traditional medicine systems like Ayurveda for thousands of years [1]. Various medicinal plants have been found in India due to its rich biodiversity. There are a variety of pharmacological properties in Eclipta Prostrata. A traditional use of the plant was for hepatoprotection, anti-inflammation, and antioxidant effects [2]. Recent studies have explored its potential in managing metabolic disorders. There is a significant global health concern associated with hyperlipidemia, which involves elevated blood lipid levels. Cardiovascular diseases, the leading cause of death worldwide, are strongly linked to it [3]. Changing lifestyles and dietary habits, as well as genetics, are factors contributing to the rise in hyperlipidemia in India. In order to identify and characterize plant material, pharmacognostical studies are crucial. It takes a thorough examination to establish quality standards for *Eclipta Prostrata* and to differentiate it from closely related species due to its unique morphological characteristics. It is essential to understand macroscopic and microscopic characteristics, as well as physiochemical constants, to identify a plant botanically and chemically [4]. Bioactive compounds present in *Eclipta Prostrata* are being explored through phytochemical studies. There are several compounds in the plant that contribute to its therapeutic properties, including alkaloids, flavonoids, and polyphenols [5]. Extraction processes, such as those using hexane, chloroform, and ethanol, enable researchers to obtain different extracts for further study.

A growing need for safe and effective therapeutic interventions is evident due to the rising prevalence of hyperlipidemia and related disorders [6]. A key enzyme involved in cholesterol biosynthesis, HMG-CoA reductase, has been evaluated in-vitro in the study. It is crucial to inhibit this enzyme in order to manage hyperlipidemia Due to the limitations and side effects that conventional pharmacological interventions have, exploring natural sources for hypolipidemic agents is important

Corresponding Author: **F. Johnsy Mary Email: -** Sharonangela2011@gmail.com

With its traditional history, *Eclipta Prostrata* has the potential to provide an alternative or complementary approach to lipid disorders. By integrating pharmacognostical, phytochemical, and pharmacological aspects of *Eclipta Prostrata*, this study will contribute to scientific understanding of the plant. A novel hypolipidemic agent derived from this valuable medicinal plant could potentially be developed as a result of the findings.

MATERIALS AND METHOD

Collection of Plant

The fresh healthy stem of *Eclipta Prostrata* Linn belonging to the family Asteraceae were collected from [Porur, Chennai] Tamil Nadu, India during the month of October 2023. The plant was identified and Authenticated by Dr. K.N. Sunil Kumar, Research Officer and H.O.D of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai. (E03102302P)

Pharmacognostical studies

Morphological studies

The fresh healthy stem of *Eclipta Prostrata* are studied for its morphological characters like color, odour, taste, stem length, stem width by means of organoleptic test.

Microscopical studies

Collection of specimens

The plants of specimens were collected from the (Porur, Chennai) Tamil Nadu.

Sectioning

A sectioning of plant was added fresh water as needed until the cutting of fully stem. A small section of thin layer of stem is taken without affecting the part of cell or tissues, after placing the slide.

Staining and microscope

Staining of the solutions are 0.1% Phloroglucinol, dil. HCL, glycerine + water, added to over the cover slip. Under the microscope to show different cell component such as crystals, starch grains and lignified cells was employed.

Powder microscopy

The powdered plant material was used for powder microscopy analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagent were used. Powder was stained with 1% Phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed through microscope. All lignified with cell.

Physicochemical studies [7, 8]

A variety of physicochemical parameters were tested on the powdered leaf material, including Ash value, loss on drying, swelling index, foaming index and extractive values.

Phytochemical studies

Extraction [9]

Previously dried, powdered, sieved and stored crude drugs of the stem of *E.prostrata* were taken. It was extracted by 3 different solvents such as hexane, chloroform and ethanol by means of Soxhlet apparatus. Then the entire extracts were concentrated to dryness using a buchi rotary evaporator under reduced pressure. The final yields of individual extracts were calculated and were stored in labeled sterile bottles under appropriate temperature.

Preliminary phytochemical screening

In botanical terms, phytochemicals are chemicals derived from plants and are used to describe a broad range of secondary metabolites. Bioactive compounds are screened for phytochemical activity using phytochemical screening assays, which are a simple, quick, and inexpensive method. The various chemical tests were performed on this hexane, chloroform and ethanol extracts of stem of *E.prostrata* for the identification of Carbohydrate, Alkaloids, Saponins, Sterols, Flavonoids, Tannins and Protein, Fixed oils by using standard procedure.

