CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.7215496

Available online at: http://www.iajps.com *Research Article*

FREE RADICAL SCAVENGING PROPERTIES OF COLEUS VETTEVEROIDES LEAF EXTRACTS AND DNA PROTECTION AGAINST HYDROGEN PEROXIDE RADICAL DAMAGE: A PRELIMINARY ANALYSIS OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS

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Article Received: September 2022 **Accepted:** September 2022 **Published: October** 2022

Abstract:

Background*: For thousands of years, nature provides medicinal agents, and an incredible number of therapeutic drugs have been derived from natural sources, many based on their usage in traditional medicine.* ***Objective:*** *The goal of this study was to examine the antioxidant properties of Coleus vetteveroides leaves.* ***Methodology****: Enzymic antioxidants-POD, CAT, and GST, Non-enzymic antioxidants- ascorbic acid, flavonoids, tocopherol, chlorophyll, phenols, carotenoids, and lycopene. The free radical scavenging effects and chelating properties were observed by extracting the leaves with three different solvents (Hexane, Ethylacetate, and Isoproponol). Agarose gel electrophoresis was used to test the DNA protective activity of various leaf extracts against radicals.* ***Result:*** *The plant leaves exhibited strong antioxidant and radical scavenging properties.* ***Conclusion:*** *The leaves of Coleus vetteveroides have a potential content of both enzymatic and non-enzymatic antioxidants that could protect against oxidants and free radical damage. The phytochemical screening revealed the presence of flavonoids, alkaloids, and phenols.*

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Please cite this article in *Kiruthika Balasubramanian et al,* ***Free Radical Scavenging Properties Of Coleus vetteveroides Leaf Extracts And DNA Protection Against Hydrogen Peroxide Radical Damage: A Preliminary Analysis Of Enzymatic And Non-Enzymatic Antioxidants., Indo Am. J. P. Sci, 2022; 09(10).***

INTRODUCTION:

The demand for medicinal plants has grown exponentially as a result of their significance and has been used to treat illnesses from the earliest times. India is well known as the "EMPORIUM OF MEDICINAL PLANTS"[1-3]. Unlike humans plants and other animals, lack a distinct immune system. Plants use a mix of chemicals to protect themselves from predators, and parasites attack plants with similar pathways that attack humans. As a result, substances used by plants to defend themselves may be useful to humans in the treatment of diseases. Popular perceptions of medicinal plant use and efficacy play an important role in the revelation of their therapeutic capabilities, thus they are regularly prescribed, even if their chemical ingredients are not always fully understood [4-7].

Antioxidants are also referred to as phytochemicals, however, not all phytochemicals are antioxidants. Phytochemical consumption has been linked to a variety of health advantages. Non-infectious disorders such as cardiovascular disease, cancer, and diabetes are leading causes of death, however, phytochemicals in the diet may help to reduce the risk of these conditions. [8-9].

A single free radical can harm millions of cells in the human body, preventing it from functioning properly [*10*]. Antioxidants are essential chemicals that protect the body from damage caused by oxidative stress generated by free radicals [11]. Many enzyme systems in the human body produce reactive oxygen species (ROS) such as hydroxyl radicals, and hydrogen peroxide as a result of oxygen consumption [12]. These ROS are useful as signal transducers and growth regulators at modest levels [13]. A large range of plants has been shown to have potent antioxidant and free radical scavenging properties all around the world [14]. Antimicrobial activity testing of phytochemicals has revealed that higher plants could be a source of novel antibiotic prototypes [15].

The purpose of this study was to look into the antioxidant capabilities, and free radical scavenging activity of *Coelus vetteveroides* leaves. It is a small and succulent herb, with pubescent leaves and fibrous roots, which are strongly aromatic. The plant's leaves can be utilized in a variety of ways, including juice mixed with honey to alleviate stomach distress and crushed leaves to treat headaches [16].

MATERIALS AND METHODS:

The goal of the study was to analyze the antioxidant levels in the *Coleus vetteveroides* leaves. For Enzymatic, Non-enzymatic, Radical Scavenging, Chelating, and Phytochemical screening, leaves were collected from the University of Agricultural Science, GKVK, Karnataka.

For each parameter, fresh leaves were procured, washed in running water to remove any surface contaminants, and blotted dry between the folds of soft tissue papers.

