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PHYTOCHEMICAL SCREENING AND DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS OF ECLIPTA ALBA HASSK. AND LIPPIA NODIFLORA LINN.

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ABSTRACT

This research aims to identify the Phytochemical screening and *in vitro* total phenolic and flavonoid contents of hexane, chloroform, ethyl acetate and ethanolic extracts of *Eclipta alba* and *Lippia nodiflora.* All the main phytoconstituents were present in ethyl acetate soluble fraction of ethanolic extracts as compared to petroleum ether extract. The total phenolic content and total flavonoid content were determined by Folin-Ciocalteau and aluminium chloride methods respectively. Measurement of total phenolic content by Folin-Ciocalteu assay in hexane, chloroform, ethyl acetate and ethanolic extract of *Eclipta alba* was found to be 0.0, 6.02 ± 0.18, 12.23 ± 0.05 and 6.05 ± 0.15 mE GAE/mg of the extract respectively and in hexane, chloroform, ethyl acetate and ethanolic extract of *Lippia nodiflora* was found to be 5.82 ± 0.09, 6.10 ± 0.08, 15.77 ± 0.12 and 13.71 ± 0.05 mE GAE/mg of the extract respectively [with the equation y= 0.025x + 0.0580 (r^2 = 0.9952)]. Flavanoid contentin hexane, chloroform, ethyl acetate and ethanolic extract of *Eclipta alba* was found to be 2.85 ± 0.11, 4.3 ± 0.063, 5.5 ± 0.051 and 6.15 ± 0.058 mEQuercetin/mg of the extract respectively and in hexane, chloroform, ethyl acetate and ethanolic extract of *Lippia nodiflora* was found to be 3.89 ± 0.032, 4.15 ± 0.15, 7.09 ± 0.032 and 5.05 ± 0.15 mEQuercetin/mg of the extract respectively [with the equation y= 0.0391x + 0.040 (r^2 = 0.9830)]. The results are in accordance with the phytochemical properties present in the plant.

KEYWORDS

Eclipta alba, Lippia nodiflora, Total Phenolic, Total flavonoid, Folin-Ciocalteu assay, Gallic acid and

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INTRODUCTION

Knowledge of medicinal values of plants is recognized by almost every society on earth. The inhabitants of the remote places have good knowledge about the utilization of plants because of the non- availability of synthetic drugs. In addition, for the survival, they use the plant based drugs growing nearby their villages. Based on their right

or wrong experiences they discovered the therapeutic agents of these plants in particular diseases. These experiences are transferred from parents to offspring[1]. Traditional medicine based on plants has played a key role in the health care system of many countries little- India, China etc[2]. Herbal medicine is still the main stay of about 70-80% of the world population stays on the herbal medicine.

Eclipta alba L. Hassk. Is an annual herbaceous plant, commonly known as king of hairs. Main active principles consist of coumestans like wedelolactone, desmethylwedelolactone, furanocoumarins, oleanane and taraxastane glycosides[3,4]. The plant is commonly used in hair oil all over India for healthy black and long hair[5]. It has been reported to show protective effect on experimental liver damage in rats and mice[6].In Ayurveda, the root powder is used for treating hepatitis, enlarged spleen and skin disorders. Mixed with a little oil when applied to the head, the herb relieves headache.

Lippia nodiflora is the important member of the family verbenaceae showing a variety of medicinal uses. It can be the source of the indigenous medicine. In India, it is found in the warmer parts including Andhra Pradesh, Karnataka, Kerala, and Maharashtra, some parts of Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. The plant contains a variety of constituents such as triterpenoids**,** flavonoids, phenols, steroids, and many others**.** Among these flavonoids were the most commonly found constituent. Nodifloretin (3), *β*-sitosterol glycoside and stigma sterol glycoside from the leaves of *L. nodiflora*)[7]. Nodifloridin A (1) and Nodifloridin B (2) along with lactose, maltose, glucose, fructose, and xylose were isolated from the plant. The plant is used as gastro protective effect[8], anti-inflammatory, antineoplastic[9], antioxidant[10] and diuretic[11]. The plant is used for the treatment of diuretic, aphrodisiac, diseases of heart, ulcers, bronchitis, fever and colds.

