ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antioxidant Activity and Hydroxyl Radical Induced DNA Damage Protection Effect of Aqueous Extract of *Curcuma amada* ROXB

Vishnupriya M[1], Nishaa S[1], Sasikumar JM[1], Gopalakrishnan VK[2*]

[1]Department of Biotechnology, Karpagam University, Coimbatore, Tamil Nadu, India – 641021.
[2]Department of Biochemistry and Bioinformatics, Karpagam University, Coimbatore, Tamil Nadu, India – 641021

ABSTRACT

Mango ginger (*Curcuma amada* Roxb.) has been extensively used in South India to make pickles. The present study was aimed at determining the antioxidant activity and DNA protecting activity of aqueous extract of C.amada. The antioxidant activity of the aqueous extract of C.amada was determined by using its Iron chelating capacity and Hydroxyl radical scavenging assay. The DNA damage protection potential was also determined using Hydrogen peroxide (H_2O_2) induced damage on herring sperm DNA. Results revealed a strong antioxidant activity with IC_{50} value of 297.3 µg/ml on iron chelating capacity and IC_{50} value of 323.8 µg/ml on hydroxyl radical scavenging activity. The extract also showed a concentration dependent DNA damage protecting effect. These results clearly demonstrates the strong antioxidant and DNA damage protecting potential of C.amada aqueous extract and marks its use as a potential source of natural antioxidant.

Key words: *Curcuma amada* Roxb., Antioxidant activity, DNA damage protection activity, Hydrogen peroxide.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

DNA is under constant attack from many sources: Radiation, ultraviolet light, and contaminants in our food and in our environment can all wreak havoc on our genetic material, potentially leading to cancer and other diseases [1]. DNA damage induction is a fundamental unavoidable process and plays a key role in cancer development and the induction of heritable genetic defect. DNA damaging agents (genotoxins) are nearly always identified as carcinogens [2]. Humans are constantly exposed to natural DNA damaging agents such as sunlight, dietary agents (such as cooked meat, acrylamide) and endogenously formed oxygen free radicals. Reactive oxygen species are cytotoxic and disturb normal metabolisim through oxidative damage to lipids, proteins and nucleic acids[3].Over production of ROS are also capable of damaging a wide range of essential cellular biomolecules such as proteins, enzymes, DNA, RNA, lipids and carbohydrates through oxidative modification, consequently may adversely affect immune functions and contributing to the pathological conditions including, aging, gastric ulcer, diabetes, carcinogenesis, neurodegenerative diseases, rheumatic joint inflammation and AIDS [4].

DNA Damages are physical abnormalities in the DNA, such as single and double strand breaks, 8-hydroxydeoxyguanosine residues and polycyclic aromatic hydrocarbon adducts. Enzymes can recognize DNA damages, and thus they can be correctly repaired if information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. DNA damage is caused due to interaction of DNA with ROS or RNS. DNA damage accumulates in brain, muscle, liver, kidney, and in long-lived stem cell. These accumulated DNA damages are the likely cause of the decline in gene expression and loss of functional capacity observed with increasing age [5]. Many research studies have identified potent plants that are effective against DNA damage. Herbs contain some of the most powerful natural antioxidants and are highly prized for their antioxidant and anti-ageing effects. Natural products offer an untold diversity of chemical structures. These compounds often serve as lead molecules, the activities of which can be enhanced by chemical manipulation and by de novo synthesis [6]. The beneficial effect of antioxidants on promoting health is believed to be achieved through several possible mechanisms, such as directly reacting with and quenching free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme system [7]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ), often used in foods to prevent oxidative degradation, are known to have toxic and carcinogenic effects on human health [8]. To date, many medicinal plants have proved successful in combating various ailments, leading to mass screening for their therapeutic components. In the present study the DNA damage protecting potential was investigated by means of synthetic DNA damage inducing agent.

Curcuma amada Roxb. has been used in traditional Indian system of medicine for various ailments including gastric disorders, sore throat and the plant has also been reported to possess good antioxidant activity by Policegourd et al, [9]. In South India the rhizomes of the

plant has been used in making pickles. The plant rhizomes yield a raw mango flavor and this aroma makes it well suited for making its use in food processing industries. The present study was aimed to determine the antioxidant potential and hydrogen peroxide induced DNA damage preventive potential of aqueous extract from *Curcuma amada* Roxb.

