

Antioxidant potential of *Eclipta alba*, a traditional medicinal herb attenuates oxidative DNA damage in vitro

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Abstract. Purushothaman A, Ganesh A, Meenatchi P, Sundaram R, Venkataramanan. 2020. Antioxidant potential of *Eclipta alba*, a traditional medicinal herb attenuates oxidative DNA damage in vitro. *Nusantara Bioscience* 12: 73-78. The plant *Eclipta alba* (L.) Hassk. is an important plant used in the traditional Ayurvedic, Unani systems of holistic health and herbal medicine of the East. This study aimed to evaluate the antioxidant and DNA damage protection activities of ethanolic extract of *E. alba*. Quantitative analysis of total phenolic content (TPC) and identification of bioactive components using Gas Chromatography-Mass Spectroscopy (GC-MS) was performed to provide scientific basis for traditional usage of this plant. To investigate the antioxidant potential, extracts were tested for their capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH·), hydrogen peroxide (H₂O₂) and Superoxide radicals (O₂^{•-}). DNA damage protective activity of ethanol extract of *E. alba* was checked on pBluescript M13+ plasmid DNA. The Plasmid DNA was oxidized with H₂O₂ + UV treatment in the absence and presence of different concentrations of *E. alba* extract (75, 150, and 300 µg/mL). Electrophoresis was performed using 1% agarose at 40 V for 3 h in the presence of ethidium bromide. Gel was scanned on a Gel documentation system. Bands on the gels corresponding to supercoiled circular, circular relaxed, and linearized DNA were quantified. The results of preliminary phytochemical screening of *E. alba* extract showed the presence of flavonoids, saponins, steroids, terpenoids, and tannins. The extract was found to have rich phenolics content of 26.38 ± 2.45 milligram of gallic acid equivalents (mg GAE/g). The extract exhibited excellent antioxidant activities. GC-MS analysis of the extract confirmed the presence of major active principles. Furthermore, the extract significantly inhibited DNA damage induced by reactive oxygen species (ROS). Altogether, the results of current study revealed that *E. alba* is a potential source of antioxidants and provides pharmacological credibility to the ethnomedicinal use of this plant in traditional system of medicine, also justifying its therapeutic application in oxidative damage induced diseases such as cancer, diabetes, and neurological disorders.

Keywords: DNA damage, *Eclipta alba*, free radicals, natural antioxidants, Reactive Oxygen Species, ROS

INTRODUCTION

Drugs of plant origin play a significant role in the public health care system of every nation (WHO 2019). Complementary and Alternative Medicine (CAM) systems *viz.* Siddha, Ayurveda, Kaempo, Unani, Folk, Sowa Rigpa, and Chinese medicines have gained their attractiveness in recent years (Golla et al. 2011). The demand for herbal medicines is bigger than ever because of their safety, efficacy, fewer side effects, and good belief of society in herbal medicines and their products (Martins Ekor 2013). Medicinal plants are significant source of natural antioxidants and herbal drugs have been used for the treatment or prevention of diseases and for the promotion of good health since ancient times (Sofowora et al. 2013). Plants are the main source of remedies for the world during 1770s as there were only a few or no chemically synthesized drugs available (Duke 1990). For instance, *Andrographis paniculata* (Burm. f.) Nees (Family Acanthaceae) was the only known remedy against malarial fevers until the discovery of quinine from *Cinchona* trees (Sanjutha et al. 2008). Many of the drug molecules in

modern pharmacology are derived from plant sources (Veeresham 2012). Some medicinal plants have noteworthy potential as source of natural antioxidants and are used to safeguard against disorders related to oxidative stress (Ting et al. 2011). However, increasing needs for natural antioxidants have provoked great importance for the discovery of potent antioxidants from plant sources (Khodaie et al. 2012). Certain plants with excellent free radical scavenging capacity proved to have a protective effect on H₂O₂-induced cytotoxicity and DNA damage (Russo et al. 2001).

