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PHYSIOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF *ECLIPTA ALBA*

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ABSTRACT

The present study comprises physicochemical and phytochemical evaluation of arial parts of *Eclipta alba* in different extracts using ethanol, petroleum ether, benzene, chloroform and aqueous solvents by using standard methods. The physical evaluation was carried out for the determination physiochemical profile such as ash values and extractive values, whereas phytochemical evaluation was carried out for the screening of bioactive compounds in different extracts of *E. alba* (arial parts). Preliminary phytochemical analysis test showed the presence of carbohydrates, protein, glycosides, saponin, steroids, flavonoids, alkaloids and tannins. Chloroform extract of plant showed the presence of proteins terpenoids, saponin, tannin and steroids. Petroleum ether extract of plant leaf showed the presence of carbohydrates, protein, terpenoids, saponin and steroids. This study will help in development of a suitable monograph, determining the quality and purity of a crude drug and laying down pharmacopoieal standards for *E. alba*.

KEYWORDS: ***Eclipta alba,*** **Phytochemical, Physiochemical and Bioactive compounds.**

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INTRODUCTION

Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the World's population relies on plants and its extracts for health care[1]. India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. The medicinal value of plants lies in some active ingredients that produce a definite physiological action on the human body. The active ingredients in medicinal plants are defined as chemical compounds that act directly or indirectly prevent or treat disease[2]. The most important of these bioactive chemical constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. These chemical compounds have countless benefits to humans, which are exploited as natural pesticides, flavoring, fragrances, fibers and beverages and also acts as a precursor for bioactive compounds used as therapeutic drugs[3]. Knowledge of the biologically active constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

Eclipta alba Linn, Family

Compositae (Asteraceae) is a very common medicinal plant known by other names: Bhangra, Bhringraj, Mochkand, and Maka and is found throughout India[4]. This tropical annual is a creeping and moisture-loving herb; stem are short and rounded bears small flowers that can be white, yellow or blue, leaves are opposite and lance-shaped. *E. alba* is reported in literature for its various biological activities such as: calm the mind, removes memory disorders, relieve swollen glands, strengthens spleen, works as a general tonic, useful for treatment of edema, fevers and rheumatic joint pains, stimulate digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders[5]. *E. alba* is also reported for hepatoprotective[6], antioxidant properties[7], antihyperglycemic, antimicrobial, antinociceptive, analgesic, antiinflammatory, antiviral, immunomodulatory and nootropic activity[8]. This plant known to possess important medicinal properties, as it is a rich source of anthraquinones, flavonoids, polysaccharides, sterols, phenolic compounds and coumarins[9]. The objective of the present investigation was to screen the physicochemical, behavioral characteristic and also the major phytochemicals of the various extracts of the *Eclipta alba* that would be attributed to the biological activity.

MATERIALS AND METHODS

Plant material identification and Collection

The plant material (*E. alba*) which is used for study was collected and was identified by the taxonomist of depts., Department of Botany, University of Rajasthan Jaipur. The voucher number was RUBL 20252.

Preparation of plant material

The collected *E. alba* aerial parts were washed with tap water. The plants were cut in to small pieces and air-dried thoroughly under shade (at room temperature) for 2 months. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder, weighed and stored in air-tight container for furthers analysis. This powder material was used for the analysis of physicochemical and phytochemical constituents.

Extract preparation

The coarse powder material was subjected to soxhlet extraction separately and successively with petroleum ether, benzene, chloroform and ethanol. Finally the marc was allowed for maceration about 24 hours with distilled water to obtain aqueous extract. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). The % yield of extracts was calculated (Table No. 1). All the extracts were stored in a refrigerator in air tight containers for further analysis.

Physicochemical Analysis

Physico chemical constants such as the percentage of total ash content, acid insoluble ash, water soluble ash, water and alcohol soluble extractives and loss of weight on drying, were calculated based upon standard procedures prescribed in Pharmacoeia as follows[10].