MATERIALS AND METHODS

The rhizomes of Mango ginger (*Curcuma amada* Roxb.) were collected from the native farms of Coimbatore and were authenticated by, Dr.C.Kunhikannan, Scientist E, Division of Biodiversity, Institute of Forest Genetics and Tree Breeding, Ministry of Environment and Forest, Government of India, Coimbatore.

Preparation of the Extract

The rhizomes were then cleaned and crushed to extract the juice in it by using distilled water. The collected extract was then concentrated under reduced pressure and controlled temperature (40-50°C) using a rotary evaporator.

Hydroxyl Radical Scavenging Assay (Deoxyribose Assay)

Deoxyribose is oxidized when exposed to hydroxyl radicals; such degradation can be detected by heating the products in the presence of thiobarbituric acid under acidic conditions, which leads to development of a pink chromogen, by the method of Halliwell (1987)[11], with slight modifications. The assay mixtures, containing the sample, used a final volume of 1 mL, 1mM in deoxyribose, 24mM in sodium phosphate (containing 15mM NaCl, pH 7.4), 0.1mM in $FeCl_3$, 0.1mM in EDTA, 1mM in H_2O_2, and 0.1mM in ascorbic acid. After incubation at 37^{o}C for 1 h, colour development was promoted by addition of 1.5 mL of 28% (w/v) TCA and 1.0 mL of 1% (w/v) TBA in 0.05M NaOH, followed by heating at 100^{o}C for 15 min. Inhibition of deoxyribose degradation was expressed as percent decrease in absorbance, when compared to the BHT control (assay without sample).

$$\text{Hydroxy Radical Scavenging activity (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100.$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test which is compared to that of the reference standard BHT. The colour was read at 560nm against blank samples. The hydroxyl radical scavenging activity was expressed as the IC_{50} value. BHT was used as a positive control.

Iron Chelating Capacity (ICC)

The Iron chelating capacity (ICC) was investigated using the method of Dinis et al. 1994[10].Briefly, different concentrations of both the sample extracts and standards (50-250 µg mL) were mixed with $FeCl_2$ (2mM) and ferrozine (5mM).The mixture was made up to 0.8ml with

ISSN: 0975-8585

deionised water. After 10min incubation at room temperature, the absorbance of ferrous ion-ferrozine complex was measured at 560nm in UV spectrophotometer. EDTA was used as control for iron chelating assay. The percentage of iron chelation was calculated as follows:

$$\text{Chelating capacity of ferrous ion (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100.$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the aqueous extract. The colour was read at 560nm against blank samples.