Eclipta alba (L.) commonly referred to as False Daisy is a medicinal plant belonging to the family Asteraceae. The plant *E. alba* is a pantropical species found in Indonesia, Sri Lanka, India, Philippines, Nepal, Laos, Kampuchea, Pakistan, Thailand, Vietnam, Malaysia, Myanmar, and also in Brazil and the United States. It has been declared as an endangered plant by the United State's Department of Agriculture (Soni and Soni 2017). The ethnobotanical and ethnomedicinal survey of *E. alba* reported that the plant is used in the treatment of a variety of ailments in traditional and folk medicine in many

tropical and sub-tropical countries (Jahan et al. 2014). It is also an important medicinal herb found in traditional medicine of the Eastern countries and the Indian Materia Medica that includes about 2000 drugs of natural origin. (Satheesh Naik et al. 2019). According to Saint Vallalaar, *Eclipta* is the number one herb in hierarchy and has been reported to possess hepatoprotective/anti-hepatotoxic, antimicrobial, anti-inflammatory, anti-diabetic activity, anti-cancer, anti-malarial, and antioxidant properties and promote hair follicle growth (Soni and Soni 2017).

Oxidative damage by reactive oxygen species (ROS) to cellular macromolecules (nucleic acids, lipids, and proteins) is implicated in the development of many diseases, including neurodegenerative disorders, cancer, liver cirrhosis, cardiovascular diseases, atherosclerosis, cataracts, diabetes, and inflammation (Aruoma 1998). Emerging research evidence has suggested that antioxidants can control the auto-oxidation by interrupting the propagation of free radicals or by inhibiting the formation of free radicals and subsequently reduce oxidative stress, improve immune function, and increase healthy longevity (Kirakosyan et al. 2003; Tan et al. 2018). It has been reported that the medicinal plants containing a wide variety of natural antioxidants, such as phenolic acids, flavonoids are of great value in preventing the onset and/or progression of many human diseases (Halliwell et al. 1992) including oxidative DNA damage.

So far, there are no reports related to the protective effect of *E. alba* against free radicals and oxidative DNA damage. The present study aimed to analyze the phytochemical components of ethanol extract of *E. alba* and to determine its protective effect against oxidative DNA damage induced by UV-photolysis of H₂O₂.

MATERIALS AND METHODS

Plant samples and extraction

Eclipta alba was collected from a local garden in Puducherry, Tamil Nadu, India. The plant was identified and authenticated by IMPCOPS (Indian Medical Practitioner's Co-operative Pharmacy and Stores Ltd.), Thiruvanniyur, Chennai-600-041, India. The dried leaves were pulverized, using a sterile electric blender, to obtain a powdered form. The powdered form was stored in airtight glass containers, protected from sunlight until required for analysis. Powdered plant material (50 g) was extracted three times with 500 mL of 80% ethanol (EtOH)/H₂O while being macerated at room temperature for 24 h each time. The extracts were filtered under vacuum using Buchner funnel lined up with Whatman No.1 filter paper. The solvent was eliminated under vacuum using a rotary evaporator at 40 °C. The extracts were used for analysis of phytochemical components, total phenolic content, GC-MS identification of bioactive compounds, antioxidant, and protective effect of *E. alba* extract against oxidative DNA damage.

Phytochemical screening and total phenolic content

Preliminary phytochemical tests were performed on the ethanol extract using standard procedures to identify the components as described Edeoga et al. (2005). The total phenolic contents were determined using Folin-Ciocalteu reagent as described by Singleton et al. (1999) with slight modifications. The extract (200 µL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and allowed to stand at room temperature for 5 min. 1.5 mL sodium bicarbonate solution (60 g/L) was added to the mixture and after incubation for 90 min at room temperature, the absorbance level was measured at 750 nm using a UV-Visible spectrophotometer (SICAN 2310/Incarp, Japan). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions. The results were calculated as gallic acid equivalent (GAE) per gram extract and reported as mean value ± SD.

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the extract, as well as positive control butylated hydroxyanisole (BHA) was measured using the method of Koleva et al. (2002) with slight modifications. Briefly, 2.0 mL of methanol solution of DPPH (2.0×10⁻⁴ mol/l) was mixed with equivalent aliquot of different concentrations of samples (10, 20, 40, 60 and 80 µg /mL). The tubes were incubated for 30 min at 37°C. The solvent alone was considered "blank." The decrease in absorbance of test mixtures (due to quenching of DPPH free radicals) was determined at 517 nm using UV-Visible spectrophotometer; Butylated hydroxyanisole (BHA) was used as the positive control. The inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{A}_{517} \text{ of control} - \text{A}_{517} \text{ of Sample})}{\text{A}_{517} \text{ of control}} \times 100$$

Assay of H₂O₂ scavenging activity

The ability of ethanolic extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (0.1M, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Samples in different concentrations (10, 20, 40, 60 and 80 µg/mL) were added to a hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide was calculated using the formula:

$$\% \text{ Scavenged H}_2\text{O}_2 = ([\text{Ac} - \text{As}] / \text{Ac}) \times 100$$

