Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3): 260-264

Research Article ISSN 0975-248X

Possible Mechanism of *Murraya koenigii* and *Cinnamomum tamala* With Reference to Antioxidants Activity

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ABSTRACT
Antioxidants are one of the most important nutraceutical compounds that have emerged from the recent decades of research in food science. The advances in this field have allowed a better understanding of the free radical damage of cellular constituents, such as lipids, proteins and DNA. Antioxidants and radical scavengers have a crucial role in the treatment or prevention of several diseases such as type 2 diabetes, atherosclerosis, cancer, cardiovascular disorders and neurodegenerative disorders. Restrictions on the use of synthetic antioxidants are being imposed because of their toxic properties. The present study is the continuation of a program aimed at investigation on antioxidant activity of extracts from medicinal plants and to identify alternative natural and safe sources of food antioxidant especially from plant origin. In this report the anti-peroxidative effect of alcoholic extract of *Murraya koenigii* and *Cinnamomum tamala* have been studied in rat liver homogenate where ferrous sulphate has been used as inducer to induce lipid per oxidation. On the basis of results, it could be concluded that TBARS production in normal condition group is very slow and it is very high in $FeSO_4$ treated groups. Results further revealed that at lower doses, the rate of formation of TBARS is slow but grows as the level of dose is increased. Significant and moderate results were found from 0.40 mM to 0.80 mM of ferrous sulphate. The mechanisms underlying the beneficial effects may be related to the antioxidant effects of the polyphenols resulting in decreased free radical production.

Keywords: *Murraya koenigii*, *Cinnamomum tamala,* anti-peroxidative effect, lipid peroxidation, ferryl- perferryl complex, TBARS production.

INTRODUCTION

Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases. According to World Health Organization, 65-80% of the world populations rely on traditional medicine to treat various diseases. To date, many plants have been claimed to pose beneficial health effects such as antioxidant and antimicrobial properties. With the emergence of multiple strains of antibiotic resistance microorganism, great interest has been generated in search for potential compounds from plants for therapeutic, medicinal, aromatic and aesthetic uses. [1] Free radicals are capable of inducing lipid peroxidation in biological membranes. Lipid peroxidation induced damages and it's involved in ageing and pathological disorders, atherosclerosis, lipofuscinosis, intermittent oxygen toxicity and liver injury caused by oratic acid and ethanol. [2-9] The effects of free radicals on human beings have recently been considered as their close toxicity, diseases and ageing. [10-11] Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. [12] Besides, well known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices, some natural antioxidants (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements. [13] Active oxygen species can easily initiate the lipids causing damage of the cell membrane constituents i.e. phospholipids, lipoproteins by propagating a reaction cycle. [14] It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds. [15] Flavonoids and a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action. [16] *Murraya koenigii*, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Srilanka and other south Asian countries. Leaves are rich in minerals, vitamin A, vitamin B, and are a rich source

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of carbohydrates, proteins, amino acids and alkaloids. [17-18] The plant has also been used in traditional Indian medicine systems for a variety of ailments. [19-20] It was found that reduction in total serum cholesterol and an increase in the HDL and lower release of lipoproteins into the circulation take place when rats were fed with a standard diet along with curry leaves. [21] Curry leaves also exhibited strong antioxidant property on liver and heart. It was found that phenolic antioxidant is present in *Murraya koenigii* and other herbs. [22] Hypoglycemic activity of *Murraya koenigii* on normal and diabetic rats was found. [23] The beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta cells in experimental diabetes in rats was studied. [24] *Cinnamomum tamala* Nees and Ebern. (Hindi- Tejpat) is an evergreen tropical tree, belonging Lauraceae family. It is mainly used for flavoring food and widely used in pharmaceutical preparation because of its hypoglycemic, stimulant and carminative properties**.** Essential oil extracted from the leaves contains monoterpenoids including phellandrene, eugenol, linalool and some traces of α-pinene, *p*cymene, ß-pinene and limonene, phenylpropanoids. This plant is frequently mentioned in various Ayurvedic literatures for its various medicinal values. It is also used in Indian system of traditional medicines. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhoea, nausea and vomiting. Ancient literature has revealed that in the first century A.D. dried leaves and bark of this plant were prescribed for fever, anemia and body odour. Its seeds were crushed and mixed with honey or sugar and administered to children for dysentery. The available in vitro and animal in vivo evidence suggests that cinnamon has anti-inflammatory, antimicrobial, antioxidant, antitumor, cardiovascular, cholesterol-lowering, and immunomodulatory effects. In vitro studies have demonstrated that cinnamon may act as an insulin mimetic, to potentiate insulin activity or to stimulate cellular glucose metabolism. [25] Therefore, this work was designed to investigate antioxidant activity of *Murraya koenigii and Cinnamomum tamala* and their plausible mechanism.