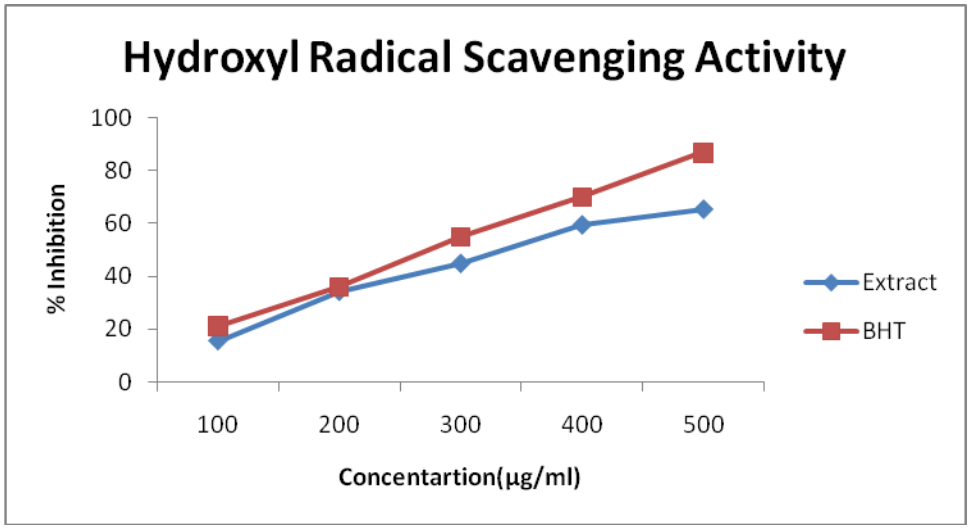
DNA Damage Protecting Assay

The protective effect of extract on oxidative DNA strand breakage was evaluated with Herring sperm DNA by Lin et al, [12] method with slight modifications. Each aliquot of the reaction mixture was blended with aqueous extract of C.amada (10 - 50µg/ml), 50µl Herring sperm DNA solution (HIMEDIA Laborotaries, Mumbai), 9µl 180 mM $FeSO_4$ and 36µl 600mM hydrogen peroxide and incubated at room temperature for 15 min. Then, 10µl of 1mM EDTA was added to stop the reaction. The blank was the Herring sperm DNA solution. The control was the reaction mixture without extract. Each 10µl of the aliquot was applied to 1% agarose gel containing 0.1% Ethidium bromide. Electrophoresis was carried out on 1% agarose in 1× TAE buffer (2M Tris, 1M sodium acetate, 50mM EDTA, and pH 8.0) at room temperature using an electrophoresis system. Subsequently, the gel containing 15µL of ethidium bromide (10 mg/mL) was observed under ultraviolet light, using a transilluminator and photographed.

RESULT AND DISCUSSION

Antioxidant Activity

Fig 1-Hydroxyl Radical Scavenging Activity

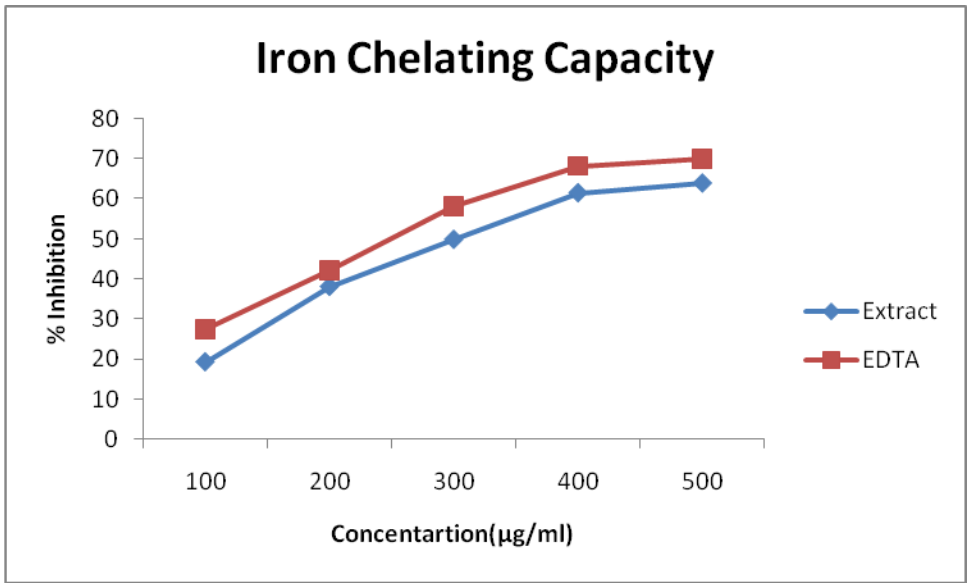


Values are expressed as Mean ± SD for three samples

ISSN: 0975-8585

Figure 1 shows the hydroxyl radical scavenging activity of the extract. It was observed that the percentage of inhibition of the hydroxyl radical in the presence of aqueous extract of C. amada was dose dependent. The maximum quenching capability of the hydroxyl radical was found to be 65.20% at the highest concentration tested (500µg/ml). The IC_{50} value of the plant extract was found to be 323.8 µg/ml which was comparable with that of the standard BHT with IC_{50} value of 262.3µg/ml. Hydroxyl radical species is considered as one of the quick initiators of the lipid oxidation process, abstracting hydrogen atoms from unsaturated fatty acids [13]. Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases.