Where; Ac is Absorbance of the control, and As is absorbance of the sample or standard (BHA)

Assay of superoxide radical-scavenging activity

Measurement of superoxide anion-scavenging activity of the extracts was based on the method described by Liu et al. (1997) with slight modification. The reaction mixture consisted of 1.0 mL of NBT (156 mM in 100 mM potassium phosphate buffer pH 7.4), 1.0 mL of NADH (468 mM in 100 mM potassium phosphate buffer pH 7.4) and 0.5 mL of an appropriately diluted sample (50-250 µg/mL). The reaction was initiated by addition of 100 µL of phenazine meta sulfate (PMS) (60 mM in 100 mM potassium phosphate buffer pH 7.4) to the mixture. The tubes were incubated at ambient temperature for 5 min and the absorbance was measured at 560 nm. L-ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = ([Ac - As] / Ac) \times 100$$

Where; Ac is absorbance of the control, and As is absorbance of the sample or standard (BHA)

Protective effect on DNA damage

DNA damage protective activities of ethanol extract of *E. alba* was checked on p Bluescript M13+ plasmid DNA. Plasmid DNA was oxidized with H₂O₂ + UV treatment in the presence of different concentrations of *E. alba* extract and checked on 1% agarose according to Attaguile et al. (2000) In brief, the experiments were performed in a volume of 10 in a microfuge tube containing 200 ng of plasmid DNA in phosphate buffer (7.14 mmol phosphate and 14.29 mmol NaCl), pH 7.4, H₂O₂ was added at a final concentration of 2.5 mmol/L with and without 1 µL of (75, 150 and 300, µg/mL) ethanol extract of *E. alba*. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator at 300 nm under room temperature. After irradiation, the reaction mixture (10 µL) with gel loading dye was placed on 1% agarose gel for electrophoresis. Electrophoresis was performed at 40 V for 3 h in the presence of ethidium bromide (10 mg/mL). Untreated pBluescript M13+ plasmid DNA was used as a control in each run of gel electrophoresis along with partial treatment (i.e. only UV treatment and only H₂O₂). Percent inhibition of the DNA strand scission was calculated as follows:

$$\text{Inhibition (\%)} = I - [(Sm + a - Sc) / (Sm - Sc)]$$

Where; Sm+a is the percentage remaining supercoiled after treatment with mix plus agent, Sc is the percentage remaining supercoiled in control untreated plasmid and Sm is the percentage remaining supercoiled with mix without agent (Fukuhara and Miyata 1998). Gel was scanned on a Gel documentation system (Gel-Doc-XR; BioRad, Hercules, CA, USA). Bands on the gels were quantified discovery series Quantity One program (BioRad, USA).

Data analysis

Data are presented as the mean ± standard deviation (SD). Significant differences among the groups were

determined by one-way analysis of variance (ANOVA) using SPSS 16.0 software package program.

RESULTS AND DISCUSSION

Phytochemical screening and TPC

The results of preliminary phytochemical screening of *E. alba* (Table 1) revealed the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids, and steroids. The total phenolic content (TPC) of *E. alba* extract was found to be 26.38 ± 2.45 mg gallic acid equivalent (GAE) per gram plant extract (Table 2).

Phytochemical screening of plants is of paramount importance in identifying new sources of active compounds having medicinal significance and benefit from the utilization of available natural sources (Mungole Arvind et al. 2010). In the present study, phytochemical screening of the *E. alba* revealed that the plant is rich in tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids, and steroids. The phenolic compounds are one of the largest and the most ubiquitous plant metabolites, which attracted great attention in relation to their potential for beneficial health effects. The total phenolic content of *E. alba* was found to be 26.38 ± 2.45 mg Gallic acid equivalent/g of extract. The high amount of total phenolic content in the extract justified its high antioxidant activities. Therefore, the antioxidant activity observed in the present study may be due to the presence of rich phenolic compounds in the plant extract.

GC-MS identification of bioactive components

The ethanol extract of *E. alba* contained rich phytochemical components which in turn resulted in the identification of fifteen different compounds by GC-MS analysis. The prevailing bioactive components (peak area greater than or equal to 2%) with their Retention time (RT) are presented in Table 3 and Figure 1.