In Vitro Hypolipidemic Activity

HMG COA Inhibitor [10, 11]

Inhibition assay of HMG-CoA Reductase enzyme:

The test and standard drug solution was made into 5 concentration series (10 µg/ml to 160µg/ml) , and their reaction mixture contained 200 µL, consisting HMG-CoA of 12 µL substrate, 164 µL buffer, and 5 µL reductase enzyme, NADPH (Nicotinamide adenosine dinospotide hydrogen phosphate) 4 µL, and 15 µL test solution. In addition, it contains a mixture of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in triplicate, incubated at 37°C for 10 mins, and the absorbance was measured at 340 nm wavelength using microplate reader. Atorvastatin used as standard drug.

% inhibition = [(OD of control - OD of test) / (OD of control)] × 100

Result and Discussion

Pharmacognostical Studies

The Result of the Pharmacognostical studies is as follows,

Morphology

Color	:	Green to Reddish brown or Purple
Odor	:	Aromatic
Taste	:	Bitter
Stem length	:	2-15.5 cm
Stem width	:	0.5-1.5 cm

Transverse Section of Stem

- Mature stem has single layered epidermis, externally covered with cuticle.

- Round or irregular shaped parenchymatous cells having wide air spaces.
- Vascular bundle in a ring, collateral, endarch of varying sizes transferred by medullary rays.
- Xylem consists of large numbers of vessels, xylem fibres and xylem parenchyma; Xylem vessels appear evenly distributed throughout the xylem.
- Aerenchyma is a modified parenchymatous tissue containing air chamber between cells

Powder Microscopic

The powder preparation includes the following,

1) Epidermis

It is made of a single layer of cell and the cells are closely packed to each other without any intercellular spaces. Epidermis is a protective tissue that covers the entire surface of the plant.

2) Tracheid

A tracheid is a long, lignified cell in the xylem of vascular plants. There are of pits (also known as pupils or guide hales) or decorative on the cell walls of tube cells. When mature, tracheids do not have a protoplast. The main functions are to transport water and inorganic salt, and to provide structural support for trees.

3) Lignified Fibres

Lignin enhance plant cell wall rigidity, hydrophobic properties and promotes minerals transport through the vascular bundle in plant. Lignin provides mechanical strength and resistance against pathogens, and make the cell walls impermeable to water.

4) Oil Glands

These are multicellular epidermal glands. The normal function of sebaceous glands is to produce and secrete sebum.

5) Cork Cell

Cork consists of the irregularly shaped, thin-walled. It protects plants from external injury to some extent.

Physio-Chemical Studies

The physio-chemical constant analysis of Stem of *Eclipta Prostrata* Linn.

Anti – Hypolipidemic Activity

HMG COA Inhibitor

Inhibition assay of HMG-CoA Reductase enzyme:

The test and standard drug solution was made into 5 concentration series (10 µg/ml to 160µg/ml) , and their reaction mixture contained 200 µL, consisting HMG-CoA of 12 µL substrate, 164 µL buffer, and 5 µL reductase enzyme, NADPH (Nicotinamide adenosine dinospotide hydrogen phosphate) 4 µL, and 15 µL test solution. In addition, it contains a mixture of the enzyme reaction, control, and the test solution. The reaction mixture was conducted in triplicate, incubated at 37°C for 10 mins, and the absorbance was measured at 340 nm wavelength using microplate reader. Atorvastatin used as standard drug.

% inhibition = [(OD of control - OD of test) / (OD of control)] × 100

The IC_{50} values of the given test sample (HCAS) and the standard drug (**Atorvastatin**) were found to be **46.04 µg/ml** and **10.89 µg/ml**, respectively.

Table 1. Physio-Chemical Characteristics of *Eclipta Prostrata*

SI.NO	PHYSIO CHEMICAL CONSTANT	PERCENTAGE (% W/W)
1.	Ash Values	
	Total Ash	44 ± 0.3
	Water Soluble Ash	77.7 ± 0.3
	Acid Soluble Ash	26 ± 0.4
	Sulphated Ash	35 ± 5.0
2.	Extractive Value	
	Alcohol Soluble Extractive Value	12 ± 0.2
	Water Soluble Extractive Value	5 ± 0.5
3.	Loss on Drying	22 ± 0.5
4.	Foaming Index	>100
5.	Swelling Index	>100

Table 2: Percentage yield of successive extract of Stem of *Eclipta Prostrata* Linn

