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Phytochemical Screening and Spectroscopic Determination of Total Phenolic and Flavonoid Contents of *Eclipta Alba* Linn

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ABSTRACT

This research aims to detect the Phytochemical Screening and in-vitro total phenolic and flavonoids contents of chloroform and methanolic extracts of Eclipta Alba. All the main phytoconstituents were present in methanolic extract as compared to chloroform extract. The total phenolic content and total flavonoid content were determined by Folin–Ciocalteu and aluminum chloride methods respectively. The total phenolic contents of methanolic extract (269.5±0.5mg/g) and chloroform (182 ±0.5 mg/g), were expressed as Gallic acid equivalents The flavonoid contents were found to be (139.5±0.5mg/g) and (85.666±0.2886 mg/g) in methanol and chloroform extracts respectively using rutin equivalents. The results are in concurrence with the phytochemical properties present in the plant.

Key words Phytochemical, *Eclipta Alba*, phytoconstituents, Folin–Ciocalteu

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INTRODUCTION

In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 - 7,500 plants for curing different diseases [1] *Eclipta alba* **(L.)** (Asteraceae) is an annual herbaceous plant, commonly known as false daisy, Bhringaraj. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate which is found a common weed throughout India ascending up to 6000 ft [2] It has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation. [3]

It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds [4] Polyphenols are considered to be important ingredients in human diet and exert a lot of biological effects such as antioxidant activity and inhibitory effects on carbohydrates hydrolyzing enzymes due to their ability to binds with protein [5] The decrease in the cases of oxidative-stress associated diseases like cancer, diabetes[6] Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [7]

MATERIALS AND METHODS

***Plant material*:** The whole plant of *Eclipta alba* was collected from the local surroundings at Bhopal city of M.P, during the month of September to October 2011. The plant was acknowledged by Dr. Zia-Ul-Hassan Head of the PG Department of Botany, Safia Science College Bhopal MP. The voucher specimens (herbarium; V. No. 05EA, 25/2010) are kept in the herbarium of Bhoj Mahavidyalaya Bhopal (M.P.) future reference.

***Preparation of extract*:** The dried powdered of *Eclipta alba* (2kg) was successively Soxhlet extracted using Petroleum Ether, chloroform and methanol for 72 hours. The extracts were dried under reduced pressure using rotator evaporator to get the crude and were stored below 4ºC until further used. When needed, the extract was suspended/dissolved in desired solvent and used. Folin-ciocalteau reagent and all other chemicals used were Merck products

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the standard procedures of Trease. [8] The Petroleum ether, chloroform and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. Alkaloids, Terpinoids, Glycosides, Steroids, Triterpenoids, Flavonoids, Carbohydrates, Saponins and Tannins.

Test for carbohydrates

Molish test

Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of Conc. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

Test for alkaloids

Mayer's test: Crude extract was mixed with Mayer's reagent (Potassium mercuric iodide solution) Cream color ppt. was formed showing the presence of alkaloids

Hager's Test: To the 2-3 ml of filtrate, Hager's reagent was added. Yellow precipitate was formed showing the presence of alkaloids

Test for Terpinoids

Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 were added. The solution was shaken well. A reddish brown coloration of the interference indicated the presence of terpinoids.

Teat for flavonoids:

Shinoda test: Crude extract was mixed with few fragments of magnesium ribbons and conc. hydrochloric acid was added drop wise. Pink scarlet color appears after few minutes, indicated the presence of flavanoids.

Zinc hydrochloride test

To the test solution, add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes**.**

Test for triterpenes: To the extract, chloroform and conc. H_2SO_4 was added. Appearance of red color indicated the presence of triterpenes.

Test for tannins.

$FeCl_3$ Solution Test: On addition of 5% $FeCl_3$ solution to the crude extract, deep blue black color appeared, indicated the presence of tannins

Test for saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins.

Teat for Amino acids

Ninhydrin test: To the 3ml of crude sample, 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish color indicated presence of amino acids.

TOTAL PHENOLIC CONTENT (TPC)

The methanolic extract was analyzed for total phenolic content (TPC) using spectrophotometer by Folin-Ciocalteu method. Gallic acid was used as the reference standard. All determinations were carried out in triplicate

and the results were expressed as mg/g gallic acid equivalent (GAE). Different concentrations i.e, 0.01, 0.02, 0.03, 0.04, 0.05 mg/ml of gallic acid, was prepared in methanol. Concentrations of 0.1 – 1mg/ml of plant extract was also prepared in methanol. 0.5ml of each extract sample was taken, mixed with 2.5 ml of (a 10 fold) dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate solution. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature. The absorbance at 765 nm was measured after 30 minutes. at 20º C and the calibration curve was drawn. To the similar reagent, 1 ml methanolic extracts (40 mg/ml) was mixed as described above and after 1 hr. the absorbance was measured. [9, 10]