Table 1. Qualitative analysis of phytochemical components of *Eclipta alba*

Phytochemical components	Present/absent
Tannins	+
Saponins	+
Flavonoids	+
Terpenoids	+
Cardiac glycosides	+
Alkaloids	+
Steroids	+

Note: + indicates presence

Table 2. Yield of ethanolic extract and total phenolic contents in ethanolic extract of *Eclipta alba* leaves

Yield of ethanol extract (%)	Total phenolic contents (TPC) (mg gallic acid equivalent /g of extract)*
14.47 ± 2.36	26.38 ± 2.45

Note: *Values are expressed as mean ± standard deviation

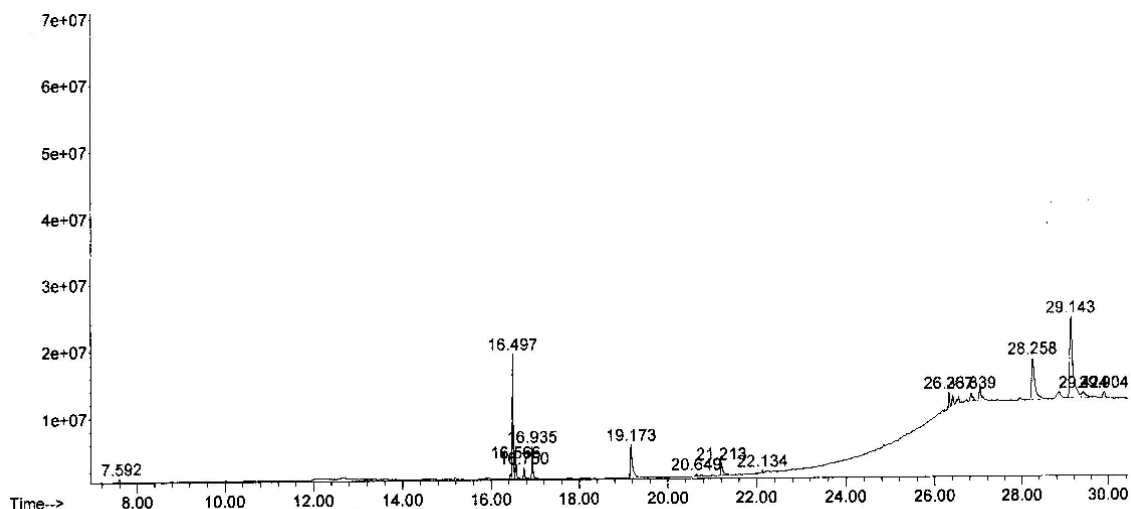


Figure 1. A representative GC-MS chromatogram of ethanol extract of *Eclipta alba*

Table 3. The prevailing bioactive components with their Retention time (RT), Peak area (%), and name of the compound

Retention time	Peak area %	Name of the compound
16.492	15.55	Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-Bicyclo[10.8.0]eicosane, (E)-
16.564	3.06	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-cyclohexane, 1,2,3-trimethyl-trans-1-Butyl-2-methylcyclopropane
16.753	2.11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
16.942	5.08	1,13-Tetradecadiene
19.178	8.71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
21.212	3.58	2-cyclohexen-1-one, 4,4-dimethyl-cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-Phytol
26.832	2.30	Benzo[b]naphtho[2,3-d]thiophene, 6,8-dimethyl-7-Methyl-5-oxo-2-p-tolyl-3,5-dihydroindolizine-6-carbonitrile
28.255	17.89	2H-1,3-benzoxazine, octahydro-2-(4-nitrophenyl)-, cis-Methyltris (trimethylsiloxy)silane
29.141	32.71	Hydrocinnamic acid, benzyldimethylsilyl ester
29.432	3.36	4-Methyl-2-trimethylsilyloxy-acetophenone
		1,4-Bis (trimethylsilyl)benzene
		1,4-Benzenediol, 2,5-bis (1,1-dimethylethyl)-Hydrocinnamic acid, benzyldimethylsilyl ester
		1-Ethynyl-3,5-dimethyladamantane-2-Trifluoromethylbenzoic acid, 2-methyloct-5-yn-4-yl ester
		Diallylphenylvinylsilane
		5-Methyl-2-trimethylsilyloxy-acetophenone
		Methyltris (trimethylsiloxy)silane cyclotrisiloxane, hexamethyl-