MATERIAL AND METHODS

Preparation of Alcoholic Extract

One kg of *Murraya koenigii and Cinnamomum tamala* was dried, powdered and the material was extracted with ethanol by cold percolation method (material was dipped into ethanol for 7 days) and ethanol was collected. The extract was freed from solvent under reduced pressure to give a red brown, highly viscous syrup. The yield was 21.4% and 11.2% respectively. The ethanolic extract of *Murraya koenigii and Cinnamomum tamala* was tested for its anti peroxidative property in animal system.

Preparation of Tissue Homogenate

Rats were fixed on the operation table with ventral side up and then dissected. Liver was perfused with normal saline through hepatic portal vein. Liver was harvested and its lobes were briefly dried between filter papers (to remove excess of blood) and were cut thin with a heavy-duty blade. These small pieces were then transferred to the glass Teflon homogenizing tube to prepare homogenate (1 g, w/v) in phosphate buffer saline (pH 7.4) in cold condition. It was centrifuged at 2000 g, for ten minutes. Supernatant was collected and finally suspended in PBS to contain approximately 0.8-1.5 mg protein in 0.1 ml of suspension to perform the *in vitro* experiment.

Estimation of Lipid peroxidation in terms of TBA –RS

0.1 ml of reaction mixture (5% homogenate with or without toxin treated/drug treated) was transferred to a tube containing 1.5 ml of 10% trichloroacetic acid (TCA). After 10 minutes tubes were centrifuged and TCA soluble fraction was fully separated to develop the colour reaction. Now the tube containing TCA soluble fraction was added to 1.5 ml thiobarbituric acid (TBA) in 50% acetic acid and mixed well. It was heated in boiling water bath for 30 min, to complete the reaction. The tubes were cooled to determine the absorbance at 535 nm. The values were evaluated on the standard curve using 1, 1, 3, 3-tetra ethoxy propane (TEP).

Statistical Evaluation

The results, given here are the mean ± SD of six separate experiments. Level of significance has been evaluated by using student's test.

Table 1: Effect of different concentration of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

S. No.	$FeSO_4$ (mM)	TBA-RS (n mole/100mg protein)
1	0.00	72.97 ± 10.17
2	0.10	123.60 ±12.68
3	0.20	220.82 ± 13.38
4	0.30	310.40 ± 10.18
5	0.40	405.69 ± 20.28
6	0.60	430.52 ±14.09
7	0.80	575.23 ± 16.42
8	1.25	660.26 ± 18.37

Values are mean ± SD of six different experiments

Table 2: Effect of Incubation period of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

S. No	Drug (µg/ml)	TBA-RS (n mole/100 mg protein)	
		M. Koenigii	*C. Tamala*
1	0	79.23±18.20	81.16±32.10
2	$FeSO_4$	405.83±20.18	405.83±20.18
3	40	400.10±10.20	400.10±10.20
4	80	345.18±16.20	402.18±16.28
5	160	280.10±10.20	395.14±9.24
6	320	225.18±8.20	397.14±8.20
7	600	190.24±8.80	402.14±8.14
8	1000	125.23±6.40	394.12±6.40

Values are mean ± SD of six different experiments

Table 3: Comparative study of *Murraya koenigii* and *Cinnamomum tamala* on ferrous sulphate induced lipid peroxidation in rat liver homogenate

