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RADICAL SCAVENGING ACTIVITY OF LEAVES AND RHIZOMES OF *Curcuma amada*

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ABSTRACT

Free radicals are formed continuously as normal by-products of oxygen metabolism during mitochondrial oxidative phosphorylation, which will be scavenged by antioxidants present in the system. Plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. The need for antioxidants becomes even more critical with increased exposure to free radicals. The present study was conducted to assess the free radical scavenging activity of aqueous, methanolic and chloroform extracts obtained from the rhizomes and leaves of the under exploited medicinal plant *Curcuma amada* against battery of free radicals in cell free systems and the results were compared. The methanolic extract of both rhizomes and leaves showed ability to scavenge free radicals followed by chloroform and aqueous extracts.

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Key Words

Free radical, antioxidant,
secondary metabolites, oxidative
stress

INTRODUCTION

During the past years, the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in the oxidative deterioration of food products as well as in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer. The putative protective effects of antioxidants against these deleterious oxidative-induced reactions have received increasing attention lately, especially within biological, medical, nutritional, and agrochemical fields^[1].

Plants produce an extensive range of chemicals, including “secondary metabolites”, which may exert beneficial health effects when consumed by man. Many of the plant secondary metabolites act as antioxidants in animals. On the other hand, these metabolites of plant origin may be used to prevent food deterioration via inhibition of lipid oxidation. Antioxidant action is a combination of several distinct chemical events such as metal chelation, quenching free radicals by hydrogen donation from phenolic groups; oxidation to a non-propagating radical; redox potential and enzyme inhibition^[2].

When oxidation caused by free radicals and reactive oxygen species emerges in food or in biological systems, antioxidants can prevent or delay this process via single or combination of forementioned mechanisms. Hence, antioxidants may help the body to protect itself from various types of oxidative damage which are linked to diseases such as cancer, diabetes, cardiovascular disorders and aging^[3]. Consequently, search for food and drinks with high antioxidant content and enhancement of their antioxidant properties for nutritional purposes are currently of major interest. Food such as common vegetables and fruits which are consumed all over the world as well as wild plants that are consumed by local inhabitants are screened for their antioxidant capacity. The assessment of antioxidant capacity of one of such plant was *Curcuma amada* which remains an interesting and useful task for finding new sources of natural antioxidants.

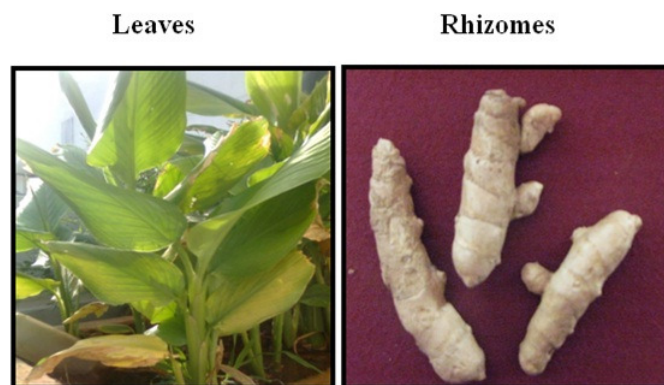
Synthetic antioxidants, like butylated hydroxyanisole, are good free radical scavengers; however, the synthetic antioxidants can be carcinogenic. Therefore, there is an increasing interest in searching for antioxidants of natural origin. We report here the results of a screening for antioxidant and free radical scavenging activity of aqueous, methanol and chloroform extracts of the leaves and rhizomes of *Curcuma amada* (Zingiberaceae), commonly known as mango-ginger^[4,5].

MATERIALS AND METHODS

Plant material

Curcuma amada rhizomes were procured from Arya Vaidya Pharmacy, Centre for Indian medicinal Plant heritage, Kanjikode, Kerala and were grown as pot culture in our University herbal garden. Both leaves and rhizomes were collected fresh for the study.