Enzymatic Antioxidants

The enzymatic antioxidants analyzed were Catalase, Peroxidase, and Glutathione S-transferase. Catalase was analyzed by the method of Luck (1974)[17], Peroxidase by the method of Reddy et al. (1985)[18], and Glutathione-S-Transferase by Habig et al. (1974)[19] methods proposed respectively.

Non-Enzymatic antioxidants

Ascorbic acid, Tocopherol, Total Carotenoids and Lycopene, Total Phenols, Flavonoids, Reduced Glutathione, and Chlorophyll were analyzed using the spectrophotometric methods of Roe and Keuther (1943)[20], Rosenberg (1992)[21], Zakaria *et al.* (1979)[22], Mallick and Singh (1980)[23], Cameron *et al.* (1943)[24], Moron *et al.* (1979)[25] and Witham et al. (1971)[26] respectively.

Radical Scavenging effects

The free radical scavenging effects of the leaves were analyzed against Hydrogen peroxide and Hydroxyl radicals

Preparation of plant extract:

Fresh *Coleus vetteveroides* leaves were collected, washed in water to remove surface pollutants, and air-dried for 10 days in the shade. The dried leaves were ground into a fine powder, which was then used to make three distinct solvent extracts using a sequential extraction process.

Sequential extraction/Successive extraction

A shaker incubator was used to dissolve 10g of powder in 100ml of hexane for 24 hours. The solvent was then filtered, and the resulting filtrate was used to create the hexane extract, which was then stored in an airtight container. The residue was air-dried, weighed, and dissolved in 100mL Ethyl acetate solvent before being incubated in a shaker for 24 hours. Ethyl acetate extract was created by filtering the solvent and storing it. The leftover residue was air-dried and weighed before being dissolved in Isopropanol solvent and the same procedure was repeated. After drying all three solvent extracts (hexane, ethyl acetate, and isopropanol), 100mg was utilized for free radical scavenging and phytochemical assays.

The H_2O_2 scavenging effect of the leaf extracts was estimated according to the method of Ruch *et al.* (1989)[27]. The scavenging capacity for hydroxyl radical was measured according to the method of Elizabeth and Rao (1990)[28] and the chelating property of *Coleus vetteveroides* leaf extracts was determined based on the method of Brown *et al.* (1998)[29].

Agarose gel electrophoresis for assessing the DNA protective effect of *Coleus vetteveroides :*
Sample preparation

The procedure of Marmur (1961)[30] was used to isolate goat liver DNA: 2 g liver tissue was weighed and homogenized in 8 ml SSC solution. The pellet was separated by centrifuging the suspensions at 300 rpm for 10 minutes. The pellet was re-centrifuged at 10,000rpm for 10 minutes after adding 4ml of 0.2M NaCl. Cold ethanol was added to the supernatant twice its capacity. The precipitated DNA pellet was dissolved in TE buffer and used as part of the experiment.

1% agarose gel was prepared, and samples were loaded in the wells by mixing 2ul of 6x gel loading dye with 15ul of control DNA. 15 ul of different plant extracts were combined with 15ul of DNA and loaded in individual wells with 6x gel loading dye. DNA was damaged by adding hydrogen peroxide to generate free radicals, which were then loaded into the appropriate wells. The DNA was treated with extracts and hydrogen peroxide before being loaded into the migration pattern well. 100 volts were used for the gel electrophoresis.

Preliminary phytochemical screening of *Coleus vetteveroides* leaves
The extracts of *Coleus vetteveroides* leaves were tested for the presence of various phytochemicals using three different tests (A) Mayer's test: An aliquot of the extract was treated with Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml of distilled water) and were observed for the formation of cream coloured precipitate. (B) Dragendroff's test: A fraction of the extract was treated with Dragendroff's reagent and observed for the formation of a reddish-orange coloured precipitate. (C) Wagner's test: A small fraction of the extract was treated with Wagner's reagent (1.27g of iodine and 2g of KI in 100ml of distilled water) and observed for the formation of a reddish-brown coloured precipitate.