S. No	$FeSO_4$ (mM)	TBA-RS (n mole/100 mg protein) Time (minutes)			
		15 (A)	30 (B)	45 (C)	60 (D)
1	0.00	69.62 ±10.13	72.97 ±16.13	77.29 ±23.13	81.09 ±10.12
2	0.10	150.60 ±14.63	123.24 ±12.18	325.50 ±16.64	340.24 ±16.24
3	0.20	190.07 ±19.51	220.62 ±13.28	330.24 ±8.74	440.64 ±11.84
4	0.30	213.74 ±18.74	310.78 ±10.13	380.44 ±9.79	450.24 ±10.18
5	0.40	240.24 ±24.20	405.62 ±12.28	479.58 ±12.13	570.50 ±10.11
6	0.60	280.34 ±14.13	430.52 ±15.90	609.62 ±10.09	716.08 ±9.13
7	0.80	325.93 ±16.28	575.20 ±8.13	610.24 ±22.13	650.74 ±19.45
8	1.25	421.24 ±18.14	660.26 ±9.14	750.05 ±20.13	839.15 ±13.14

Values are mean ± SD of six different experiments

RESULTS

Effect of different concentrations of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

This experiment was aimed to determine the optimum dose of ferrous sulphate for induction of lipid peroxidation in our experimental conditions. 3 ml of rat liver homogenate (5% in phosphate buffer saline, pH 7.4) was taken to each 35 mm Petridis. To these plates, different concentrations of ferrous sulphate were added as given in Table 1. Plates were mixed gently and incubated for 30 minutes. At the end of incubation time, 0.1 ml of aliquots was taken out from each plate to estimate TBARS, produced. Results were compared with the normal control value, obtained under similar conditions.

Dose dependent increased in lipid peroxidation has been seen (Fig 1). Results show that at lower doses, the rate of formation of TBARS is slow which increases with dose. Significant and moderate results have been found from 0.40 mM to 0.80 mM of ferrous sulphate.

Effect of incubation period on ferrous sulphate induced lipid peroxidation in Rat liver homogenate

As mentioned in the literature, TBARS production in various systems depends upon the incubation time, concentration of inducers and the presence of antioxidants. These observations led us to find out the proper incubation time to induce optimum lipid peroxidation in our laboratory conditions.

Fig. 2 indicates that in control conditions, 69.63 nmoles of TBARS is formed at the time of 15 minutes which increases to 81.09 nmoles at 60 minutes. In ferrous sulphate treated dishes formation of TBARS increases 2 to 3 folds over the control value at the same time points. The optimum point was selected as 30 minutes.

Comparative study of *Murraya koenijii* and *Cinnamomum tamala* on ferrous sulphate induced lipid peroxidation in rat liver homogenate

The aim of this study is to compare the optimum dose and time of the antioxidative effect of the alcoholic extract of *Murraya koenigii* and *Cinnamomum tamala* (Table 1).

Fig. 3 clearly indicates that protection is very high and significant in case of *Murraya koenigii* than *Cinnamomum tamala.*

