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Research Article **Biotechnology**

Nutritional Value of *Eclipta prostata* and evaluvation of its efficacy in rats

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ABSTRACT

In the present study aimed to evaluate the nutritional value of *Eclipta prostata* and evaluvation of its efficacy in rats. The preliminary phytochemical analysis of the leaves of *Eclipto prostrata* revealed presence of Flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids , phlobatannins. Steroids, alkaloids and anthroquinones. Phytochemicals further confirmed by histochemicals. Significant amount of minerals were found in the *Eclipto prostrata* leaf. Vitamin C and E were found to be in *Eclipto prostrata* leaf. Improved the antioxidant and hematological parameters in rats. Overall, the *Eclipto prostrata* leaves are a rich source of phytochemicals that can be important in disease preservation. Some of these phytochemical such as Alkaloids, Flavonoids, Tannins, Saponin, Glycosides, Cardiac glycosides, Terpenoids and polyphenolic compounds have possessed biological activity.

Keywords: *Eclipto prostrata*, Nutrition, Phytochemicals, Minerals, Vitamins, Antioxidant

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INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al*., 1998: Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics Meagher and Thomson,2000) and About 150 phytochemicals have been studied in detail.

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, Because of this property; many researchers have been performed to reveal the beneficial health effects of phytochemicals. The purpose of the present review is to provide an overview of the extremely diverse phytochemicals presents in medicinal plants.

Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases (Akunyili). Plant and plant products play a wide range of biological properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. Keeping in view, the present study to investigate the phytochemical analysis of *Eclipto prostrata* leaves.

MATERIALS AND METHODS

Plant materials:

The fully mature *Eclipto prostrata* plant leaves were collected in January 2015 from New Bus stand, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of alcoholic extract:

The leaves of *Eclipto prostrata* plant were first washed well and dust was removed from the rhizome. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 50% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method. Alkaloid determine by the method of Harborne (1973). Tannin determination by method of Van-Burden and Robinson (1981). Saponin determine by the method of of Obadoni and Ochuko (2001). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994).

Histochemical tests

The powder of *Eclipto prostrata* leaves were treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The *Eclipto prostrata* leaf powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavanoids and treated with Mayers reagent gave reddish brown colour indicates alkaloids.

Qualitative analysis of Inorganic elements

Ash of drug material (500mg) was prepared and treated with HNO_3 and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the following tests (Khandelwal 2006):

Pharmacological studies

Animals

Male albino rats of Wistar strain approximately weighing 100-125g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and watter were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silical (1.02%).

Chemicals

Thiobarbituric acid (TBA), 2,4, Dinitophenylhydrazine (DTNPH), reduced glutathione were purchased for sigma chemical company, mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Preparation of extract

The leaf of Plant name (yours) was collected from *Eclipto prostrata*. The collected leaf/root/rhizome was cut into small pieces and shade dried at room temperature and makes a fine powder using pestle and mortar. The powder material of leaf/root/rhizome was macerated with 50% methanol at room temperature for 24 hours. After 24 hours, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

Experimental design

Body weight of animals was recorded and they were divided into four groups of six animals each as follows. Group I: Normal animal received with standard fed and water to allow *ad libitum.* Group II: Normal rats treated with *Eclipto prostrata* leaf powder (500mg/kg) for 7 consecutive days.

Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood was collected by cardiac puncture into serum separator tubes. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

Biochemicals estimation

The level of ascorbic acid was estimated by the method of Omaye *et al* (1979).α-tocopherol was estimated by the method of Baker *et al* (1980). Vitamin A was estimated by the method of Bayfield and Cole (1980). Superoxide dismutase activity was determined by the procedure of Kakkar *et al* (1984). The activity of catalase was assayed by the method of Beers and Sizer (1952). The activity of mitochondrial glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). RBC and WBC counted by the method of Ochei and Kolhatkar, (2000).

Statistical Analysis:

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by studeetn "t" test and $p< 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Liu, 2003).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Eclipto prostrata* leaves investigated and summarized in Table-1. The phytochemical screening *Eclipto prostrata* leaves showed that the presence of flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids phlobatannins. Steroids, alkaloids and anthroquinones.