SI.NO	EXTRACT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	Hexane	Soxhlet Extraction	Semi solid	Greenish Black	2.02 %
2.	Chloroform		Semi solid	Greenish Black	5.75 %
3.	Ethanol		Semi sticky	Greenish Yellow	6.02 %

Table 3: Preliminary Phytochemical screening on Stem of *Eclipta Prostrata* Linn

SI.NO	PHYTO CONSTITUENTS	POWDER	HEXANE	CHLOROFORM	ETHANOL
1.	Carbohydrates	+	+	+	+
2.	Alkaloids	+	+	-	+
3.	Steroids & Sterols	+	+	+	+
4.	Glycosides	+	-	+	+
5.	Saponins	+	+	-	-
6.	Flavanoids	+	-	-	+
7.	Phenolics / Tannins	+	+	+	+

8.	Protein	+	+	-	-
9.	Amino acids	+	+	-	-
10.	Fixed oil	+	+	+	+

[+] = POSITIVE; [-] = NEGATIVE

(Vas – Vascular bundle, Xyl – Xylem, Aer – Aernchyma, Paren – Parenchyma, Epi – Epidermis).

Figure 1

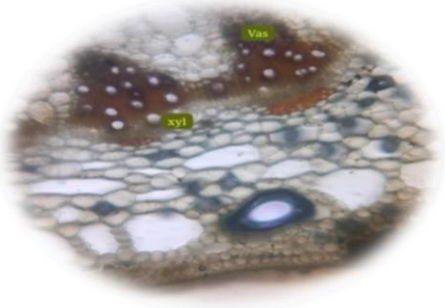


Figure 2

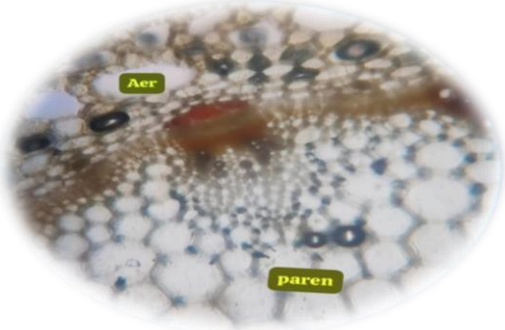


Figure 3

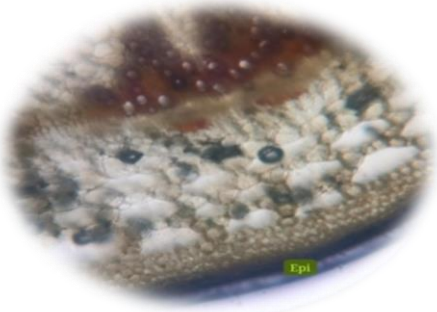


Figure 4- Cork cell

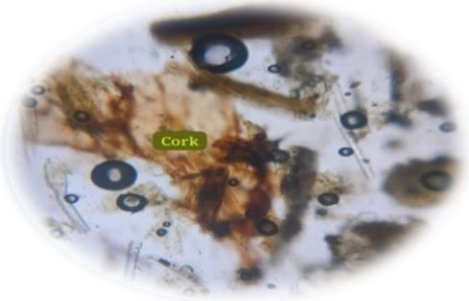


Figure 5- Lignified fibers

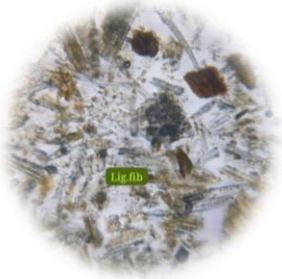


Figure 6- Starch grains, Simplified fibers,

Figure 7- Oil glandsEpi- Epidermis

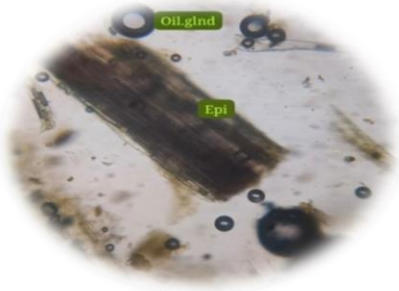


Figure 8- Tracheids

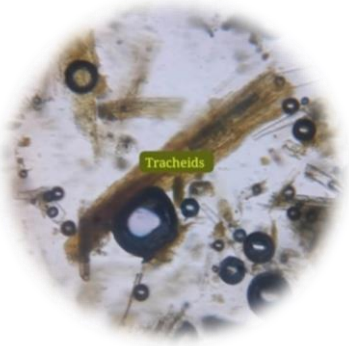
