

# DNA Protection Against UV Rays: A Study on Polyphenols of Curry Leaves (*Murraya koenigii*)

Dinesha Ramadas<sup>1</sup>, Vedamurthy Joshi<sup>2</sup>, B. Ramesh<sup>3</sup>, Santhosh Kumar Nune<sup>4</sup> and Rajesh Kowti<sup>5\*</sup>

1. Adichunchanagiri Institute for Molecular Medicine, AIMS-Central Research Laboratory, Adichunchanagiri Institute of Medical Sciences.

CORMIL & CMPAT, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara, Mandya District, Karnataka, India

2. Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara, Mandya District, Karnataka, India

3. Department of Pharmaceutical Chemistry, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara, Mandya District, Karnataka, India.

4. Department of Clinical Biochemistry, Shridevi Institute of Medical Sciences, Tumakuru, Karnataka, India

5. Department of Pharmacology, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara, Mandya District, Karnataka, India.

**Abstract:-** As a part of normal life, the Reactive Oxygen Species, Oxygen-derived radicals, free radicals are generated constantly and also when body exposed to few factors like strong chemicals and UV rays. In this study, the DNA protection nature of Polyphenols from Curry leaves against UV rays was studied. The above study was done using submarine gel electrophoresis in which, the DNA was damaged using UV rays of selected wavelength. A maximum of 15µg dosage of polyphenols enriched extract was taken to prevent the DNA fragmentation and the protection level was compared with positive control Ascorbic acid (400µM). It was observed that, the Polyphenols of Curry leaves and standard antioxidant like Ascorbic provides a protection of 69% and 63% respectively, which provides an impression that, polyphenols of Curry leaves provides protection to DNA.

**Keywords:-** Curry leaves extract, DNA damage, DNA protectant, Cytotoxicity, UV rays, Polyphenols, Non-toxic.

## I. INTRODUCTION

In the living systems, the oxidative damage induced by several factors like free radicals, UV rays, reactive oxygen species (ROS) and many other factors. Though the life span of above radicals are nearly nano seconds but induces stress but it also responses to activate molecular signals against which is useful. When the body exposed to UV rays, it stimulates the body to release free radicals, ROS will damage to living tissue<sup>[1-2]</sup>. The over production of ROS are harmful to living organisms, which leads to make the body under oxidative stress. The excess ROS production gives a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition and finally changes the so called programmed cell death means apoptosis pathway<sup>[3-6]</sup>. It is reported that, wide UV radiations induces oxidative stress resulting in DNA damage<sup>[7-8]</sup>. Lot of studies reported that, proteins from Star

anise, Wild Turmeric (*Curcuma aromatica*), Curry leaves (*Murraya koenigii*) and *Muntingia calabura* plant provides protection to DNA against oxidative stress<sup>[9-13]</sup>.

## II. MATERIALS AND METHODS

Deoxyribonucleic acid (DNA), well known antioxidant Ascorbic acid, and other chemicals from Sigma Chemical company USA, HIMEDIA, India. Fresh Curry leaves were procured from authentic source.

### A. Extraction

Fresh Curry leaves are washed with double distilled water, further, leaves were shade dried, powdered, sieved and stored in a dry glass container for further use. The polyphenol extracted from Curry leaves powder (25 g) was done by mixing with 250mL of methanol solvent for 72 h using Soxhlet extractor. After completion of extraction, the excess methanol was evaporated and the polyphenol enriched crude extract was obtained. Further the extract was suspended in 60 mL of water. The above crude extract further extracted with different organic solvents like hexane, chloroform, ethyl acetate and butanol and at the end all crude extracts were mixed, filtered<sup>[14]</sup>.

### B. Phytochemical analysis

The phytochemical analysis was done to ensure the presence of bioactive in the extract using standard protocols<sup>[15-19]</sup>. As per the protocols, the Protein estimation, Phenolics estimation, Ascorbic acid estimation, total Sugar estimation and Flavonoids estimation was done. In the above analysis, standard curve was used to compare.

### C. Isolation of human peripheral lymphocyte

10ml of venous blood drawn from young healthy donors to isolate human peripheral lymphocytes according to the method Smith et al<sup>[20]</sup>. In brief, the blood was collected in 5:1 ratio of citrate – dextrose solution to which hemolyzing buffer was added. The viable cells were counted

at an interval of 20th, 40th and 60th minutes of incubation [21].

$$\% \text{ viability} = \frac{\text{Total no. of viable cells}}{\text{Total no. of viable cells + dead cells}} \times 100$$

**D. Survival of lymphocytes: Effect of UV rays and protection by antioxidants**

The study of survival of lymphocytes against UV rays and the protection by Curry leaves Polyphenols and Ascorbic acid was done as explained in methods [22].

**E. Submarine agarose gel electrophoresis**

Agarose gel was prepared in a buffer mixture of Tris, Sodium acetate, NaCl, EDTA containing of Ethidium Bromide and electrophoresis conducted using suitable buffer.