Table 1: Phytochemical screening of *Eclipto prostrata* leaves

S.No	Phytochemical analysis	Results
1	Tannin	+
2	Phlobatannins	+
3	Saponin	+
4	Flavonoids	+
5	Steroids	+
6	Terepenoids	+
7	Triterpenoids	+
8	Alkaloids	+
9	Carbohydrate	+
10	Amino acid	-
11	Anthroquinone	-
12	Polyphenol	+
13	Glycoside	+

(+) Prresence (-) Absence

Table 2 Quantitative analysis of *Eclipto prostrata* leaf

Secondary metabolites	Result (mg/gm)
Alkaloids	45
Phenol	120
Tannin	65
Saponin	137

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).

Vitamin C, or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons, and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2004).

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and

margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys (Clark, 2008). The vitamins of the *Eclipto prostrata* leaves investigated and summarized in Table-3.

Table 3 Qualitative analysis of vitamins in *Eclipto prostrata* leaf

S.no	Vitamins	Observations
1	Vitamin A	–
2	Vitamin C	+
3	Vitamin D	+
4	Vitamin E	+

(+) Presence (-) Absence

Minerals

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan et al., 2005). Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects (New Wall et al., 1996). The vitamins of the *Eclipto prostrata* leaves investigated and summarized in Table-4.

Qualitative or quantitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food. The quality of many foods depends on the concentration and type of minerals what they contains, also play a very significant role against a variety of degenerative diseases and processes, they may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn, some minerals are essential to a healthy diet (e.g. Calcium, Phosphorus, Potassium and Sodium) where as some can be toxic (e.g. Lead, Mercury, Cadmium and Aluminium). It is clear that mineral nutrition is important to maintain good health and because of that determination of As, Ca, Fe, Mg, Na, K, Zn, Ni, Co etc. have been added to Ayurvedic Pharmacopoeia of India (The Ayurvedic Pharmacopoeia of India, 1999).

Table 4 Qualitative analysis of inorganic element in *Eclipto prostrata* leaf

Inorganic elements	Results
Calcium	+
Magnesium	+
Sodium	+
Potassium	+
Iron	+
Sulphate	+
Phosphate	+
Chloride	+
Carbonate	+
Nitrate	+

(+) Presence (-) Absence

Histochemical studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998) . Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan et al., 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Table 6 represents histochemical studies of *Eclipto prostrata* leaf powder

Table 6 Histochemical studies of plant powder

S.No	Charecterisation	Observation	Result
1	Lignin	Red/pink	+
2	Flavonoids	Yellow	+
3	Alkaloids	Reddish Brown	+
4	Tannin	Dark blue to Black	+
5	Starch Grain	Blue	--
6	Steroids	Violet to blue to green	+
7	Polyphenol	Blue Green/red	+

(+) Presence (-) Absence

Efficacy of *Eclipto prostrata* in rats

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase

of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis, 2003). Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plant's survival during stress. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Liu, 2003).

There is an emerging interest in use of naturally occurring antioxidants for therapeutic usage. Particularly, flavonoids and phenolics are considered as potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, aging, cancer, diabetes cardiovascular dysfunction and inflammatory diseases. Flavonoids and phenolics are widely distributed in the plant kingdom and therefore an integral part of diet, with significant amount reported in vegetables, fruits and beverages (Soobratte *et al*., 2005).

The present study was carried out to evaluate the efficacy of *Eclipto prostrata* in rats. The observations made on different groups of experimental and control animals were compared as follows. Table 7 represents the activity of SOD, GPx and Catalase in serum of normal and experimental rats. Group II rats treated with *Eclipto prostrata* showed a significant increased in the activity of SOD, GPx and Catalase when compared to Group I rats.

Table 7 represents the activity of SOD, GPx and Catalase in serum of experimental rats

S. NO	Parameters	Normal	Treatment Group
1	SOD (U/ml)	3.56±0.24	4.35±0.30
2	GPx (U/ml)	5.23±0.36	6.23±0.43
3	CAT (U/ml)	7.35±0.51	8.38±0.58

Values are expressed as Mean ± SD for six rats

Table 8 represents the content of Vitamin A, C and E in serum of normal and experimental rats. Group II rats treated with *Eclipto prostrata* showed a significant increased in the content of Vitamin A, C and E when compared to Group I rats.

Table 8 represents the content of Vitamin A, C and E in serum of experimental rats.

S. NO	Parameters	Normal	Treatment Group
1	Vitamin – A (mg/dl)	4.25±0.29	5.37±0.37
2	Vitamin – C (mg/dl)	8.25±0.57	9.65±0.67
3	Vitamin – E (mg/dl)	3.89±0.27	4.72±0.33

Values are expressed as Mean ± SD for six rats

Table 9 represents the content of hemoglobin, RBC and WBC in blood of normal and experimental rats. Group II rats treated with *Eclipto prostrata* showed a significant increased in the content of Vitamin A, C and E when compared to Group I rats.