It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds. 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. 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.

In the present study, phytochemical screening and total phenolic and total flavonoid contents of two plants *viz.*, *Ecliptaalba*and *Lippia nodiflora* were determined.

MATERIAL AND METHODS

Collection of Plant Materials

The plant specimen for the proposed study *Lippia nodiflora* (here after *L. nodiflora*) was collected from the wetland fields and other irrigated fields in and around Madurai District, Tamil Nadu, India. The herbarium of these plants was identified and authenticated by Dr. D. Stephen, Professor, Department of Botany, American college of Arts and Science, Madurai, Tamil Nadu and the specimen was deposited in Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai, Tamil Nadu, India.

Preparation of *E. alba* and *L. nodiflora* extracts

The fresh whole plant of *E. alba* and *L. nodiflora* was washed with distilled water to remove unwanted foreign materials like soil and dusts. After, washed plant material was dried under shade at room temperature without direct exposure of sunrays. It was then coarsely grounded by using mechanical device. The powdered plant material was passed through sieve no 40 and stored in an airtight container for further use.

The coarsely powdered plant materials of *E. alba* (2000 gm) and *L. nodiflora* (2000 gm) were extracted separately to exhaustion in a soxhlet apparatus for 72 hours by using petroleum ether (60 - 80˚ C), chloroform and ethanol (95 %) solvent (Merk and Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by Whatman filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40 - 50˚ C) and reduced pressure. The

extracts were preserved in airtight containers and kept at 4˚ C until further use.

Qualitative phytochemical investigation of *E. alba* and *L. nodiflora*

Plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat infectious diseases and cosmeceutics[12,13]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[14].

The powdered drugs of *Eclipta alba* and *Lippia nodiflora* were subjected to preliminary phytochemical screening for the detection of various plant constituents. The extracts obtained were then subjected to qualitative tests for their identification of various plant constituents like alkaloids, carbohydrates, glycosides, proteins, amino acids, steroids *etc*. The various tests are performed and results obtained are as under. Reagents were prepared for this tests based on the earlier research[15].

Detection of carbohydrates

1 gm of extract was dissolved with mother solvent and filtered. The filtrate was used to perform the following tests for detecting the presence of carbohydrates.

Molisch's test (general test)

2 - 3 ml of filtrate was treated with 2 – 3 drops of α - naphthol solution in an alcohol, shaked well and then 1 ml of conc. H_2SO_4 was added carefully along the sides of the test tube. Formation of violet ring at the junction of two liquid indicated the presence of carbohydrates.

Detection of alkaloids

The small residue of extract was added with small volume of dilHCl. It was then shaken well and filtered. The filtrate was used to perform following tests.

Dragondorff's test

(14 gms of KI with 5.2 gms of bismuth carbonate in 50 ml of glacial acetic acid)

Few drops of Dragondorff's reagent were added to 2 - 3 ml of the filtrate. Presence of alkaloids was indicated by formation of orange brown precipitate.

Mayer's test

(1.36 gms of $HgCl_2$ in 60 ml of distilled water + 5 gms of KI in 20 ml of distilled water, make up the volume to 100 ml)

Few drops of Mayer's reagent were added to 2 - 3 ml of the filtrate. Presence of alkaloids was indicated by formation of cream colour precipitate.

Hager's test

(Saturated solution of picric acid)

Few drops of Hager's reagent were added to 2 - 3 ml of the filtrate. Presence of alkaloids was indicated by formation of yellow colour precipitate.

DETECTION OF GLYCOSIDES

Detection of cardiac glycosides

Baljets test

A thick section showed yellow to orange colour under the microscope or mixed with sodium picrate.

Legal's test

Extracts were treated with sodium nitro prusside in pyridine and methanolic alkali. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Keller-Killiani test

One drop of 5 % ferric chloride solution was added to 2 ml of ethanolic extract and 3 ml of glacial acetic acid and this solution was transferred carefully to the surface of 2 ml of conc. H_2SO_4. Formation of reddish brown colour at the junction of the two liquid layers and bluish green colour appeared in upper layer which indicated the presence of cardiac glycosides.