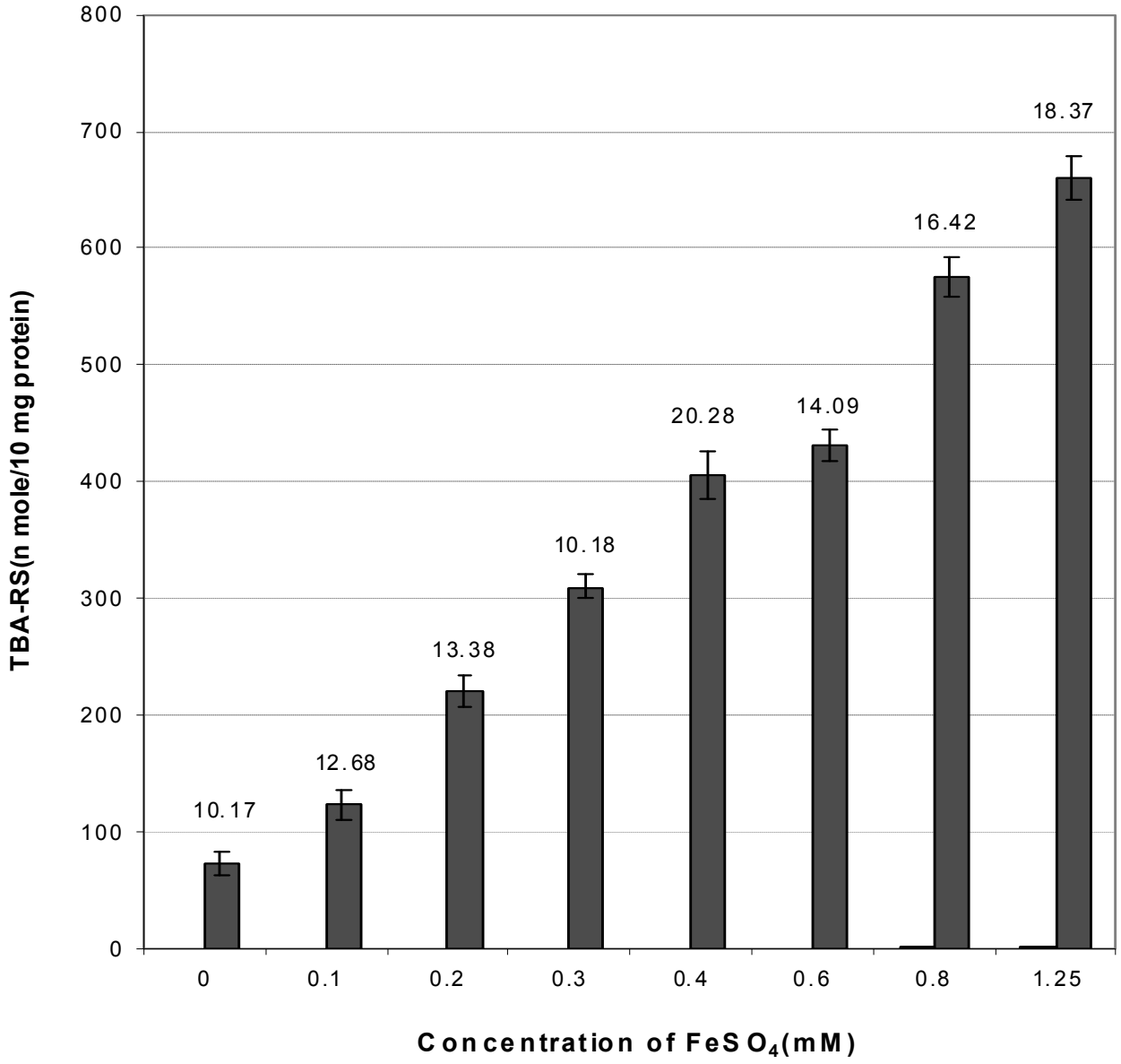

Fig. 1: Effect of different concentration of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