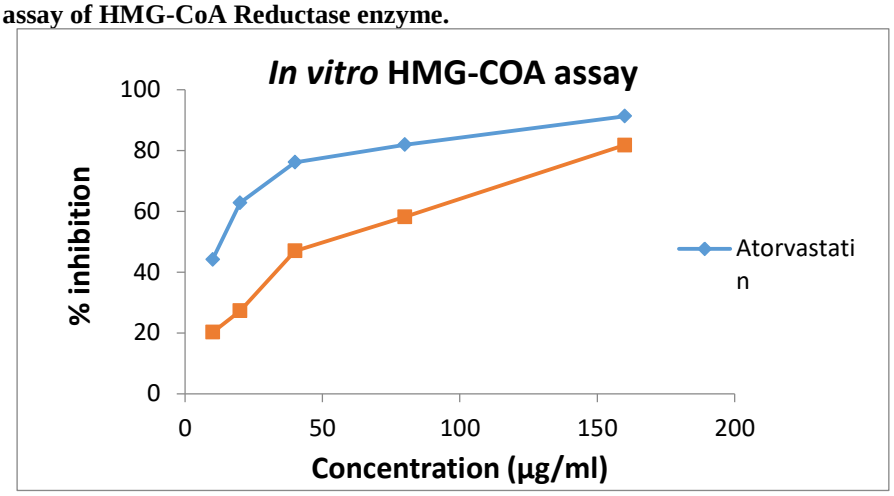
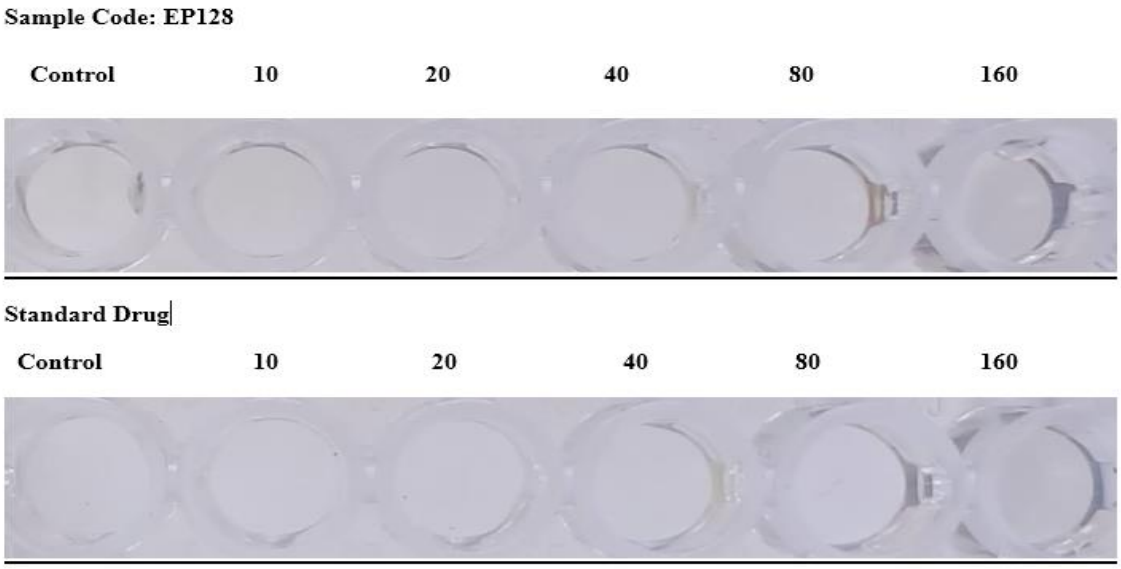


Figure: 9 Inhibition assay of HMG-CoA Reductase enzyme.





CONCLUSION

A pharmacognostical, phytochemical, and in vitro study of hypolipidemic activity in the stem of *Eclipta Prostrata* Linn. Providing an understanding of *Eclipta Prostrata's* macroscopic and microscopic characteristics, physiochemical constants, and phytochemical composition. Plant identification is accurate based on the identified pharmacognostical standards. Using alpha-amylase and alpha-glucosidase inhibitory assays, the ethanolic extract of the stem demonstrated significant yield and potential hypolipidemic activity in vitro. Comparing the extract to Atorvastatin, the IC50 values confirm the extract's effectiveness. In this study, *Eclipta Prostrata* is investigated from a pharmacological perspective, emphasizing its hypolipidemic properties in particular. Our understanding of the plant's therapeutic potential is enhanced by the generated pharmacognostical standards and pharmacological findings that will pave the way for more investigations in natural remedy development and drug development.

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