Detection of anthroquinone glycosides

Modified Borntrager's test

Extracts were treated with ferric chloride solution and boiled on water bath for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of pink colour in the ammoniacal layer indicated the presence of anthranol glycosides.

Detection of saponins

Foam or frothing test

Few grams of extracts were diluted with 20 ml of distilled water and shaken in a graduated cylinder

for 15 min. Formation of 1 dm layer of foam indicated the presence of saponins.

Detection of steroids

Salkowski test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. H_2SO_4, shaken and allowed to stand. Formation of red in the chloroform layer and acid layer showed greenish yellow fluorescence which indicated the presence of steroids.

Libermann Burchard's test

About 1 mg of extract was dissolved in 1 ml of $CHCl_3$ and filtered. The filterate was treated with few drops of acetic anhydride then boiled and cooled. Conc. $H_2SO_4$was added from the side of the test tube. Formation of green colour indicated the presence of steroids.

Detection of tannins

Gelatin test

Extracts were treated with 1 % gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.

The crude extract or dry powder was treated with alcoholic $FeCl_3$ (Ferric Chloride) reagent and formation of blue colour indicated the presence of tannins.

Detection of flavonoids

Shinoda's test

Small quantity of extract was dissolved in 5 ml of ethanol (95 %) and treated with few drops of conc. HCl and 0.5 gm magnesium turnings. Presence of flavanoids was indicated by magenta colour.

Alkaline reagent test

Extracts were treated with few drops of NaOH. Formation of intense yellow colour, which became colourless on addition of dilute acid, indicated the presence of flavanoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavanoids.

Detection of amino acids

Ninhydrin test

To the extract, 0.25 % ninhydrin reagent was added and boiled for few min. Formation of blue colour indicated the presence of amino acids.

Detection of proteins

Biuret test

The extracts were treated with 1 ml of 10 % NaOH and heated. To this a drop of 0.7 % copper sulphate solution was added. Formation of purplish violet colour indicated the presence of proteins.

Millon's test

2 to 3 ml of extract was mixed with 5 ml of Millon's reagent and formation of white precipitate indicated the presence of proteins.

Xanthoprotein test

The extracts were treated with few drops of conc. HNO_3 solution. Formation of yellow colour indicated the presence of proteins.

Quantitative phytochemical studies

Flavonoids are considered as one of the most varied and prevalent group of natural compounds and probably they are one of the most important natural phenolics. From literature point of view, it has been recognized that many flavonoid compounds demonstrated a wide range of activities including antioxidant properties and their effects on human nutrition and health were also significant. Hence it was essential to calculate the total amounts of phenolics and flavonoids in specific extract.

QUANTIFICATION OF TOTAL PHENOLIC CONTENT

Preparation of gallic acid standard solution (1mg/ml)

10 ml of gallic acid was taken in a standard flask and mixed with 10 ml of methanol solution (1mg/ml). From this 20 ml of mixed solution, 2 ml was taken and then mixed with 18 ml of methanol (0.1mg/ml). From this 5, 4,3,2, 1 ml of solution was taken in separate test tubes and made up to 10 ml with methanol.

Determination of total phenolic content

1mg/ml of extract samples (*Eclipta alba* and *Lippia nodiflora*) were prepared and then 0.1 ml of sample, 1.9 ml distilled water and 0.1 ml of Folin Ciocalteau reagent were added in a tube, and then 1 ml of 20 % Na_2CO_3 was added. The reaction mixture was incubated at room temperature in dark for 2 hrs and the absorbance of the blue colour sample was recorded at 765 nm in UV visible

spectrophotometer. The blank consist of all reagents and solvents excluding sample.

The sample was tested in triplicate and a calibration curve for gallic acid was obtained. The results were compared to gallic acid calibration curve and the total phenolic content of extracts was expressed as µg of gallic acid equivalents (GAE) per mg of dry extracts.