Figure 1: *Curcuma amada*



Extract preparation

The leaves and rhizomes collected fresh were rinsed with tap water blotted dry using a filter paper and used for extract preparation. The components present in the leaves and rhizomes were extracted using solvents of different polarity namely aqueous, methanol and chloroform. The methanolic and chloroform extracts were prepared and after evaporation of respective solvent were dissolved in DMSO (Dimethyl sulfoxide) [20mg plant extract in 50 μ l DMSO]. The aqueous extract was prepared fresh.

Free radical scavenging activity

The scavenging ability of the extracts were tested using a battery of free radicals.

Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity

The radical scavenging and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert it into Diphenyl picryl hydrazine. The degree of decolorization from purple to yellow color was measured spectrophotometrically at 517 nm^[6].

Azino bis ethyl bezthiozoline sulphonic acid (ABTS) scavenging activity

The percent inhibition of ABTS radical by plant extracts were determined by the ability of plant extract to scavenge the cationic free radical ABTS. The extent of decolorization was measured at 745nm^[7].

Hydrogen peroxide scavenging activity

The ability of the plant extracts to scavenge Hydrogen peroxide radical was determined by measuring the decrease in absorbance at 230nm spectrophotometrically^[8].

Hydroxyl scavenging activity

The extent of hydroxyl radical scavenging activity by plant extracts were measured colorimetrically by studying the reaction between Deoxyribose and the plant extracts for hydroxyl radical generation with Fe³⁺/Ascorbate/EDTA/H₂O₂ system^[9].

Inhibition of Nitric oxide generation

The nitric oxide was produced *in vitro* at physiological pH from sodium nitroprusside which interacts with oxygen to produce nitrite ions. The extent of nitric oxide generation was studied using Griess reagent method^[10].

Inhibition of Superoxide generation

The extent of superoxide generation was studied on the basis of the production of Nitroblue tetrazolium formazan of the superoxide ion by the plant sample which is measured colorimetrically at 560nm^[11].

STATISTICAL ANALYSIS

The parameters of the experiment are expressed as Mean ±S.D. Statistical evaluation of the data was

done using one way ANOVA with the level of significance at P<0.001 in sigma stat package version 3.1.

RESULTS AND DISCUSSION

Free radical-induced oxidative stress is the root cause for many human diseases. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells^[12]. Cells have developed antioxidant mechanisms to quench the free radicals but when the generation of free radicals exceeds the scavenging capacity of the cell, the excess free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells resulting in induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases^[13,14,15,16,17].

Oxygen is, no doubt, an indispensable part of aerobic life. However, under certain circumstances, it can seriously affect our well being through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species, and their potential deleterious effects such as atherosclerosis, ischemic heart disease, ageing, inflammation, diabetes, immunosuppression, neurodegenerative diseases, cancer and others^[18,19]. The most frequently encountered free radicals are the hydroxyl radical (HO•), the superoxide radical (O₂•-), the nitric oxide radical (NO•) and the lipid peroxy radical (LOO•) while non-free radical species principally being H₂O₂ and singlet oxygen^[20].

Nevertheless, almost all organisms are protected from free radical attack by defense mechanisms such as a preventive antioxidant system that reduces the rate of free radical formation, and another is a system to produce chain-breaking antioxidants that scavenge and stabilize free radicals. But, when free radical production rate exceeds the capacity of the antioxidant defense mechanisms substantial tissue injury results^[21]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of free radical mediated diseases.