Determination of total Ash value

One to two grams of the plant material was poured in pre-weighed crucibles and placed over a flame to burn the plant material completely to ash. These crucibles were covered with lids, than placed in a furnace at 600°C for 2 hours. After cooling, the crucibles were placed in desiccators and reweighed. The percentages of ash values were then calculated using following formula.

$$\text{Percentage of total ash} = \frac{\text{Net weight of ash obtained after ignition} \times 100}{\text{Net weight of plant sample taken for test before the ignition}}$$

Acid Insoluble Ash

To determine the acid insoluble ash, one gram of ash was added to 25 ml of 2 M hydrochloric acid (HCl) and boiled gently at 70-90°C for 5 minutes. It was filtered through filter paper and packed in ash less filter paper. This pack was kept in crucible and ignited for 15 minutes in oven at 700°C. The crucible was removed and cooled to room temperature. The acid insoluble ash was calculated.

$$\text{Percentage of total ash} = \frac{\text{Net weight of ash obtained after ignition} \times 100}{\text{Net weight of plant sample taken for test before the ignition}}$$

Water Soluble Ash

To determined water soluble ash total ash, one gram of ash was added to 25 ml of water and boiled at 100°C for 5 minutes. It was filtered through filter paper and packed in ash less filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15 minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight was attained. Percentage of water soluble ash was calculated.

$$\text{Percentage of total ash} = \frac{\text{Net weight of ash obtained after ignition} \times 100}{\text{Net weight of plant sample taken for test before the ignition}}$$

Preliminary Phytochemical Analysis

Qualitative analysis of various extracts aerial parts of *E. alba* was performed for the identification of various classes of active chemical constituents like alkaloids, carbohydrates, glycosides, proteins, amino acids, steroids etc. using different methods of Harborne[11] and Kokate[12].

Test for Carbohydrates

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

Test for Proteins

To 1 ml of the each extract, two drops of freshly prepared 0.2% ninhydrin reagent was added, and the mixture was heated. The development of a violet color indicates the presence of proteins.

Test for Anthraquinone

About 0.5 g of each extract was boiled with 10 % HCL for few minutes on water bath. The reaction mixture was filter and allows to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10 % ammonia was added to each mixture and heated. Rose-pink color formation indicates the presence of anthraquinone.

Test for Terpenoids
1 ml of the each extract was mixed with 2 ml of chloroform in a test tube and evaporated to dryness. 2 ml of concentrated sulphuric acid (H_2SO_4) was then carefully added to form a layer. Development of a reddish brown coloration at the interface indicated the presence of terpenoids.

Test for Flavonoids
To 1 ml of each extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for Saponins
5 ml of each extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Alkaloid
3 ml each extract was stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for Tannins
About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Glycosides
Each extract of plant was treated with 1 mL of $FeCl_3$ reagent (mixture of 1 volume of 5% $FeCl_3$ solution and 99 volumes of glacial acetic acid), followed by the addition of few drops of concentrated $H_2 SO_4$. Appearance of greenish blue color within a few minutes indicated the presence of glycosides.

Test for Steroids
About 0.5 g of the each extract fraction of plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid (H_2SO_4). Appearance of violet to blue or green colour indicated the presence of steroids.

RESULTS AND DISCUSSION

The standardization of a crude drug is an integral part of establishing its correct identity. For inclusion of a crude drug in Pharmacopoeia, pharmacognostic parameters and standards must be established[13]. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation and determination of the authenticity of the material of the plant[14].

Extractive values
Extractive values obtained from *E. alba* using different solvents were recorded in table-1. It is useful for the evaluation of a crude drug as it gives idea about the nature of chemical constituents present in it and is useful for estimation of chemical constituents, soluble in that particular solvent used for extraction[15]. The water soluble extractive value was indicating the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites present in the plant sample [16]. *E. alba* water extractive value of 2.741% showed that water permeates the cells of the aerial parts and thus, a better extractive compared to alcohol with extractive value of 2.041% and benzene with extractive value of 0.733%.

Table 1
The percentage yield of various extracts of Eclipta alba L.

Extracts	Nature of extract	Colour	Yield (%)
Alcohol	Semisolid	Greenish yellow sticky	2.041
Chloroform	Semisolid	Greenish yellow sticky	0.127
Benzene	Semisolid	Greenish yellow sticky	0.733
Petroleum ether	Semisolid	Greenish yellow sticky	0.332
Aqueous	Semisolid	Greenish yellow sticky	2.741

Physicochemical parameter

The determination of physicochemical parameter is important in determination of adulterants and improper handling of drugs. Powder analysis parameters like ash value were determined on the powder of *Eclipta alba* and reported in table-2. Ash values are important quantitative standards[17] and criterion to judge the identity and purity of crude drugs especially in the powder form[18]. Moreover the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug[19]. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. and these More water soluble ash value appears that this plant powder ash is more soluble to water compared to the other ashes.