QUANTIFICATION OF TOTAL FLAVONOID CONTENT

Preparation of quercetin standard solution

Ten mg of quercetin was taken in a standard flask and made up to 10 ml with methanol. From this 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.5 ml of the solutions were taken in separate test tubes and made up to 10 ml volume with methanol.

Determination of total flavanoid content

One mg/ml of extracts samples (*Eclipta alba* and *Lippia nodiflora*) were prepared and then extracts or standard solution (0.1 ml) were mixed with 4 ml distilled water and 0.3 ml of 5% $NaNO_2$. After 5 min, 0.3 ml of 10 % $AlCl_3$ was added. After 6 min, 2.0 ml of 1M NaOH was added to the mixture and total volume was made up to 10 ml with distilled water. Immediately the absorbance of the mixture was determined at 420 nm in UV-Visible spectrophotometer. The blank consist of all reagents except sample.

The sample was tested in triplicate and a calibration curve for quercetin was obtained. Total flavanoid content was expressed as µg quercetin equivalents (QE) per mg dry extract. Drug used was quercetin which was obtained from Sigma Aldrich, USA.

RESULT AND DISCUSSION

Preliminary Phytochemical Screening:

The extracts were subjected to preliminary phytochemical screening to find the chemical constituents present. The results of preliminary phytochemical investigation of petroleum ether and ethyl acetate fraction of ethanolic extracts of *Eclipta alba* and *Lippia nodiflora* are shown in Table 1.It found the presence of carbohydrates, amino acids, steroids, saponins and proteins in petroleum ether extract of *Eclipta alba* and alkaloids, carbohydrates, flavanoids, amino acids, cardiac glycosides, steroids, saponins, proteins and tannins in ethyl acetate fraction of ethanolic extract of *Eclipta alba.* Qualitative phytochemical screening revealed the presence of alkaloids, amino acids, steroids and proteins in petroleum ether extract of *Lippia nodiflora* and alkaloids, carbohydrates, flavanoids, amino acids, saponins, proteins and tannins in ethyl acetate fraction of ethanolic extract of *Lippia nodiflora.* Alkaloids were present in ethyl aceatate fraction of ethanolic extracts of *Eclipta alba* and *Lippia nodiflora.* Amino acids, flavonoids, cardiac glycosides, steroids, saponins, proteins and tannins were present and carbohydrates and anthraquinone glycosides were absent in ethyl acetate fraction of ethanolic extracts of *E. alba*. Carbohydrates, flavonoids, saponins and tannins were present in ethyl acetate fraction of ethanolic extracts of *L. nodiflora* and absent in petroleum ether extracts of *L. nodiflora.* Cardiac glycosides and anthraquinone glycosides were present in ethyl acetate fraction of ethanolic extracts of *E. alba* and *L. nodiflora* and absent in petroleum ether extract.

Quantitative phytochemical studies

The total phenolic content and total flavanoid contents of the hexane, chloroform, ethyl acetate and ethanol extracts of *Eclipta alba* and *Lippia nodiflora* are shown in Table No.2. The different extracts of whole plant of *E. alba* and *L. Nodiflora* were evaluated for the detection of its total phenolic and total flavanoidal content. Measurement of total phenolic content by Folin-Ciocalteu assay in hexane, chloroform, ethyl acetate and ethanolic extract of *Eclipta alba* was found to be 0.0, 6.02 ± 0.18, 12.23 ± 0.05 and 6.05 ± 0.15 mE GAE/mg of the extract respectively and in hexane, chloroform, ethyl acetate and ethanolic extract of *Lippia nodiflora* was found to be 5.82 ± 0.09, 6.10 ± 0.08, 15.77 ± 0.12 and 13.71 ± 0.05 mE GAE/mg of the extract respectively [with the equation $y= 0.025x + 0.0580$ ($r^2 = 0.9952$)].