Fig 2-Iron Chelating Capacity



Values are expressed as Mean ± SD for three samples

Fig 2 shows the Iron chelating capacity of aqueous extract of C.amada. The quenching efficacy of the plant extract toward the ions was found to be increasing on a dose dependent manner. The IC_{50} value of the plant extract and EDTA was found to be 297.3 µg/ml and 235.3 µg/ml respectively. The inhibition of the metal ions by plant extract was comparable with that of the synthetic antioxidant used (EDTA). Ferrozine can quantitatively form complexes with Fe^{2+}.The method of metal chelating activity is based on chelating of Fe^{2+} ions by the reagent ferrozine which is a quantitative formation of a complex with Fe^{2+} ions [14]. In the presence of other chelating agents, the complex formation is disrupted and as a result the red colour of the complex is decreased. Measurement of the rate of colour of reduction therefore allows estimation of the chelating activity of the coexisting chelator [15]. In this assay in comparison with the standard the extract interfered with the formation of ferrous complex with ferrozine reagent. EDTA is used as the standard as it is known metal ion chelator. Metal chelating capacity of the extract was significant as it reduced the concentration of the catalysing transition metal in lipid peroxidation [16]. Metal ions can initiate lipid peroxidation and start a

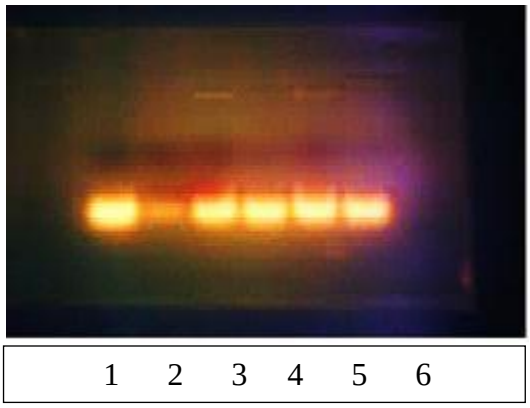
ISSN: 0975-8585

chain reaction that leads to the deterioration of food [17]. The catalysis of metal ions also correlates with incidents of cancer and arthritis [11].

DNA Damage Protection Activity

The protective effect of *Curcuma amada* Roxb. extract on DNA damage was studied using Herring sperm DNA. Fig 3 shows the electrophoretic pattern of Herring sperm DNA after the Fentons reagent induced damage both in the presence and absence of *Curcuma amada* Roxb. extract.

Fig 3- DNA Damage protecting Potential of Herring Sperm DNA



Lane 1(C) –Herring sperm DNA damaged with hydroxyl radical from Fentons reagent.
Lane 2-6-Damaged DNA with sample at various concentrations (10, 20, 30, 40, 50µg/ml) indicated as 1,2,3,4,5 respectively.

The Fentons reaction involves the reaction between hydrogen peroxide and Fe^{2+} to form hydroxyl radical. The results showed complete degradation of Herring sperm DNA treated with Fentons reagent which is indicated in lane 1. The action of plant extract on the DNA damage caused by Hydroxyl radical was indicated from lane 2 to lane 6. The intensity of the DNA damage was reduced on a concentration dependent manner of aqueous extract towards DNA which shows the protective effect of the extract towards hydrogen peroxide induced damage. At the maximum concentration of the extract used (50µg/ml) the DNA damage was almost nil with high intensity DNA band which is indicated on Fig 3.The increasing concentration of the plant extract exerted its effect towards the prevention of DNA damage. The result suggests that C.amada extract protects DNA through antioxidant activity. Most of the oxidative damage in biological systems is caused by the $OH^{\bullet}$ which is generated by the reaction between $O_2^{\bullet -}$ and H_2O_2 in the presence of metal ions [18]. Kumar et al, [19] reported that oxidative modification of DNA has been suggested to contribute to aging and various diseases including cancer and chronic inflammation.

The higher antioxidant potential is attributable to their capacity to scavenge harmful ROS and other free radicals that originate from various cellular activities and lead to oxidative

stress. Among the various free radicals, hydroxyl radicals (OH^-) are well studied for their effect on genetic material DNA. Moreover DNA damage by free radicals is an important contributor for cancer development. Hydrogen peroxide is believed to cause DNA strand breakage by generation of the hydroxyl radical (OH^-**)** close to the DNA molecule through Fenton reaction. This may result in DNA instability, mutagenesis and ultimately carcinogenesis [20-23].The antioxidant ability of the phytochemicals present in plant extract might be attributed to its ability to scavenge free radicals, quenching singlet or triplet oxygen, or decomposing peroxides hence could lower occurrence and mortality rates of several human diseases [24,25].

