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IN VITRO ANTIOXIDANT ACTIVITY OF *CURCUMA AMADA* ROXB.

Sanjay Jain, Rakesh Barik* and Arti Jain

Department of Pharmacognosy, Smriti College of Pharmaceutical Education, Indore, Madhya Pradesh, India

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Correspondence to Author:

Rakesh Barik

Department of Pharmacognosy, Smriti College of Pharmaceutical Education, 4/1 Pipliya Kakkad, Mayakhedi Road, Nipania, Dewas Naka, Indore, Madhya Pradesh, India

E-mail: barikrakesh@yahoo.co.uk

ABSTRACT

A study was undertaken to evaluate the antioxidant potential of rhizomes of *Curcuma amada* (Zingiberaceae). Aqueous and ethanolic extracts were subjected to *in vitro* antioxidant activity screening models such as DPPH, ABTS, nitric oxide and inhibition of lipid peroxidation activity. Ascorbic acid was used as the standard. In all the models studied, both the extracts showed nearly equal activities thereby justifying the traditional claims of the plant.

INTRODUCTION: Molecular oxygen is an essential component for all living organisms, but the formation of various reactive intermediates of molecular oxygen metabolize the cells aerobically, thus eventually leading to a process termed as 'oxidation' [1]. Normal physiological processes involve utilization of oxygen in which approximately 5% of the oxygen gets reduced univalently to oxygen-derived free radicals. These radicals, known as reactive oxygen species (ROS), exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second [2].

When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders such as glycated protein oxidation in diabetes mellitus, low-density lipoprotein oxidation in atherosclerosis, red blood cell hemolysis in glucose- 6- phosphate dehydrogenase deficiency, etc [3]. Natural products have been used to prevent such types of damage since long [5].

Many plants contain substantial amounts of antioxidants like vitamin C and E, carotenoids, flavonoids, tannins, etc. that can be used to scavenge the excess free radicals from human body [6].

Curcuma amada Roxb. (Zingiberaceae), commonly known as "Ama Haldi" is found all over India. The herb is rhizomatous with a leafy tuft, 60-90 cm high with white or pale yellow flowers in spikes in the center of the tuft of leaves [7]. It reported to contain ocimene, dihydro-ocimene, α-pinene, α-curcumene, β-curcumene, linalool, cuminyl alcohol, keto-alcohol, camphor, turmerone, linalyl acetate, safrole, curcumin, myristic acid, car-3-ene, myrcene, 1,8-cineol, limonene and perillene [8]. The rhizomes are used by the tribals of Madhya Pradesh for the management of diabetes mellitus [9].

MATERIALS AND METHODS: All chemicals used were of analytical grade. DPPH (1,1 di phenyl 2 picryl hydrazyl) and ABTS i.e., 2,2 azino bis (3 ethyl benzo thiazoline 6 sulphonic acid) were obtained from Sigma Chemicals, USA.

Sodium do decyl sulphate, nitro blue tetrazolium chloride, phenanthroline, naphthyl ethylene di amine di hydrochloride, potassium per sulphate, dimethyl sulphoxide, hydroxylamine hydrochloride, ammonium molybdate, sulphanilamide, ortho phosphoric acid, sodium nitroprusside, riboflavin, EDTA and sodium phosphate were obtained from Loba Chemie Private. Limited, Mumbai, India

Plant material: The rhizomes of *Curcuma amada* (CA) were collected from the local market in Indore and identified at Department of Botany, Government Agriculture College, Indore. A voucher specimen (SCOPE/PHCOG/06-08/01) has been maintained in the museum of our department for further reference.

Preparation of Extracts: The shade dried rhizomes were powdered and passed through sieve no. 40. These were extracted with ethanol for 24 h using a soxhlet extractor and macerated with distilled water to obtain ethanolic and aqueous extracts respectively. The extracts were concentrated to dryness using a freeze drier (yield: 5.2% w/w and 4.0% w/w for ethanolic and aqueous extract respectively). Both the extracts were used for the antioxidant study.

DPPH Radical Scavenging activity [11]: 15 mg of DPPH was dissolved in 10 ml of methanol. 75µl of this solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75µl of DPPH was added to a mixture of methanol and 50 µl of extract. The final volume was adjusted to 3 ml. Decrease in absorbance of the DPPH was measured 517 nm

ABTS Radical Scavenging activity [12]: ABTS 2mM and Potassium per sulphate 70mM were prepared in distilled water (0.0548g in 50 ml and 0.0189g in 1ml respectively). 200ml of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. To 0.5 ml of various concentrations of the extracts, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract, methanol for alcoholic extract and water for aqueous extract were taken. The absorbance was measured at 734 nm.