Flavanoid contentin hexane, chloroform, ethyl acetate and ethanolic extract of *Eclipta alba* was found to be 2.85 ± 0.11, 4.3 ± 0.063, 5.5 ± 0.051 and 6.15 ± 0.058 mEQuercetin/mg of the extract respectively and in hexane, chloroform, ethyl

acetate and ethanolic extract of *Lippia nodiflora* was found to be 3.89 ± 0.032, 4.15 ± 0.15, 7.09 ± 0.032 and 5.05 ± 0.15 mEQuercetin/mg of the extract respectively [with the equation y= 0.0391x + 0.040 (r^2 = 0.9830)].

The *E. alba* and *L. nodiflora* plant extracts were tested in triplicate and a calibration curve for gallic acid and quercetin are showed in Figure No.1 and 2. The absorbance of gallic acid and quercetin are showed in Table No.2 and 3. The total phenolic content of extracts was expressed as µg of equivalents (GAE) per mg of dry extracts and the total flavanoid content was expressed as µg quercetin equivalents (QE) per mg dry extract and values are shown in Table No.4.

Table No.1: Qualitative Phytochemical screening of *E. alba* and *L. nodiflora* extracts

S.No	Plant constituents	*E. alba*		*L. nodiflora*	
		Petroleumether extract	**Ethyl acetate fraction of ethanolic extract**	**Petroleumether extract**	**Ethyl acetate fraction of ethanolic extract**
1	Alkaloids	-	+	+	+
2	Carbohydrates	-	+	-	+
3	Flavonoids	-	+	-	+
4	Amino acids	+	+	+	+
5	Glycosides 1. Cardiac glycosides 2. Anthraquinoneglycosides	- -	+ -	- -	- -
6	Steroids	+	+	+	-
7	Saponins	+	+	-	+
8	Proteins	+	+	+	+
9	Tannins	-	+	-	+

\+ = Showed colour reaction

\- = Did not showed colour reaction

Table No.2: Absorbance value of gallic acid (765 nm)

S.No	Concentration (µg/ml)	Absorbance of gallic acid (765 nm)
1	2	0.11
2	4	0.26
3	6	0.34
4	8	0.42
5	10	0.50
6	12	0.59
7	14	0.62
8	16	0.76
9	18	0.81
10	20	0.95
y =0.025x+0.0580		r^2=0.9952

Table No.3: Absorbance value of quercetin (420 nm)

S.No	Concentration (µg/ml)	Absorbance of quercetin (420nm)
1	1	0.09
2	2	0.12
3	3	0.15
4	4	0.18
5	5	0.21
6	6	0.25
7	7	0.29
8	8	0.33
9	9	0.37
10	10	0.41
y =0.0391x+0.040		r^2=0.9830

Table No.4: Total phenolic and flavonoid content

S.No	Name of the extract	Total phenolic content(mE GA/mg dry extract)	Total flavonoid content (mE quercetin/mg dry extract)
Eclipta alba Hassk			
1	Hexane	-	2.85 ± 0.11
2	Chloroform	6.02 ± 0.18	4.3 ± 0.063
3	Ethyl acetate	12.23 ± 0.05	5.5±0.051
4	Ethanol	6.05 ± 0.15	6.15 ± 0.058
***Lippia nodiflora* Linn**			
1	Hexane	5.82 ± 0.09	3.89 ±0.032
2	Chloroform	6.10 ± 0.08	4.15 ± 0.15
3	Ethyl acetate	15.77 ± 0.12	7.09±0.032
4	Ethanol	13.72 ± 0.05	5.05 ± 0.15