**F. DNA damaged induced by UV rays: Protection by Polyphenols of Curry leaves (*Murraya koenigii*) and other standard antioxidant**

Finely sheared Calf thymus DNA was exposed to UV rays of wavelength 345nm with and without antioxidants for 1 hours at 37°C in phosphate buffer saline of pH 7.4 and the reaction mixture was mixed with Ethidium Bromide. The fluorescence was measured at 520nm excitation and 590nm [23]. Suitable blanks and controls were used. Finally, the bands visualized under U.V Transilluminator.

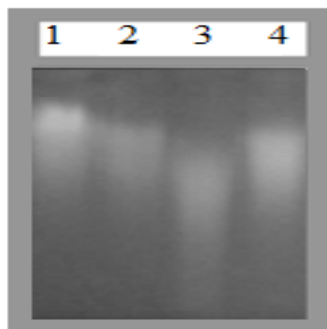
**III. RESULTS AND DISCUSSION**

**Table -1: Phyto analysis of Curry leaves extract**

Phyto-chemicals	Extract
Carbohydrates	-
Protein	-
Polyphenols	+
Flavonoids	+
Ascorbic acid	-

Values are means ± SD of triplicates

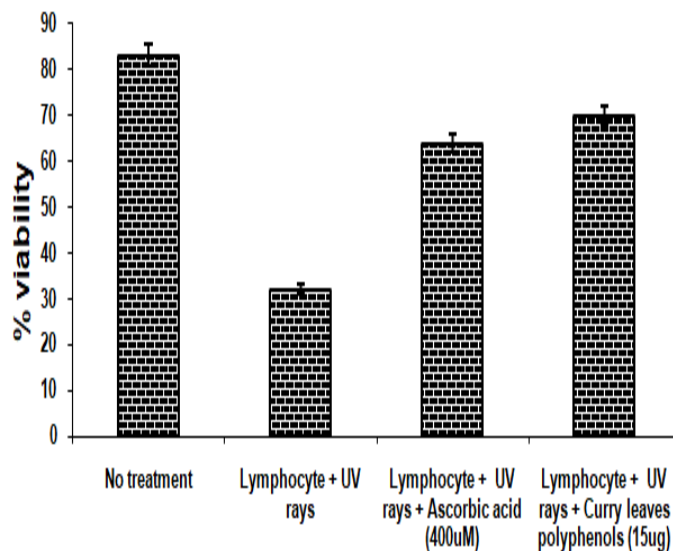
**Fig. 1: Ultraviolet rays induced DNA damage and protection by Curry leaves polyphenols and standard antioxidant**



- Lane 1:** Sheared DNA (10 µg)
- Lane 2:** DNA + UV radiation + polyphenols of Curry leaves (15µg)
- Lane 3:** DNA + UV radiation
- Lane 4:** DNA + UV radiation + Ascorbic acid (400µM)

DNA (10µg) with and without Curry leaves polyphenols (15µg) / Ascorbic acid (400µM) in Phosphate Buffer Saline – pH 7.4 - UV radiation (345nm) for 1 hours. About 4µg DNA ran on agarose gel in submarine Gel electrophoresis.

**Fig. 2: UV rays induced Cell toxicity and protection by Curry leaves polyphenols and standard antioxidant Ascorbic acid**



Lymphocytes (10<sup>6</sup>cells) pretreated with or without antioxidants and exposed to UV rays for one hour. Viability of the cells was determined by Trypan blue exclusion method.

Unprotected or extensive exposure to UV rays makes genetic defects, induces mutations and cause damages the DNA in skin cells, and also reported to cause eye damage, including cataract[24]. UV radiation also causes sunburns, wrinkles, reduces immunity, induces premature aging etc. Herbs, its preparations and other synthetic and natural antioxidants fight against negative skin changes[25]. The Polyphenols of Curry leaves when subjected to proximate analysis, it showed that, Polyphenols and Flavonoids are present. The extract was thick dense, dark coloured polyphenol enriched. As shown in fig. 1 lane 2 showed, DNA damage caused by UV radiation and protection by Curry leaves polyphenol extract, Lane 3 shows that DNA damage caused by UV rays, Lane 4 showed protection of Ascorbic against DNA damage. In the lymphocyte survival study, the result shows that, the Curry leaves polyphenols extract is non toxic. The survival of lymphocytes on pre treatment of UV rays a time course study was done and the results shows that, Polyphenols of Curry leaves showed

more protection (69%) when compared to Ascorbic acid (63%).

#### IV. CONCLUSION

The above study results confirmed that, the non-toxic and protective nature of Polyphenols of Curry leaves.

#### ACKNOWLEDGEMENT

The authors thank Adichunchanagiri University for providing facility and opportunity to conduct the above studies at Adichunchanagiri Institute for Molecular Medicine and Sri Adichunchanagiri College of Pharmacy.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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