Antioxidant and free radical scavenging activities of *Eclipta alba*

The DPPH free radical scavenging model can be used to evaluate the antioxidant activity in a relatively short time. The extract exhibited DPPH, hydrogen peroxide, and superoxide radical scavenging in a concentration-dependent manner (Table 4). The extract, which contained a considerable amount of total phenolics and flavonoids, showed a significant effect in inhibiting DPPH, reaching $68.5 \pm 4.3\%$ at a concentration of $80 \mu\text{g/mL}$. The IC_{50} value (the amount of extract/ antioxidant material required to scavenge 50% of free radical in the assay system) for DPPH was found to be $46.19 \pm 3.5 \mu\text{g/mL}$. Similarly, the extract exhibited significant hydrogen peroxide and

superoxide radical scavenging activities of $58.9 \pm 4.98\%$ and $64.2 \pm 4.9\%$, respectively at a concentration of $80 \mu\text{g/mL}$. The DPPH radical, H_2O_2 , and superoxide radical scavenging activities of *E. alba* indicates the strong antioxidant capacity of its components that can maintain sound health and protect the human body from oxidative damage.

Protective effect of *Eclipta alba* on DNA damage

Protective effect of *E. alba* on oxidative DNA damage was analyzed using on pBluescript M13+ vector (Figure 2.A-B). Figures 2.A and 2.B show the electrophoretic pattern and quantified band intensities of DNA after UV-photolysis of H_2O_2 in the absence and presence ethanol

extract of *E. alba*. DNA derived from pBluescript M13+ plasmid showed two bands on agarose gel electrophoresis (lane 1); the faster-moving band corresponded to the native form of supercoiled circular DNA (96.74% of sc DNA) and the slower moving band was the circular relaxed form (3.36% oc DNA). EcoRI digested pBluescript M13 (+) DNA showed a major band of linearized DNA (97.85% Lin DNA) and a very small proportion of Sc DNA (2.15%) in lane 2. The UV irradiation of DNA in the presence of H₂O₂ (lane 3) resulted in the cleavage of sc DNA into linear form (Lin DNA). This indicated that the hydroxy radical (OH[•]) generated from UV photolysis of H₂O₂ produced DNA strand scission, which resulted in the conversion of supercoiled circular DNA to linearized DNA. The addition of different concentrations of *E. alba* extract (in Figures 2.A and 2.B the lanes 4-6) to the reaction mixture suppressed the formation of linear DNA and induced a partial recovery of sc DNA. The percentage of Sc DNA retained on treatment with *E. alba* extract was found to be 71.4 ± 2.62%, 82 ± 3.16%, and 89.63 ± 3.35%, at the concentrations of 75, 150 and 300 µg/mL, respectively.

ROS at low or moderate concentrations exert beneficial effects on cellular responses but at high levels, free radicals can damage biologically important macromolecules including DNA, proteins, and membrane lipids (Ebrahimzadeh et al. 2010). The mechanism for DNA damage, leading to mutation, is explained by the attack of

ROS on modification at DNA bases, strand break, DNA protein cross-link, and base-free site therefore oxidative damage of DNA is regarded as the etiology of number of diseases (Zhao and Liu 2009; Saenjum et al. 2010). In the present study, DNA damage inhibition potential of *E. alba* was evaluated using pBluescript M13 (+) DNA. UV-photolysis of H₂O₂ generates hydroxyl radicals, which is responsible for DNA oxidative damage observed in the current study. Earlier studies by Guha et al. (2011), have reported that the binding of hydroxyl radicals to DNA leads to strand breakage and opening, deoxyribose sugar fragmentation, and nitrogenous base modification. The attack of DNA by OH radical opens up the circular structure and the DNA strand becomes heavier. The oxidative DNA damage is considered as the most important mechanism in the etiology of cancer, neurological disorders, and metabolic diseases such as hypertension, diabetes, etc. Our observation corroborates with the earlier studies of Reddy et al. (2005), who reported that amla (*Embllica officinalis*), drumstick leaves (*Moringa oleifera*), and raisins (*Vitis vinifera*) are more effective in controlling oxidative damage as they possess rich sources of natural antioxidants. From the results of this study, it can be inferred that the plant *E. alba* has immense potential as a natural antioxidant and free radical scavenger and may be valuable in treating diseases of oxidative stress.

Table 4. DPPH, hydrogen peroxide, and superoxide radical scavenging activities of *Eclipta alba* extract in comparison with butylated hydroxyanisole (BHA, positive standard).