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized,

TABLE 1: FREE RADICAL SCAVENGING ACTIVITY OF LEAVES AND RHIZOMES OF *Curcuma amada* AGAINST DPPH, ABTS AND H₂O₂

PE	PERCENTAGE SCAVENGING ACTIVITY OF					
	DPPH		ABTS		H ₂ O ₂	
	LEAVES	RHIZOMES	LEAVES	RHIZOMES	LEAVES	RHIZOMES
AE	22.69±0.2 ^{a,b}	30.48±0.23 ^{a,b}	21.93±0.13 ^{a,b}	31.13±0.23 ^{a,b}	20.26±0.63 ^{a,b}	12.28±1.05 ^{a,b}
ME	71.92±0.17 ^a	64.87±0.23 ^b	71.05±0.16 ^a	64.39±0.17 ^b	48.22±0.84 ^a	50.73±0.63 ^b
CE	61.69±0.12 ^{a,b}	54.27±0.23 ^{a,b}	52.44±1.58 ^{a,b}	60.25±0.3 ^{a,b}	44.08±0.83 ^{a,b}	33.87±0.63 ^{a,b}

N=3, values are expressed as Mean±standard deviation

- a- statistically significant compared to the methanolic extract of the rhizomes
- b- statistically significant compared to the methanolic extract of the leaves

An aqueous extract of *Andrographis paniculata* showed good antioxidant ability by scavenging the DPPH radical [23]. An aqueous extract from *Choerospondias axillaries* showed a potent scavenging effect on DPPH [24]. Methanol extracts of bark, fruits and leaves of *Ficus microcarpa* and *Caesapinia digyna* root exhibited strong scavenging effect on ABTS radical cation [25, 26] which are in agreement with our results.

Hydrogen peroxide (non-radical oxidant) and hydroxyl radicals are generated *in vitro* by many metabolic processes taking place inside the cell. The present study showed that the methanolic extract of both leaves and rhizomes showed higher ability to scavenge H₂O₂ followed by chloroform and aqueous

which can be quantitatively measured from the changes in absorbance [22]. The present study showed that the methanolic extract of both leaves and rhizomes showed higher ability to scavenge DPPH followed by chloroform and aqueous extracts. Among the two parts leaves showed higher activity than that of rhizomes. The plant extracts also exhibited similar activity in scavenging the cationic free radical ABTS as indicated in Table 1.

extracts. Among the two parts rhizomes showed higher activity than that of leaves as indicated in Table 1.

The scavenging effects of the medicinal tincture from *Pedilanthus tithymaloids* on H₂O₂ could be considered of great importance for the plant anti-inflammatory effect [27]. Leaf metabolism produces H₂O₂ at high rates but current concepts suggested that the potent signaling effect of this oxidant require that concentrations be controlled by battery of antioxidative enzymes [28].

In case of hydroxyl radical which is measured in terms of amount of TBARS (Thio barbituric acid reactive substances) formed. The present study showed that

administration of plant extracts reversed the damage caused by H_2O_2 with methanolic extract showing higher potential followed by chloroform and aqueous extracts in both the parts studied as indicated in Figure 2 and Figure 3. Similar studies were reported in Stilbene glycosides isolated from the ethanol extract of the roots of *Phygonum multiforum* thumb, which showed a better hydroxyl radical scavenging activity than that by resveratrol, which is structurally similar to the stilbene glycosides [29]. It have reported that *Acanthopanax senticosus* may work by providing hydrogen atoms from the phenolic hydroxyl groups to scavenge hydroxyl radicals generated from hydrogen peroxide [30].

Superoxide radical is a highly toxic species, which is generated by numerous biological and

photochemical reactions [31]. Superoxide radicals are more detrimental due to their role as second messengers in fibroblast proliferation in inflammation and mediators of tissue destruction [32]. Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as the marker for NO scavenging activity [33]. In the present study methanolic extract of both leaves and rhizomes efficiently inhibited the nitric oxide and superoxide generation *in vitro* followed by chloroform and aqueous extracts as indicated in Table 2.