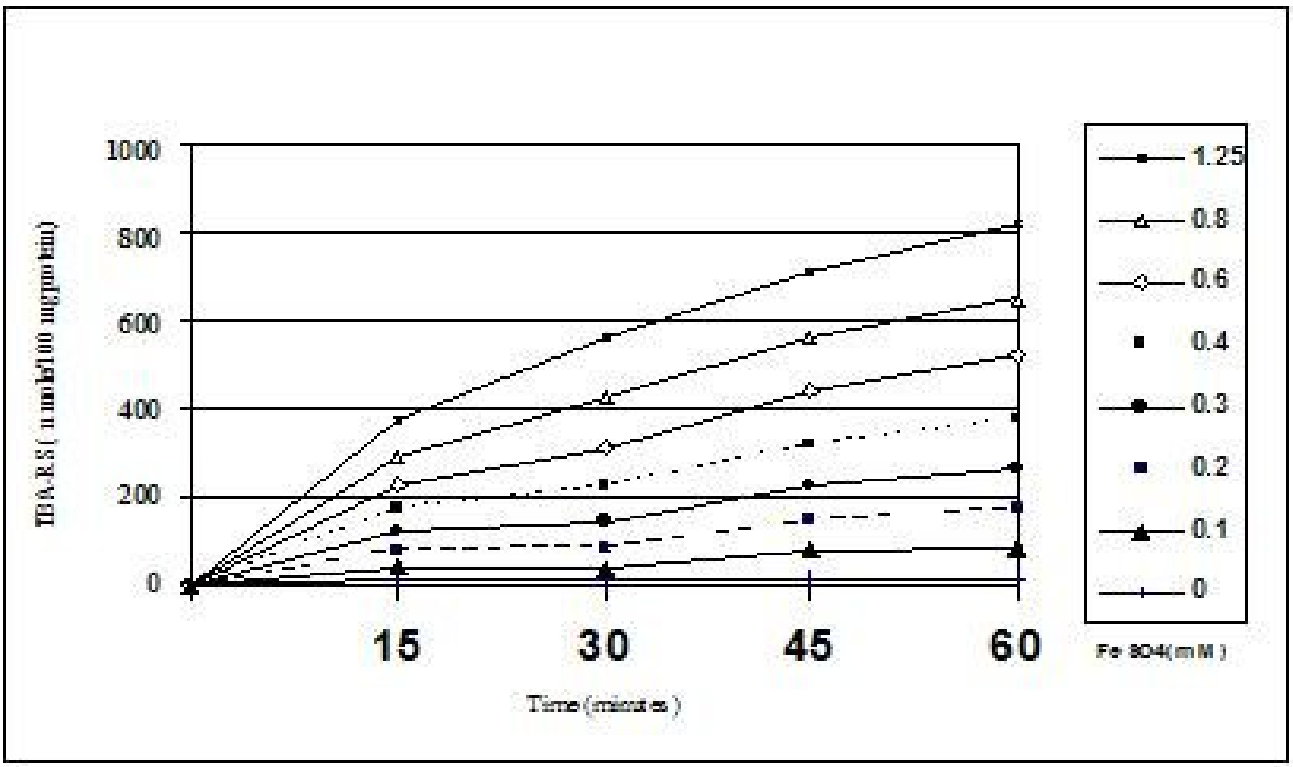

Fig. 2: Effect of Incubation period of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