TOTAL FLAVONOID CONTENT

Procedure

The amount of Total Flavonoid content in extracts was determined aluminum chloride assay through Colorimetric. A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total Flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All samples were analysed in three replications. [11, 12]

RESULTS

The Petroleum ether, chloroform and methanolic extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in the plant. The preliminary phytochemical screening revealed that carbohydrates, saponins, alkaloids, flavonoids, proteins, terpinoids and saponins were present in the whole plant. Terpinoids, tannins and saponins were present in petroleum ether extract. For chloroform extract carbohydrate, terpinoids, flavonoids and alkaloid tests were found positive. All the main constituents like flavonoids, carbohydrates, alkaloids, terpinoids glycosides and proteins were present in methanolic extract as shown in table No.I

Table I Showing preliminary phytochemical screening of crude extracts Petroleum ether, chloroform and methanol of *Elipta alba*

Phytochemicals	Tests	Petroleum ether	Chloroform	Methanol
Alkaloids	Mayer's Test	_	_	_
	Wagner's Test	_	_	+
	Hager's Test	_	+	_
Terpenoids	Salkowski test	+	+	+
Flavonoids	Shinoda Test	_	-	+
	Zinc hydrochloride test	_	+	+
	Alkaline reagent test	_	+	+
Carbohydrates	Molish test	_	+	+
	Fehling's Test:	_	+	+
Glycosides	Killer Killians test	_	+	+
Tannins	$FeCl_3$ test	+	_	_
Saponins	Froth test	+	_	_
Steroids & triterpinoid	Salkowski test	+	_	_
Amino acids	Ninhydrin test	_	+	_

+ = present, - = absent

The total phenolic content was expressed as mg/g gallic acid equivalent using the standard cure equation: $y = 0.002x - 0.003$, $R^2 = 0.968$. Graph Idepicts the variation of mean absorbance with different concentrations of Gallic acid. Table II and III describes the content of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent for the methanolic and chloroform extract. The total phenolic content was found to be 269.5±0.5 and 182±0.5 mg/g for the methanolic and chloroform extract respectively of the plant.

