

## Determination of Antioxidant Potential of *Momordica Foetida* Leaf Extract on Tissue Homogenate

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### Abstract

The antioxidant activity of *Momordica foetida* (leaves), a widely used medicinal plant is evaluated by thiobarbituric acid reactive species (TBARS) and iron chelation assays in vitro. The ethanolic extract of *Momordica foetida* leaves (3.33-330 µg/ml) inhibited lipid peroxidation induced by 60 µM iron II sulphate (FeSO<sub>4</sub>) in rat brain and liver homogenate. The result revealed maximum inhibition (47.6%) in liver at concentration of 6.66 µg/ml and maximum inhibitory effect (63.3%) in the brain at concentration of 330 µg/ml. Thus, the inhibitory effect of *Momordica foetida* leaves on lipid peroxidation and iron chelating activity were concentration dependent. Therefore the results suggest that the leaves of *Momordica foetida* could act as antioxidant agent which is associated with its medicinal use since it prevent Fe<sup>2+</sup> - induced lipid peroxidation due to its ability to chelate iron.

**Keyword:** antioxidant activity, thiobarbituric acid reactive species (TBARS) assay, *Momordica foetida*

### Introduction

*M. foetida* is a medicinal plant belonging to the family of Cucurbitaceae, kingdom plantae, phylum Schumacher, genus *Momordica*, and species *foetida*. The plant has both male and female flowers (Jeffrey, 1990). The plant (*M foetida*) is widely distributed in tropical Africa, South-Africa and also available in Flora of west tropical Africa (Burkill, 1985). The main interest in this particular plant at present appears to lie in the medicinal aspects. The insecticidal properties are only recognized in small area within its range of distribution (Baerts and Lehmann, 2002). The leaves have a bitter taste. Their nutritional composition per 100g edible portion is; energy 92kj (22kcal), protein 3.3g, fibre 3.2g, calcium 1.1mg, iron (Fe) 3.4mg, zinc (Zn) 0.4mg, Beta-carotene 5.4mg, foliate 40?g, ascorbic acid 20.6mg (Steyn, 2001). The leaves of *Momordica foetida* are collected from the wild and eaten after boiling as a vegetable in Gabon, Sudan, Uganda, Tanzania and Malawi (Olaniyi, 1975). They seem fairly unpopular and are eaten in small quantities only, usually in times of scarcity. The pulp of the ripe fruits is eaten in Ghana, Gabon, Sudan, Kenya, Uganda and Tanzania. The plants are grazed by cattle (Ruffo, 2002) and are said to be especially suitable for fattening rabbits. The effects of oxidative stress to a cell vary from cell damage apoptosis or cell suicide to necrosis (Whittaker, 1997). Some body organs are predisposed to greater levels of relative oxygen species than others. These are the lungs, brain, eye, liver, kidney and reproductive organs. The lungs are exposed to high levels of high reactive oxygen species because it is exposed to continuous high levels of oxygen. The brain on the other hand is susceptible to oxidative stress since it involves high metabolic activity. The brain is after all the body's main control mechanism; moreover it has low levels of

endogenous antioxidants to counteract reactive oxygen species. This organ is predisposed to formation of reactive oxygen species because it is constantly exposed to damaging ultraviolet. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's diseases, Parkinson's diseases, the pathologies caused by diabetes, rheumatoid arthritis and neurodegeneration in motor neuron disease (Davies, 1995). The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases (Golfrab, 1999). Antioxidants are widely recognized as ingredient and supplement in dietary process in the hope of maintaining health and preventing diseases such as cancer, coronary heart disease and even altitude sickness. The aim of this study is to determine the antioxidant potential of *M. foetida* leaf extract.

### Materials and methods

#### Materials

Thiobarbituric acid (TBA), Malondialdehyde-bis-dimethyl acetyl (MDA) sodium dodecyl sulphate (SDS), Acetic acid, Ethanol (70 %). Tris 0.1M HCl, O-phenanthroline and iron (II) sulphate were used.