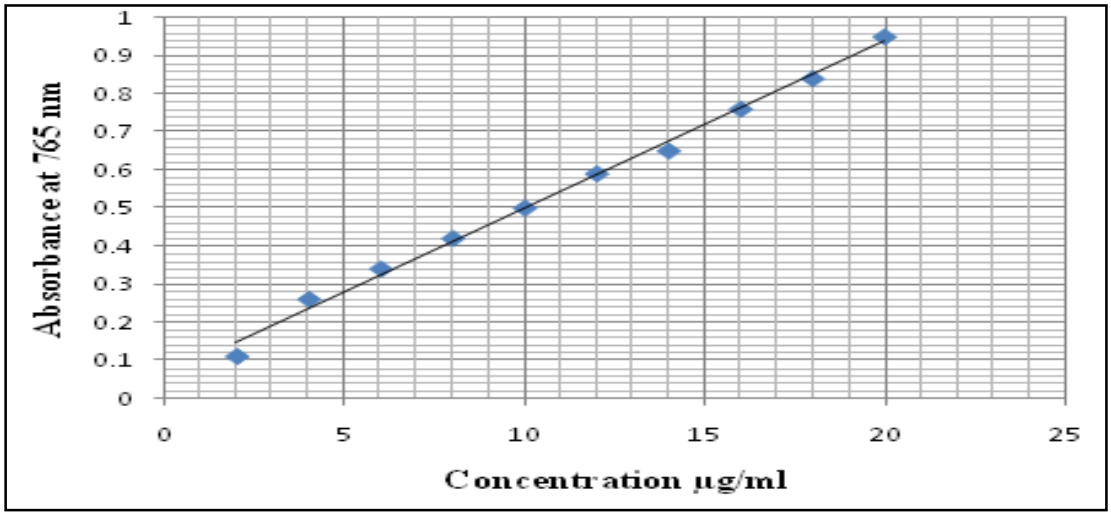

Figure No.1: Calibration curve of gallic acid

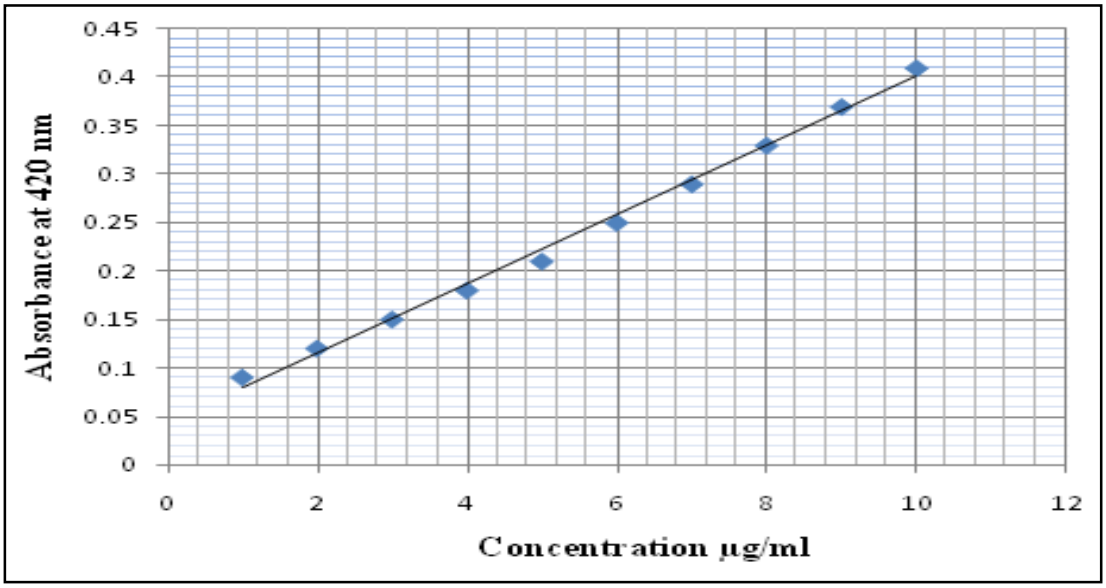

Figure No.2: Calibration curve of quercetin

CONCLUSION

Phytochemicals study have been received increasing attention because of interesting new discoveries considering their biological activities especially polyphenols and the present study confirms that the plant contains many bioactive compounds like flavonoids, tannins, terpenoids and carbohydrates etc. The study also showed that the two plants contain a large quantity of phenols and flavonoids. Flavonoids present in plants exhibit a variety of beneficial effects on human health. The plant *Eclipta alba* and *Lippia nodiflora* contain phytoconstituents like alkaloids, Flavanoids, terpenoids, saponins and tannins and this study could serve as a constructive reference to allow further *in vivo* analysis which can be carry out to evaluate the extent of protective effects of *Eclipta alba* and *Lippia nodiflora.*

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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