Phenolics detection was carried out using (D) Ferric chloride test: An aliquot of the extract was treated with 5% $FeCl_3$ reagent and observed for the formation of a deep blue-black colour and (E) Lead acetate test: A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Flavonoids were detected by using (F) aqueous sodium hydroxide test: A fraction of the extract was treated with 1N aqueous sodium hydroxide and observed for the formation of yellow-orange colouration and (G) Sulphuric acid test: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

RESULTS:

Enzymatic antioxidants
Coleus vetteveroides, the plant used for the study, has been shown to have high enzymatic antioxidant content. Table 1 shows the enzymatic activities that were obtained. Catalase, peroxidase, and glutathione-s-transferase activity were found in abundance in the leaves. These three enzymatic antioxidants act as an innate defensive mechanism in plants, helping them to withstand oxidative damage[32].

Non-Enzymatic antioxidants
Non-enzymic, low-molecular-weight antioxidants also contribute to the antioxidant spectrum. Table 2 shows the amounts of non-enzymic antioxidants found in *Coleus vettevroides* leaves. Ascorbic acid and alpha tocopherol were found in significant amounts. Total carotenoids, reduced glutathione, total phenols, flavonoids, and chlorophyll were all associated with the greatest amounts in the leaves.

Radical Scavenging activity
For radical scavenging activity, the extracted leaf samples yielded the following findings. Isopropanol and Ethyl acetate extracts of *Coleus vettveroides* leaves had significant hydrogen peroxide scavenging capacity, followed by Hexane extract, and Isopropanol extracts of *Coleus vettveroides* leaves had a strong hydroxyl radical scavenging ability, followed by Ethyl acetate and Hexane extracts. Figures 1 and 2 depict the findings of hydrogen peroxide and hydroxyl scavenging effects, respectively.

Chelating property of *Coleus vettveroides*
Plants that have iron-chelating activity are the most effective at preventing lipid peroxidation, and so play an important role in medicine. *Coleus vettveroides* extracts in Isopropanol and Ethyl acetate showed the most chelating activity, followed by Hexane extract. Figure 3 illustrates the chelating properties of three distinct extracts.

DNA protective action of *Coleus vettveroides* against radical damage

Extracts of *Coleus vetteveroides* leaves in Hexane, Ethyl acetate, and Isopropanol showed potential in protecting DNA from free radicals created by hydrogen peroxide. The migratory pattern of goat liver DNA treated with hydrogen peroxide in the presence and absence of three distinct leaf extracts is represented in Figure 4.

The presence of alkaloids, flavonoids, and phenols was observed in the phytochemical analysis of *Coleus vetteveroides* leaves of different extracts.

DISCUSSION:

Antioxidants function effectively as radical scavengers protecting cells from degenerative disease. The human body endogenously releases antioxidants to neutralize the detrimental effects of free radicals. A constant supply of external sources of antioxidants maintains the balance and achieves significant effects. Peroxides and free radicals produced by disturbances in the normal redox state of cells damage cell components leading to cytotoxicity.

The objective of this study was to discover the antioxidant and scavenging activities of *Coleus vetteveroides* leaves which have been shown to have high antioxidant properties. Previous research on the roots of *coleus vetteveroides* exhibited significant antibacterial activity. Plants produce a large number of bioactive molecules making them rich sources of different types of medicines.

The leaves of *Coleus vetteveroides* showed a considerable activity of enzymic antioxidants; catalase, peroxidase, and gluthathione-s-transferase. Catalase plays a significant role in providing antioxidant defenses to an organism. The catalase activity of *Plectranthus aramaticus* and *Catharanthus* under salinity was boosted after treatment with triazole[33]. Paclobutrazol treatment improved it, even more, allowing it to effectively scavenge H_2O_2[34]. In oxidatively challenged plants, Jaleel et al.[35] found that the H_2O_2 scavenging mechanism represented by CAT and APX is more essential than SOD in conferring tolerance.

Rhinaocanthus nasutus leaves have been reported to be the richest source of both enzymic and non-enzymic antioxidants among three underexploited medicinal plants[36]. Glutathione-s-transferase is substantially more efficient on a molar basis than other enzymes [37]. According to Zimmermann and Zentgraf (2005)[38], the components of both the enzymic and non-enzymic antioxidant systems correlate well with oxidative stress during plant development and senescence.