Graph I Showing absorbance of Gallic acid in different concentration

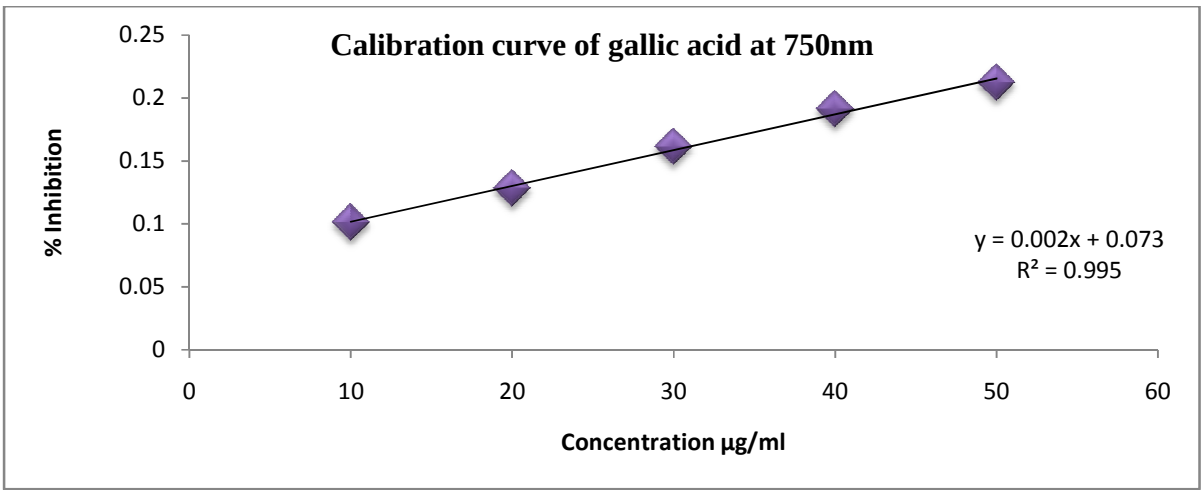


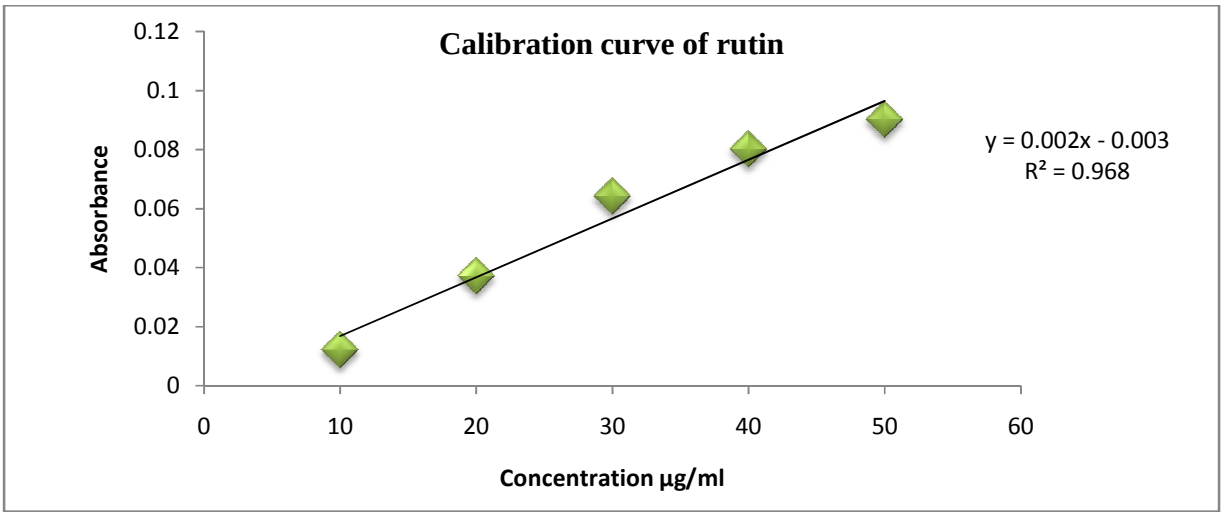
Table II shows Total Phenolic Content of methanolic extract in mg/g equiv. to Gallic Acid

S.No.	Absorbance of Extract	Conc. Of Extract	Total Phenolic Content mg/g equiv. to Gallic Acid
1	0.612	1mg/ml	269.5
2	0.613	1mg/ml	270
3	0.611	1mg/ml	269
MEAN±SD			**269.5±0.5**

Table III shows Total Phenolic Content of chloroform extract in mg/g equiv. to Gallic Acid

S.No.	Absorbance of extract	Conc. Of Extract	Total Phenolic Content mg/g equiv. to Gallic Acid
1	0.437	1mg/ml	182
2	0.436	1mg/ml	181.5
3	0.438	1mg/ml	182.5
MEAN±SD			182**±0.5**

Graph II Showing absorbance of rutin in different concentrations



The amount of total flavonoids content of methanolic and chloroform extracts were expressed in milligram of rutin equivalent using the standard curve equation: $y = 0.002x - 0.003$, $R^2 = 0.968$, Graph II illustrated the variance of

mean absorbance with different concentrations of rutin. The total flavonoid content present was found to be 139.5±0.5 and 85.666±0.2886mg/gm equiv. to Rutin for methanolic and chloroform extracts respectively (Table IV and V)

Table IV shows Total Flavonoid Content methanolic extract in mg/g equiv. to Rutin

S. No.	Absorbance of Extract	Conc. Of Extract	Total Flavonoid Content mg/g equiv. to Rutin
01	0.275	1mg/ml	139
02	0.276	1mg/ml	139.5
03	0.277	1mg/ml	140
	MEAN±SD		**139.5±0.5**

Table V shows Total Flavonoid Content chloroform extract in mg/g equiv. to Rutin

S. No.	Absorbance of Extract	Conc. Of Extract	Total Flavonoid Content mg/g equiv. to Rutin
01	0.169	1mg/ml	86
02	0.168	1mg/ml	85.5
03	0.168	1mg/ml	85.5
	MEAN±SD		**85.666±0.2886**

DISCUSSION

Phytochemicals have received increasing attention because of interesting new discoveries considering their biological activities especially polyphenols [13] and the present study confirms that the plant contains many bioactive compounds like flavonoids tannins terpinoids carbohydrates etc. It has been reported that tannins were known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention. [14, 15] Saponins, known to produce inhibitory effect on inflammation. [16] Phenols and steroids were also found responsible for anticancer activity [17] and may serve as a treatment of cancer. The study also showed that the plant contains a large quantity of phenols and flavonoids. Flavonoids have been shown to exhibit their actions on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase[18] and this property may explain the mechanisms of antioxidative action of *Eclipta alba*. They serve as health promoting compound as a results of its anion radicals. [19] Flavonoids present in plants exhibit a variety of beneficial effects on human health. The anti-inflammatory properties of various flavonoid glycosides have been studied in order to establish and characterize their potential utility as therapeutic agents for the treatment of inflammatory diseases [20, 21, 22] The results were in concurrence with the phytochemical properties present in the plant.

CONCLUSION

The plant *Eclipta alba* contain phytoconstituets like alkaloids, flavanoids, terpinoids, carbohydrates, saponins and tannins and this study could serve as a constructive reference to allow further in-vivo analysis which can be conducted to evaluate the extent of protective effects of *Eclipta alba* against chemically induced cellular damage. The present investigations revealed that the methanol and chloroform extracts of *Eclipta alba* contain significant amount of phenols and flavonoids. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; the methanolic extract manifested greater power of extraction for phenolic compounds from *Eclipta alba*. Further intention of this study was to correlate relationship of these secondary metabolites to possible biological activities and evaluate *Eclipta alba* as a potential source of natural bioactive chemicals.

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