#### Preparation of plant extracts

Fresh leaves of *M. foetida* were collected and authenticated in Plant Science Department University of Ado-Ekiti, Nigeria. The leaves were sliced, dried at room temperature and powdered. The powdered sample was extracted with cold 70% ethanol at room temperature (27°C) for 48 hours. The filtrate (ethanolic extract) was evaporated to dryness using rotary evaporator giving a percent yield of 5.7%.

#### Test Animals

All animal procedures were in strict accordance with the NIH guide for the care and use of laboratory animals. Two to three months old Wistar rats (200-250g) were used for the invitro studies.

#### Production of Thiobarbituric Acid reactive species (TBARS)

The production of TBARS was determined using a modified method of Ohkawa *et al*, 1979 as described by Puntel *et al*, 2005. The rats were killed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice. One gram of tissues was homogenized in cold 0.1M Tris buffer pH 7.4 (1:10 w/v) in an up and down stroke in a Teflon homogenizer. The homogenates were centrifuged for 10

minutes at 3000g to yield a pellet that was discarded and the supernatant was used for the assay. The supernatant (100 $\mu$ L) with or without 50 $\mu$ L of the freshly prepared prooxidant (Iron II sulphate), different concentrations (3.33-330 $\mu$ g/ml) of the plant extracts and an appropriate volume of distilled water which gives a total volume of 300  $\mu$ L were incubated at 37<sup>o</sup>c for 1hour.

The colour reaction was carried out by adding 200,500 and 500 $\mu$ L each of the 8.1% sodium dodecyl sulphate (SDS), 1.33M acetic acid (pH 3.4) and 0.6% TBA respectively. The reaction mixtures including those of serial dilutions of 0.03mM standard malondialdehyde (MDA) were incubated at 97<sup>o</sup>c for 1hour. The absorbance was read after cooling at a wavelength of 532nm in a visible / UV spectrophotometer.

### Iron chelating assay

The ability of the ethanolic extracts to chelate iron II Sulphate ( FeSO<sub>4</sub> ) was determined using a modified method of Puntel et al, 2005. Briefly 20 l of freshly prepared 1mM FeSO<sub>4</sub> were added to a reaction mixture containing 168 l of 0.1M Tris Hcl (pH 7.4), 218 l saline (0.9 NaCl) and the ethanolic extract of the plant (1-10 g/ml). The reaction mixture was left for 5minutes before the addition of 13 l of 0.25% phenanthroline (w/v). The absorbance was subsequently measured at 510nm in the spectrophotometer

### Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Duncan's multiple range test when appropriate and regression analysis.

### Results and Discussion

Oxidative stress, the consequence of an imbalance of pro-oxidants in the organisms rapidly gains recognitions as a key phenomenon in chronic diseases. It is directly involved in the pathogenic mechanism of risk factors and in the protection exerted by various environmental factors (Ines, 2000), and the magnitude of environmental risk factors. Oxidative stress is now recognized to be associated with diseases as well as with normal aging process (Ghasanfari *et al*, 2006). There is a strong correlation between thiobarbituric acid reactive specie (TBARS) substances as a marker of lipid peroxidation and products that reflect oxidative damage to DNA. Iron II sulphate (FeSO<sub>4</sub>) was used as a tool to induce lipid peroxidation. The ethanolic extract of *M. foetida* exhibited good antioxidant against pro-oxidant in tissue studied (liver and brain). Increase in the formation of TBARS in FeSO<sub>4</sub> 60 $\mu$ M - induced oxidative stress as compared to the normal suggests possible damage of tissues with an overload of iron. Free iron in the cytosol and mitochondria can cause considerable oxidative damage by increasing superoxide production which can react with iron II (Fe<sup>2+</sup>) to generate iron III (Fe<sup>3+</sup>) (Fraga and Oteiza, 2002).