Concentration (µg/mL)	% Inhibition		% Scavenged			
	DPPH		Hydrogen peroxide		Superoxide	
	Extract	BHA	Extract	BHA	Extract	BHA
10	14.3 ± 0.86	27.4 ± 1.2	14.8 ± 1.73	38.9 ± 2.7	16.7 ± 3.2	31.8 ± 2.6
20	35.6 ± 1.8	46.7 ± 2.6	28.4 ± 2.48	56.7 ± 4.5	29.6 ± 3.9	56.4 ± 5.4
40	47.2 ± 3.4	58.9 ± 3.5	32.7 ± 3.11	69.6 ± 5.2	42.3 ± 4.8	61.8 ± 4.5
60	56.8 ± 4.2	70.2 ± 4.2	49.6 ± 4.76	81.4 ± 4.9	53.4 ± 5.7	77.3 ± 4.8
80	68.5 ± 4.3	91.6 ± 3.7	58.9 ± 4.98	98.2 ± 4.6	64.2 ± 4.9	86.2 ± 3.8

Note: BHA was used as a reference antioxidant. Values are expressed as mean ± standard deviation of three independent experiments.

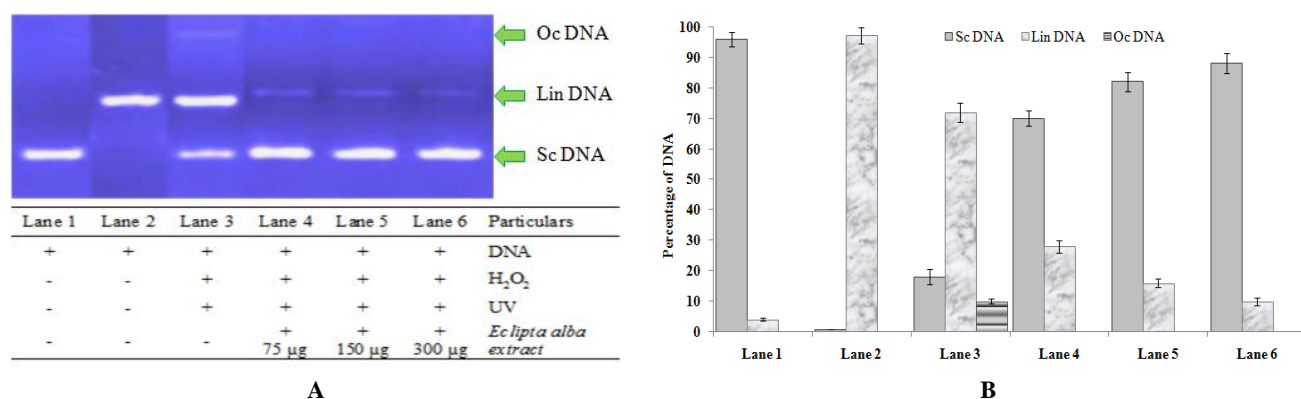


Figure 2.A. The electrophoretic pattern of DNA after UV-photolysis of H₂O₂ in the absence and presence of different concentrations of *Eclipta alba* Extract. **B.** Quantified band intensity for the Sc-DNA, Lin DNA and Oc-DNA in different lanes. Lane 1: Control DNA, Lane 2: Linearized pBluescript M13 (+) DNA (EcoRI digest), Lane 3: DNA + H₂O₂ (2.5 mM) + UV, Lane 4: DNA + 75 µg/mL of EaE+ H₂O₂ (2.5 mM) + UV, Lane 5: DNA + 150 µg/mL of EaE+ H₂O₂ (2.5 mM) + UV, Lane 6: DNA + 300 µg/mL of EaE+ H₂O₂ (2.5 mM) + UV

In conclusion, this study is the first to report the DNA damage protective effect of *E. alba* in a comprehensive manner employing a range of in vitro assays. The results of the present study confirm that the ethanolic extract of *E. alba* possesses DNA protective properties and antioxidant activity evidenced by an excellent in vitro DPPH, H₂O₂, and superoxide radical scavenging activities. This work has gathered experimental evidence on the commonly used *E. alba* as natural antioxidant for its capacity to protect organisms and cells from oxidative DNA damage associated with aging, cancer, and degenerative diseases. Thus, *E. alba* may serve as an ideal candidate for a cost-effective, readily utilizable natural polyphenolic phytochemicals. However, further research is under progress in our laboratory to identify individual components forming the antioxidative system and their detailed mechanism to develop their application for food and pharmaceutical industries.

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