The leaves of *Coleus vettevroides* contain significant amounts of non-enzymic antioxidants. Many plants have been tested for non-enzymic antioxidants and the results have been published. In comparison to the leaf, stem, and roots of *Moringa oleifera*, total levels of ascorbic acid were found to be highest in the pods[39]. These antioxidant activities were also observed to be dependent on the sample concentration[40]. In a comparative study of a few plants, the highest α-tocopherol content was reported in *Sauropus androgynus* leaves, followed by *Citrus hystrix* leaves etc.,[41]

Carotenoids are a good non-enzymatic antioxidant that protects against free radical damage by quenching singlet oxygen. According to a study, lycopene levels in the leaves and β-Carotene levels in the tubers of *Planctranthus aromaticus* were much higher than in other parts of the plant.

Antioxidant, antimutagenic, and anticancer effects are all prevalent in phenols. As a result, their presence in *Coleus vetteveroides* leaves is credited with the plant's remarkable antioxidant qualities. In the *Plectranthus aromaticus* plant, the total phenol concentration was found to be substantially higher in the tubers than in the roots and stem[42]

Amorphophallus commutatus, for example, was shown to have a significant amount of reduced glutathione in developed leaves, followed by juvenile leaves[43]. The *Coleus vetteveroides* leaves show a high level of reduced glutathione. According to Karthika et al., 2014[44], the chlorophyll concentration of healthy and tender papaya leaves was higher than that of dried leaves. Our findings support the findings of earlier research and imply that *Coleus vetteveroides* leaves are a rich source of both enzymic and non-enzymic antioxidants.

Although hydrogen peroxide is not particularly reactive, it can occasionally be hazardous to cells because it can produce hydroxyl radicals in the cells. By reducing unsaturated bonds in biomolecules, the most physiologically reactive hydroxyl radical produces hydroxylation (Lipinshi *et al.,* 2011)[45]. Thus, eliminating H_2O_2 from cells or dietary systems is critical for antioxidant defense.

The water and ethanol extracts of *Crataegus monogyna* exhibited hydrogen peroxide scavenging

ability[46]. All of the extracts of *Pouzolzia zeylanica* leaves had good hydroxyl radical scavenging activity, Cold ethyl extract showed the most effective hydroxyl radical scavenger[47]. About the above literature, the Hexane and Ethyl acetate extracts of *Coleus vettveroides* leaves exhibited strong radical scavenging effects against Hydrogen peroxide and hydroxyl radicals.

Plants that have iron-chelating activity are the most effective at preventing lipid peroxidation and thus play an important role in medicine. Isopropanol and Ethyl acetate extracts of *Coleus vetteveroides* leaves exhibited high levels of the chelating property followed by Hexane extract. In the study of *Coriander sativum* and *Petroselium crispum (stem and leaves)* Iron chelating activity of *Coriander sativum* extracts were reported to be higher than *Petroselium crispum* plant[48].

The rhizome extract of *Dioscorea alata* possessed radical scavenging activity and showed a protective effect on calf thymus DNA and plasmid DNA as evaluated by EtBr (Wang *et al.*, 2004)[49]. The alcohol: water (1:1) extract of curry leaves (*Murraya koenigii L.*) showed the highest antioxidant as reflected by the inhibition of ferrous sulphate:ascorbate-induced fragmentation and sugar oxidation of calf thymus DNA (Ningappa *et al*., 2008)[50]. The leaf extracts of *Coleus vetteveroides* showed potential in protecting DNA from hydrogen peroxide-induced radicals.

Studies with leaves of *Adathoda vasica* and *Carissa carandas linn* plants[51-54], along with *Coleus vetteroides*, revealed high quantities of enzymatic and non-enzymatic antioxidants, as well as free radical scavenging activity against hydroxyl and hydrogen peroxide radicals in the leaf extracts. Both plants have a high level of DNA protection against hydrogen peroxide radicals.

CONCLUSION:

The medicinal efficacy of the Coleus genus, which is widely utilized in Indian medicine, has been proven by modern testing and evaluation in a variety of illness states. These findings make this indigenous medication a promising candidate for bioprospecting and therapeutic development for wound, microbial infection, and liver disorders, among other conditions. The medical benefits of these plants, as well as a variety of research opportunities, are yet relatively unexplored aspects of their function.