Hydroxyl radicals have very short life span than any other free radicals and these free radicals attack the DNA double strands and breaks into single strand. So the quenching capability of the hydroxyl radical will helps to prevent the DNA double strands into single strand. The oxidative damage of DNA is one of the most important mechanisms in the initiation of cancer and this damage is usually caused by hydroxyl radicals [26]. The activity of these radicals can be reduced by natural antioxidants found in plants including herbs [27]. Fenton reaction involves the reaction between hydrogen peroxide and Fe^{2+} to form hydroxyl radicals. Scavengers of hydroxyl radicals inhibit this reaction through the reduction of Fe^{2+} [14]. Hence to assess the DNA damage protecting potential it's very important to check the hydroxyl radical quenching capability.

CONCLUSION

Dietary and other components of plants form major sources of antioxidants. Oxidants can react with DNA bases or sugars. Guanine is the most sensitive base towards oxidative attack. Increased understanding of the conditions under which each of various oxidants are produced and methods of inhibiting their formation will likely prove very important in preventing cancer and other diseases of previously unknown origin. Recent research centers on various strategies to protect crucial DNA from oxidative damage induced by free radicals [22]. In conclusion, this plant (*Curcuma amada* Roxb.) can be explored further as potential sources of natural antioxidants.

ACKNOWLEDGEMENT

We, the authors are thankful to Chancellor, Advisor, Vice-Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

REFERENCES

[1] Kang DH. AACN Clin Issues 2002; 13:540-549.
[2] Kalim MD, Bhattacharyya D, Banerjee A, Chattopadhyay S. BMC Complement Altern Med 2010; 10:77.
[3] Parida AK, Das AB. Ecotoxicol Environ Saf 2004; 60: 324-349.
[4] Moskovitz J, Yim MB, Chock PB. Arch Biochem Biophys 2002; 397:354–359.

[5] Olive PL, Banath JP, Durand RE. Radiat Res 1990; 122:86-94.
[6] Baker D, Mocek U, Garr C. The Royal Society of Chemistry 2000; 66-72.
[7] Zhou K, Laux JJ, Yu L. J Agric Food Chem 2004; 52:1118–1123.
[8] Barlow SM. Food Antioxidants. New York Elsevier 1990; 253.
[9] Policegoudra RS, Abiraj K, Channe Gowda D, Aradhya SM. J Chrom B 2007; 852:40-48.
[10] Dinis TCP, Madeira VMC, Almeida MLM. Arch Biochem Biophys 1994; 315: 161-169.
[11] Halliwell B, Murcia HA, Chirco S, Aruoma OI. CRC Crit Rev Food Sci Nutr 1995; 35: 7-20.
[12] Yu-Wen Lin, Yuh Tai Wang, Hung-Min Chang and James Swi-Bea Wu. J Food Drug Anal 2008; 16:63-69.
[13] Aruoma O I. J Am Oil Chem Soc 1998; 75:199.
[14] Halliwell B, Gutteridge J M C & Aruoma O I. Anal Biochem 1987; 165:215.
[15] Pin-Der-Duh X, Pin-Chan-Du X & Gow-Chin Yen X. Food Chem Toxicol 1999; 37:1055.
[16] Yamaguchi F, Ariga T, Yoshimira, Y & Nakazawa H. Food Chem 2000; 48:180.
[17] Gordon MH. Elsevier Appl Sci, London New York 1990; 1-18.
[18] Guitteridge JM. Biochem J 1984; 224: 761–767.
[19] Kumar K & Chattopadhyay S. Food Chem 2007; 100:1377.
[20] Athukorala Y, Nam K, Jeon Y. Food Chem Toxicol 2006: 44:1065-1074.
[21] Meneghini R. Mutation Res 1988; 195: 215–230.
[22] Schraufstatter I, Hyslop PA, Jackson JH, Cochrane CG. J Clin Invest 1988; 82: 1040–1050.
[23] Halliwell B, Aruoma OI. FEBS Lett 1991; 281: 9–19.
[24] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Food Chem 2006; 97:654-660.
[25] Balasundram N, Sundram K, Samman S. Food Chem 2006; 99: 191-203.
[26] Reddy V, Urooj A, Kumar A. Food Chem 2005; 90: 317-321.
[27] Collins AR. Am J Clin Nutr 2005; 81:261 –267.