DMSO Radical Scavenging activity [13]: To 0.5 ml of different concentration of the extracts, 1 ml alkaline DMSO and 0.2 ml NBT 20mM (50 mg in 10ml phosphate buffer pH 7.4) were added. The absorbance was measured at 560 nm.

Nitric Oxide Radical Scavenging activity [14]: Griess reagent was prepared accordingly: Solution A: 1% Sulphanilamide in 5% ortho Phosphoric acid or 25% v/v Hydrochloric acid. Solution B: 0.01% Napthyl ethylene diamine in distilled water. Solution A and Solution B were in mixed equal volumes within 12 hrs of use. Sodium nitroprusside 5mM was prepared in phosphate buffer PH 7.4 (0.0373g in 25 ml). To 1ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25^{o}C for 5hr. 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm.

Lipid Peroxidation Assay [15]: 15% w/v Trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25N Hydrochloric acid were mixed to form the stock Thio-barbituric acid (TBA) – Tri-choloro acetic acid (TCA) – HCl reagent. This solution was mildly heated to assist the dissolution of TBA]. Albino rats (180-200g) of either sex were used for the study. After decapitation, the brain was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15%w/v KCl to make 10%v/v homogenate.

The homogenate (0.5ml) was added to 1 ml of various concentrations of the extracts. Then the mixture was incubated for 30 min. The per-oxidation was terminated by the addition of 2 ml of TBA-TCA –HCl reagent. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm.

The % inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula.

$$\% \text{ inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

The institutional animal ethical committee approved the use of animals for lipid peroxidation assay (IAEC /SCOPE/07-08/01). All experiments were performed in triplicate and the results averaged. Linear regression analysis was used to calculate the IC_{50} values [16].

RESULTS AND DISCUSSION: Several concentrations ranging from 50-150 µg/ml of the ethanolic and aqueous extracts were compared for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models. On a comparative basis, both the extracts showed almost near values (**Table 1**).

TABLE 1: IC_{50} VALUES OF *IN VITRO* ANTIOXIDANT STUDY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CURCUMA AMADA* AND STANDARD (ASCORBIC ACID) [Values are mean ± SEM of 3 replicates]

Models studied	IC_{50} (µg/ml)		
	Aqueous extract	Ethanolic extract	Standard
DPPH activity	157.50 ± 2.20	141.39 ± 5.92	141.53 ± 5.63
ABTS activity	177.67 ± 4.60	174.34 ± 8.11	154.98 ± 9.90
DMSO activity	147.27 ± 11.33	151.2 ± 10.43	121.66 ± 5.4
Nitric oxide scavenging	209.72 ± 8.03	246.86 ± 5.47	169.34 ± 27.54
Lipid peroxidation activity	147.06 ± 1.79	119.07 ± 3.54	122.11 ± 3.34

Free radicals are chemical entities that can exist separately with one or more unpaired electrons [17]. The propagation of free radicals brings about a myriad of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by these free radicals. The oxidative stress exerted due to these free radicals has been implicated in the pathology of various diseases like diabetes, inflammations, cardiovascular complications, cancer and ageing [18]. Antioxidants offer resistance against the oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease [19]. In our study, both the extracts showed scavenging of free radicals in all the *in vitro* models studied.

DPPH is a stable free radical. The *in vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical $DPPH^{\bullet}$ [20]. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up [21]. From the present results, it may be concluded that *Curcuma amada* reduces the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles.

ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$ which has a long wavelength absorption spectrum [22]. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical [23]. It is a decolorization assay, thus the radical cation is performed prior to the addition of antioxidant test system, rather than the generation of the radical taking place continuously in presence of the antioxidant [24]. The results obtained imply the activity of the extracts either by inhibiting or scavenging the $ABTS^{+}$ radical as reported in earlier works.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes [25]. However, its excess production is associated with several diseases [26]. It is a very unstable species that reacts with free radicals thereby producing the highly damaging peroxy-nitrite [27]. In the present study, the nitrite produced by the incubation of solution of sodium nitrprusside in phosphate buffer was reduced by both the extracts of both species. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with nitric oxide and thus inhibit the generation of nitrite [28].

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like liver and brain [29]. In this study, lipid peroxidation was induced *in vitro* and the extracts showed concentration dependent prevention towards generation of lipid peroxides. Preliminary phytochemical screening revealed the presence of phenolic compounds, tannins and flavonoids in both the species [30]. Phenolics, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants as reported earlier [40 - 42]. Hence, these may be responsible for the observed activity in both the extracts.

The present study proved *Curcuma amada* as an antioxidant thereby justifying its traditional claims and its use in the present day system of medicine.

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