Table 1: Enzymatic antioxidant levels in the leaves of *Coleus vetteveroides*

Parameters	Amount of enzymatic antioxidants present in *Coleus vetteveroides* leaf
CAT (U*/g)	17.70 ± 0.196
POD ($U^{\#/}$ /g)	1.548 ± 0.062
GST($U^{\$}$/g)	0.16 ± 5.88

The values are Mean ± SD of triplicates
*1 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05units/minute
1 Unit = Change in absorbance at 430 nm /minute
$1 Unit = nmoles of CDNB conjugated / minute

Table-2: Levels of Non-enzymatic antioxidants in the *Coleus vetteveroides* leaves

Parameters	Non-enzymatic antioxidant levels *Coleus vetteveroides* leaves
Ascorbic acid	4.476 ± 0.025 mg/gram
Tocopherol	227.3 ± 1.12 µg/gram
Total carotenoides	120.6 ± 0.763 mg/gram
Total lycopene	2.34 ± 0.0472 mg/gram
Reduced glutathione	102.5 ± 0.05 nM/gram
Total phenol	1.44 ± 0.040 mg/gram
Total chlorophyll	103.5 ± 1.01 mg/gram

Values are mean ± SD of triplets

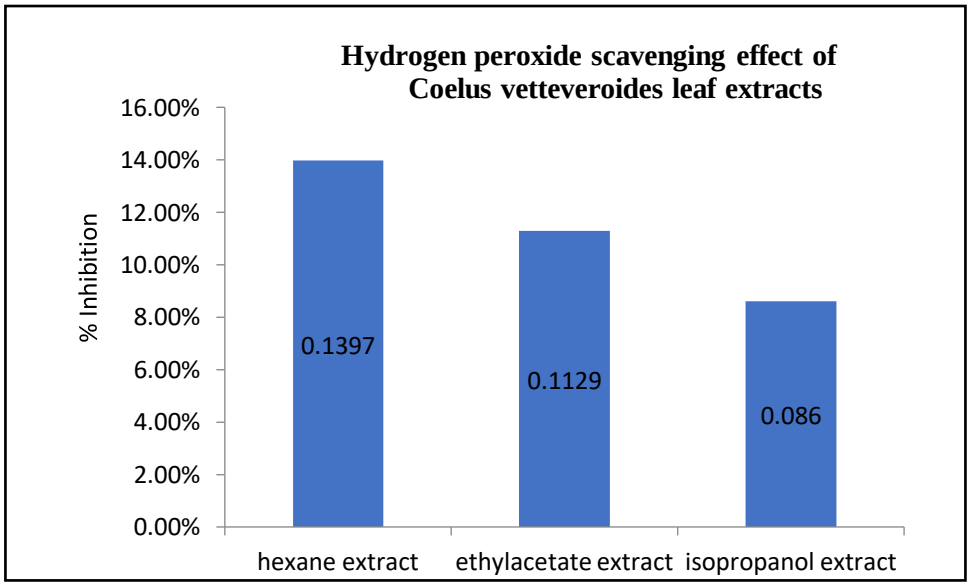

Figure 1: Hydrogen peroxide scavenging effect of *Coleus vetteveroides* leaf extracts

