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IN VITRO ANTIOXIDANT ACTIVITY OF *MANGO GINGER* RHIZOME

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ABSTRACT

Free radicals implicated more than 100 diseases including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases. Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis but observed the side effects for long term use. For this reason, interest in the use of natural antioxidants has increased. The phenolic compounds in plants act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Mango ginger* rhizome. Hence, the free radical scavenging activity of *Mango ginger* rhizome were not evaluated. Therefore, the present study were to investigate the free radical scavenging activity of *Mango ginger* rhizome through DPPH, total antioxidant assay, superoxide, metal chelation and iron reducing power activity at different concentrations (20, 40, 60 and 80µg/ml). Throughout the studies flower extract showed marked antioxidant activity. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in the flower of *Mango ginger* rhizome. Overall, the *Mango ginger* rhizome extract is a source of natural antioxidants which might be

helpful in preventing the progress of various oxidative stress mediated diseases including aging.

KEYWORDS: Free radical, *Mango ginger* rhizome, Antioxidants, Oxidative stress.

1. INTRODUCTION

In living organisms, various reactive oxygen and nitrogen species (ROS/RNS) e.g., superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), nitric oxide radicals ($NO^{\bullet}$) and non-radical compounds, can be formed by different mechanisms. It is unavoidable one because of they are continuously produced by the body's normal use of oxygen. Such species are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, and cardiovascular diseases and the aging process (Velavan, 2011; Alma *et al*, 2003). This effect was significantly reversed by prior administration of antioxidant providing a close relationship between free radical scavenging activity (FRSA) and the involvement of endocrinological responses (Wiseman and Halliwell, 1996).

The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and general public to the role of natural antioxidants in the maintenance of human health and prevention and treatment of diseases (Niki, 2010). Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential (Velavan *et al*, 2007). The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthrocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003). With this background and abundant source of unique active components harbored in plants. Therefore, the present study were to investigate the free radical scavenging activity of *Mango ginger* rhizome through the free radical scavenging such as DPPH scavenging, nitric oxide, superoxide anion radical scavenging, metal chelation, reducing power activity and total antioxidant assay.

MATERIALS AND METHODS

Chemicals

Nitroblue tetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), Sodium nitroprusside (SNP), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Potassium hexa cyano ferrate

$[K_3Fe(CN)_6]$, and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant materials

Mango ginger rhizome was collected from various gardens in Keelavandanviduthy Village, Pudukkottai district, Tamil Nadu, India. The powdered rhizome material (20 g) was soaked in 50 ml of 80% alcohol for 12 hours and then filtered through a Whatmann filter paper along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and concentrated to 1 ml. A semi solid extract was obtained after complete elimination of alcohol. The *Mango ginger* rhizome extract was stored in refrigerator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity.

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992). The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*, (1999). The superoxide anion radicals scavenging activity was measured by the method of Liu *et al.*, (1997). The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.*, (1994). The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986).

RESULTS AND DISCUSSION

Table 1 and 2 depicts the radical scavenging effect of *Mango ginger* and IC_{50} values. Similar to the antioxidant activity, the reducing power of *Mango ginger* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Mango ginger* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

DPPH Assay

Recently, the use of the $DPPH^{\bullet}$ reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The

molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH$^{\bullet}$ free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH$^{\bullet}$ is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). DPPH radical scavenging activity of plant extract of *Mango ginger* and standard as ascorbic acid are presented in Table 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.*, 2003). The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid (Table 1).

Total antioxidant activity

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.*, 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The yield of the ethanol extract of the plant extract and its total antioxidant capacity are given in Table 1. Total antioxidant capacity of *Mango ginger* is expressed as the number of equivalents of ascorbic acid (Table 1). The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract.

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the extract from *Mango ginger* assayed by the PMS-NADH system were shown in Table 1. The superoxide scavenging activity of *Mango ginger* was increased markedly with the increase of concentrations. These results suggested that *Mango ginger* had notably superior superoxide radical scavenging effects.

Table 1: Shows the Radical scavenging activity of *Mango ginger*

Concentration (µg/ml)	DPPH	Standard (Ascorbic acid)	Total Antioxidant Assay	Standard (Ascorbic acid)	Superoxide anion radical scavenging	Standard (Ascorbic acid)
20	31.81±2.22	25.6±2.04	40.62±2.84	22.35± 1.80	22.12 ±1.23	31.25 ± 2.50
40	45.48±3.18	61.26±4.90	50.00±3.5	51.23± 4.09	43.15 ±3.02	64.23 ± 5.13
60	54.00±3.78	88.98±7.11	56.25±3.93	72.54± 5.80	61.23 ±3.89	89.54 ± 7.16
80	63.61±4.45	99.34±7.94	62.50±4.37	86.35± 6.91	79.56 ±5.23	98.51 ± 7.88
IC_{50}	52.46	35.03	43.53	42.41	48.51	31.62

Values are expressed as Mean ± SD for triplicates

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of aqueous extract of *Mango ginger*, indicating that have chelating activity (Table 2). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell, 1991; Fridovich, 1995). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form s bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Mango ginger* demonstrates a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Reducing power activity

The measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Mango ginger*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Table 2). However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al*, 2000).

Table 2: Shows the Radical scavenging activity of *Mango ginger*

Concentration (µg/ml)	S.No	Reducing power	Standard (Ascorbic acid)	Fe^{2+}chelating	Standard (Ascorbic acid)
20	1	0.22±0.01	0.41± 0.03	23.07±1.61	35.23 ± 2.81
40	2	0.27±0.018	0.71 ± 0.05	46.15±3.23	65.21 ± 5.28
60	3	0.32±0.02	0.89 0.07±	80.78±5.65	78.51± 6.28
80	4	0.75±0.05	0.98 ± 0.08	92.30±6.46	98.65 ± 7.89
IC_{50}	IC_{50}	-----	-----	41.32	30.96

Values are expressed as Mean ± SD for triplicates

CONCLUSION

On the basis of the results of this study, it clearly indicates that *Mango ginger* rhizome had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, superoxide anion scavenging and metal chelator. From our results, the antioxidant activity of *Mango ginger* rhizome was concentration dependent. The extracts could exhibit antioxidant properties approximately comparable to commercial synthetic antioxidants as ascorbic acid. From the above assays, the possible mechanism of antioxidant activity of *Mango ginger* rhizome includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.

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