Table 9 represents the content of hemoglobin, RBC and WBC in blood of experimental rats.

S. NO	Parameters	Normal	Treatment Group
1	Hemoglobin (gm/dl)	14.56±1.01	16.78±1.17
2	RBC (Million/cu.mm)	5.04±0.35	7.45 ±0.52
3	WBC (cu.mm)	4276.6±299.36	7895.2 ±552.66

Values are expressed as Mean ± SD for six rats

The first enzyme involved in the antioxidant defense is the superoxide dismutase: a metalloprotein found in eukaryotic cells. Superoxide dismutase (SOD) converts the superoxide anion ($O_2^{\bullet -}$) into a less toxic product, namely H_2O_2 and O_2. Two forms of SOD exist, a copper-containing enzyme and the zinc-containing enzyme is located in the cytosol. The second type is the manganese containing SOD found in the mitochondrial matrix. The biosynthesis of SOD is mainly controlled by its substrate as superoxide anion ($O_2^{\bullet -}$). Induction of SOD by increased intracellular fluxes of $O_2^{\bullet -}$ has been observed in higher organisms (Halliwell and Gutteridge, 1990).

Catalase present in almost all the mammalian cells is localized in the peroxidomes or the micoperoxisomes and cytosol. It is a hemoprotein and catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and $OH^{\bullet}$ (Bandyopadhyay *et al*., 1999). Glutathione peroxidase catalyses the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. This enzyme is specific for its hydrogen donor, GSH and nonspecific for the hydroperoxides ranging from H_2O_2 to organic hydroperoxides. It is a seleno-enzyme; two third of which is present in the cytosol and one-third in the mitochondria. Glutathione peroxidase enzyme is a well-known first line of defence against oxidative stress (Ursini *et al*., 1986).

Vitamin C (ascorbic acid) is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic function of the body (Jaffe, 1984). As an antioxidant, vitamin C is primary role is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damages. Free radicals seek out an electron pair to regain their stability. Vitamin C is an excellent source of electron therefore it "can donate

electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity" (Bendich, 1990). Vitamin C can rejuvenate vitamin E, making it an indirect contributor to the fight against free radical damage in the lipids. Vitamin E and vitamin C are effective partners in reducing the destructive process of lipid peroxidation. It is a water-soluble and is regularly excreted in urine if excess (Iqbal *et al*., 2004).

Vitamin E is lipid soluble vitamin in the body and derived from diet. It is thought to be an important chain-breaking antioxidant and directly scavenge reactive oxygen members (ROMs). It is the major lipid-soluble antioxidant present in all cellular membranes, which protects against lipid peroxidation (Frrei, 1994). Vitamin E can directly act with a variety of oxyradicals, including the peroxy radical ($ROO^{\bullet}$), $OH^{\bullet}$, $O_2^{\bullet -}$ and singlet oxygen (Machlin and Bendich, 1987). The major function of vitamin E is its role as a physiological membrane bound antioxidant, protecting cell membrane lipids from oxidative damage initiated by ROMs. Vitamin E donates hydrogen from the 6^{th} position of its chromonal ring to free radical to form oxidized vitamin E (tocopheroxyl radical) (Packer *et al*., 1997). The oxidized vitamin E can be reduced by glutathione or ascorbic acid (Vitamin C) (McCay, 1985).

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients, oxygen and transports of waste products away from those of same cells. Blood is the most important body fluid that governs vital functions of the body like respiration, circulation, excretion, osmotic balance and the transport of metabolic substance. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs (Baynes *et al*., 2005). Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition (Hymavathi and Rao, 2000). The importance of haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals (Calabrese *et al*., 1975).

The following conclusion obtained from the study. The preliminary phytochemical analysis of the leaves of *Eclipto prostrata* revealed presence of Flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids , phlobatannins. Steroids, alkaloids and anthroquinones. Phytochemicals further confirmed by histochemicals. Significant amount of minerals were found in the *Eclipto prostrata* leaf. Vitamin C and E were found to be in *Eclipto prostrata* leaf. Improved the antioxidant and hematological parameters in rats. Overall, the of *Eclipto prostrata* leaves are a rich source of phytochemicals and possesses antimicrobial activity that can be important in infectious disease preservation.

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