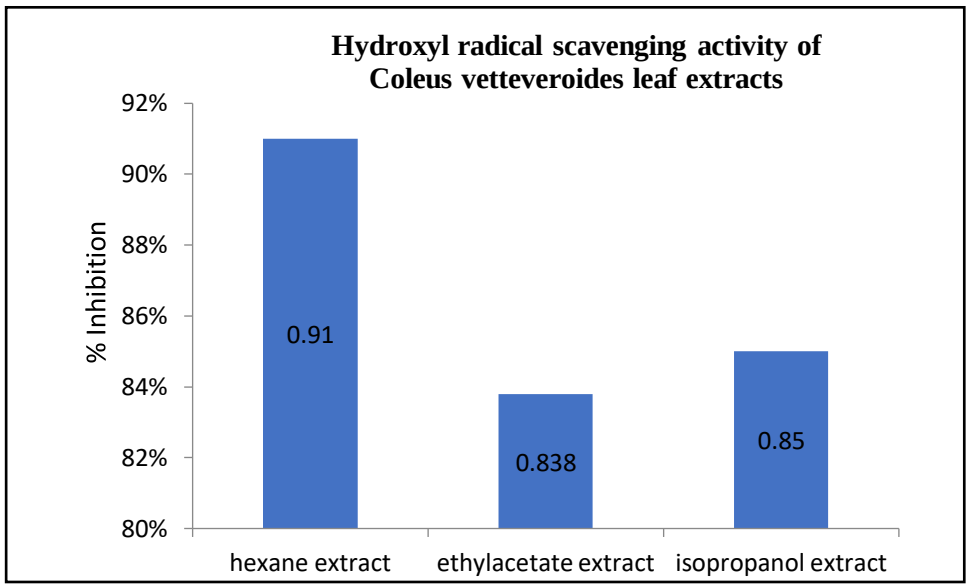

Figure 2: Hydroxyl radical scavenging effect of *Coleus vetteveroides* leaf extracts

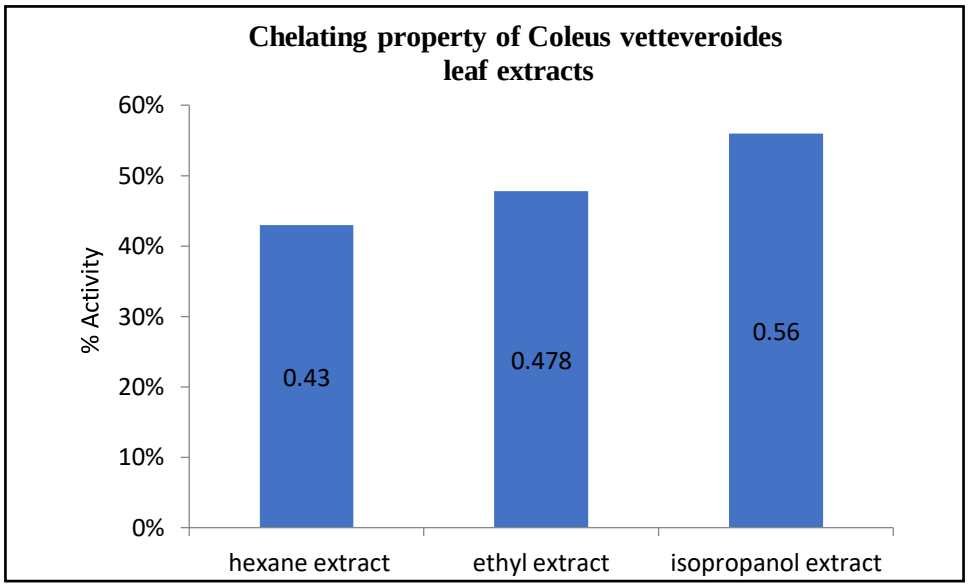

Figure 3: Chelating property of *Coleus vetteveroides* leaf extracts

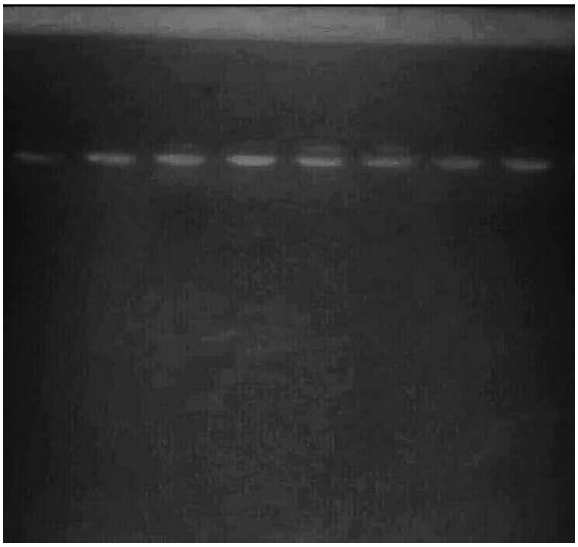

Figure 4: Migration pattern of goat liver DNA treated with H_2O_2 with and without the leaf extracts (Starting from left side of the figure) Lane1: DNA + H_2O_2, Lane 2: DNA, Lane 3: DNA + Hexane extract, Lane 4: DNA + Ethyl acetate extract, Lane 5: DNA + Isopropanol extract, Lane 6: DNA + H_2O_2 + Hexane extract, Lane 7: DNA + H_2O_2 + Ethyl acetate extract, Lane 8: DNA + H_2O_2 + Isopropanol extract

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