TABLE 2: INHIBITON OF NITRIC OXIDE AND SUPEROXIDE GENERATION BY LEAVES AND RHIZOMES OF *Curcuma amada*

PE	PERCENT INHIBITION OF			
	SO		NO	
	LEAVES	RHIZOMES	LEAVES	RHIZOMES
AE	20.61±2.16 ^{a,b}	29±0.54 ^{a,b}	13.85±0.25 ^{a,b}	17.09±0.25 ^{a,b}
ME	47.7±1.62	47.71±0.54	40.74±0.64 ^a	31.83±0.25 ^b
CE	38.54±1.62 ^{a,b}	46.94±3.78	34.71±0.51 ^{a,b}	28.15±0.64 ^{a,b}

N=3, values are expressed as Mean±standard deviation

a- statistically significant compared to the methanolic extract of the rhizomes

b- statistically significant compared to the methanolic extract of the leaves

FIGURE 2: HYDROXYL RADICAL SCAVENGING ACTIVITY OF LEAVES OF *Curcuma amada*

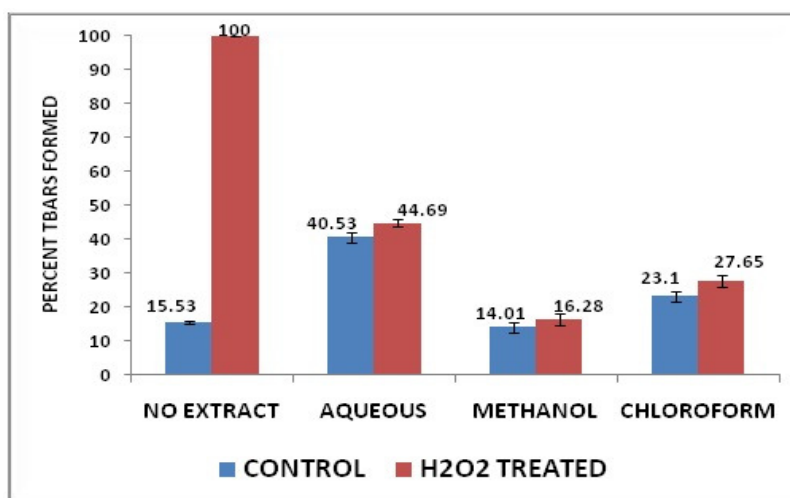
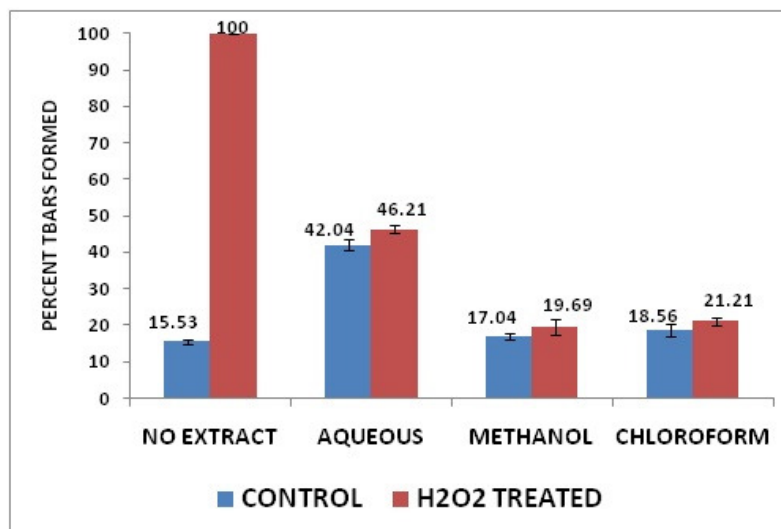


FIGURE 3: HYDROXYL RADICAL SCAVENGING ACTIVITY OF RHIZOMES OF *Curcuma amada*

Sarcodon imbricatum had strong superoxide radical scavenging activity, higher than that of references antioxidants at the same concentrations^[34]. The dose-response results of superoxide anion scavenging of the methanol extracts of the leaves of *S. macrostema* showed significant superoxide scavenging activity (76.12 %) at 100µg/ml^[35] which are in agreement with our results. A study showed that a 70% acetone extract of stem of *Caragravia hibetica* was found to be very effective in blocking superoxide radicals^[36]. Fresh homogenates of *Roseus domoscena* flower (Achuthan *et al.*, 2003) have been shown to possess strong superoxide scavenging activity^[37].

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