Table 2
Ash Values of E. alba powder

Parameters	Determined value % w/w
Total ash	16.1
Acid insoluble ash	8.98
Water soluble ash	17.95

The behavior of the aerial powder upon treatment with different chemical reagents was also observed and reported in table-3. When the powders were treated with chemicals like HCl, HNO_3, Iodine solution, etc various colours were obtained.

Table- 3
Powdered drug reaction of Eclipta alba aerial parts with different chemicals

Treatment	Colour after Treatment in Daylight
As such	Light greenish gray
Powder treated with: Conc.H_2SO_4	Dark - Red
Acetic Acid	Dark – Red
1N HNO_3	Green - Yellow
5% I_2 Solution	Black - Yellow
5% $FeCl_3$ Solution	Dark - Green
10% NaOH followed by a drop of $CuSO_4$	Dark - Red
Acetic Acid + Con. H_2SO_4	Orange - Black
Con. HNO_3 and excess of ammonia	Red- Black
Acetic Acid + a trace of $FeCl_3$ and transferred to the surface of Con.H_2SO_4	Dark - Green
Few drops of dil.NH_3 + $K_4Fe(CN)_6$	Black - Red
40% NaOH + Few drops of 10% lead acetate solution	Black -Red

Phytochemical screening

The results of phytochemical screening of different extracts of *E. alba* plant were reported in table 4. The phytochemical study revealed the presence of various phytocompounds in the solvent extracts.

Table -4
Preliminary Phtochemical screening of powder of E.alba

S. no	Test	Ethanol	Chloroform	Benzene	Petroleum ether	Water
1	Carbohydrates	-	-	-	+	+
2	Proteins	-	-	+	+	+
3	Anthraquinones	-	-	-	-	-
4	Terpenoids	+	+	+	+	+
5	Flavonoids	+	+	-	-	+
6	Saponins	+	-	+	+	+
7	Alkaloids	+	-	-	-	+
8	Tannins	+	-	+	-	+
9.	Glycosides	+	-	-	-	-
10	Steroids	+	+	+	+	-

Phytochemical screening of various extracts of the *E. alba* aerial parts showed the presence of terpenoids, flavonoids, saponins, alkaloids, tannins, glycosides and steroids while it gave the negative results for carbohydrate, protein and anthraquinones. However in chloroform solvent terpenoids, flavonoids and steroids were present and other compounds were found to be absent. Our results showed correlation with the finding of Hussain et al., who reported that in chloroform extract of *E. alba* the, terpenoids and flavonoids tests were positive [20]. In benzene extract only proteins, terpenoids, saponins, steroids and tannins were found to be present, while the rest of the compounds were found to be absent. In the petroleum ether solvent extract carbohydrates, protein, terpenoids, saponins and steroids were present where as anthraquinones, flavonoids, alkaloids, tannins and glycosides were tested absent. Aqueous extract showed the presence of carbohydrates, protein, terpenoids, flavonoids, saponins, alkaloid and tannins while glycosides and steroids were absent. From this analysis, ethanolic and aqueous extract found to have more constituents compare to other extracts. Ethanol and aqueous extract shows the presence of seven compounds each, followed by benzene and petroleum ether had five compounds each, while chloroform showed the presence of 3 compounds. Patel et al., also reported that alcoholic extract of *E. alba* was found to be rich in glycoside, saponin, flavonoid, tannin and alkaloid[21]. Plant synthesizes phytochemical (lipid, protein, starch, sugars, phenol etc.) for the normal growth and development of itself[22]. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds[23]. Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. The medicinal value of the title plant can be correlated due to the presence of various bioactive chemical constituents. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides, terpenoid quinones, and alkaloids. These findings give credence to the traditional medicinal application of the plant as remedies for measles, internal and external wounds and infections. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties whereas steroids and saponins were responsible for central nervous

system activities[24]. Further work could also be possible to investigate the specific phytoconstituents responsible for these activities.

CONCLUSION

In the present study aerial part of *E. alba* was thoroughly investigated for their physicochemical characters and major active constituents to analyze their quality, safety and standardization for their safe use. The generated information of the present study will provide data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration. Further research on this species may help in the isolation of therapeutically potent compounds.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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