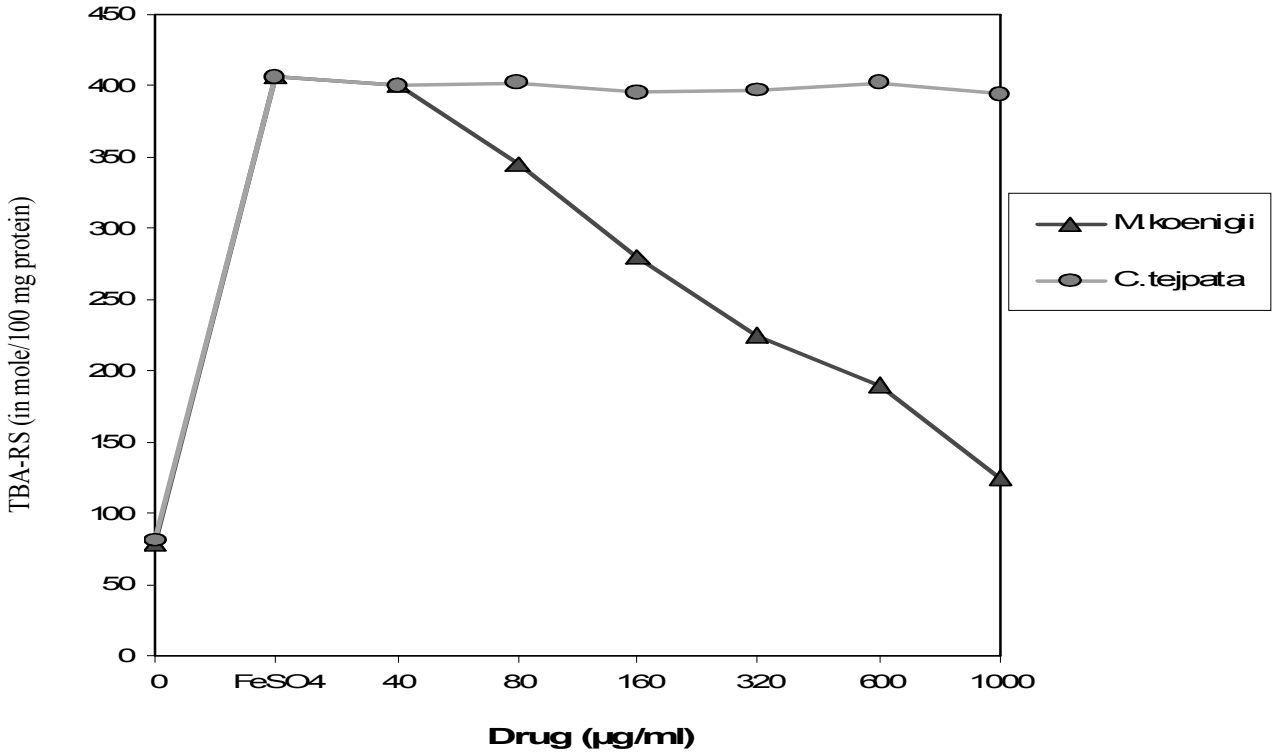

Fig. 3: Comparative study of *Murraya koenigii* and *Cinnamomum tamala* on ferrous sulphate induced lipid peroxidation in rat liver homogenate

DISCUSSION

Oxidative stress which is increased in obesity plays an important role in the development of diabetes and cardiovascular diseases in people. The objective of study was to explore possible mechanism of antioxidant activity of *Murraya koenigii* and *Cinnamomum tamala.* Cinnamon, a natural product with a long history of safety is rich in polyphenolic components that have been shown to improve the action of insulin *in-vitro*, in animal studies [26] and to possess in vitro antioxidant activity. In the present study, cinnamon extracts at 500 mg/d for twelve weeks decreased oxidative stress and improved impaired fasting glucose.

Moreover, % fat mass decreased 0.7% for the subjects consuming the capsules containing the cinnamon extract and lean body mass increased 0.6 kg. [26]

Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation etc. Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine 'Ayurveda'. Many natural and synthetic antioxidants are in use to prevent lipid peroxidation. In this report, the alcoholic extract of *Murraya koenigii* has been investigated for its protective response. Plant extracts can be characterized by polyvalent formulations and interpreted as additive, or in some cases; potentiating. [27] Anti-lipid peroxidative property of *Murraya koenigii* might be either due to chelating or redox activity. The specific ratio of ferrous to ferric is important for induction of lipid peroxidation. It has been reported that at least 1:1 ratio of ferrous to ferric is critical for initiation of lipid peroxidation. Therefore, antioxidant activity of *Murraya koenigii* may result from multiple factors involving hydrogen or electron transfer, metal chelating activity and synergistic activity and appear to be the result of many different activities.

It is appeared that essential oils and flavonoids polymers found in cinnamon with insulin-like biological and antioxidant activities could improve plasma fasting glucose and oxidative stress markers in people at high risk of oxidative stress. Considering the activities of free radicals and concentrations of substrates, the phenolic compounds from natural sources are promising candidates for drugs for atherosclerosis, diabetes etc. depending on their reactivity towards free radicals, localization, mobility in lipoprotein and fate of its radicals.

It can be concluded that high activity of *Murraya koenigii* than *Cinnamomum tamala* may result from multiple factors involving hydrogen or electron transfer, metal chelating activity and synergistic activity due to high content of polyphenols. Phytochemical analysis revealed that carbohydrate, tannin, alkaloid, steroid, triterpenoid, and flavonoid were present in the extracts *of M. koenigii* leaves and appears to be the result of many different activities.

ACKNOWLEDGMENT

The authors would like to thank IWWET Society for funding and technical support as well as it's Investigation team and all our colleagues, students who contributed technical and moral supports to make this Research successful.

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