The results clearly showed that incubation of the rat's brain in the presence of 60 $\mu$ M Fe<sup>2+</sup> caused a significant increase in the malonaldehyde (MDA) contents of the brain when compared with the normal brain homogenate. The increased lipid peroxidation in the presence of Fe<sup>2+</sup> could

be attributed to the fact that Fe<sup>2+</sup> can catalyze one electron transfer reaction that generate reactive oxygen species. Table 1 shows the inhibitory effect of ethanolic extract of *M. foetida* on Fe<sup>2+</sup> induced lipid peroxidation in rat liver homogenate. The result revealed that incubation of liver tissues in the presence of (60 $\mu$ M) Iron II sulphate cause 87% significant increase in the malonaldehyde (MDA) content of the liver when compared to normal. The results established that inhibitory effect of ethanol extract significantly reduce the lipid peroxidation in liver tissue in a concentration dependent manner from 3.33 $\mu$ g/ml to 40  $\mu$ g/ml. The ethanolic extract caused 34.6%, 35.0% and 47.6% inhibition in the liver at a concentration of 13.3  $\mu$ g/ml, 33.3 $\mu$ g/ml and 6.66 $\mu$ g/ml respectively (IC<sub>50</sub> = 8.10  $\mu$ g/ml  $\pm$  9.61).

Table 2 established the inhibitory effect of ethanolic extract of *M. foetida* on Fe<sup>2+</sup> induced lipid peroxidation in rat's brain homogenate. The results suggest that there is 75% inhibition and significant increase in TBARS content of the brain homogenate respectively. The ethanolic extract shows 51.7%, 59.9% inhibitory effect of lipid peroxidation at a concentration of 33.3 $\mu$ g/ml and 40 $\mu$ g/ml respectively (IC<sub>50</sub> 3.98  $\pm$  2.00 $\mu$ g/ml).

In order to provide an explanation for the inhibition of iron (Fe<sup>2+</sup>) induced lipid peroxidation in rat's brain and liver homogenate, the Fe<sup>2+</sup> chelating ability of *M. foetida* leaf were determined and the results were shown in figure 1. The results revealed the chelating ability of ethanolic extract of *M. foetida* on 1mMFeSO<sub>4</sub> in a concentration dependent manner. Ethanolic extract of foetida leaf showed an excellent chelating ability of 94.4% at a concentration of 1 $\mu$ g/ml and minimum chelating ability of 26.9% at 1000 $\mu$ g/ml. The chelating ability of the ethanolic extract of *M. foetida* have accounted for the higher inhibition of Fe<sup>2+</sup> induced lipid peroxidation in tissue homogenate (liver and brain), The chelating ability of the *M. foetida* increases as the concentration of the extract increases. Also the solvent used (ethanol) as a vehicle demonstrates no antioxidant potential as seen in tables 1 and 2 and as such there is statistical difference between the test samples and the solvent. This study indicate that iron chelating ability could be a major contributory mechanism by which *M. foetida* prevent lipid peroxidation in addition to inhibitory effect on thiobarbituric acid reactive species.

### Conclusion

In conclusion, We have demonstrated that extracts of Momordica leaf contain high levels of antioxidant and is capable of inhibiting lipid peroxidation directly by quenching free radicals to terminate the radical chain reaction acting as a reducing agent and chelating transition metals to suppress the initiation of radical formation as well as inhibiting reactive oxygen species. In addition, the data on extraction procedures and antioxidant activity assessment obtained in these experiments single out ethanol as the most promising sources for the isolation of natural antioxidative compounds from the leaves of Momordica foetida, although the antioxidant activity found in an invitro experiment is only indicative of the potential

health benefit, these results remain important as the first step in screening antioxidant activity of *M. foetida* leaves and can be used as an accessible source of natural antioxidants.

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**TABLE 1:** The inhibitory effect of ethanolic extract of *Momordica foetida* on Iron II sulphate induced lipid peroxidation in rat liver homogenate.

Concentration ( $\mu\text{g/ml}$ )	MDA (nmole/g tissue)	% Inhibition	Logarithm equation ( $r^2$ )	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
Normal	79.35 $\pm$ 16.5	10.63 $\pm$ 18.4		8.10 $\pm$ 9.61
Control	517.56 $\pm$ 7.2	-		
Solvent	317.39 $\pm$ 22.8	5.4 $\pm$ 20.6		
3.33	366.08 $\pm$ 15.8	31.6 $\pm$ 24.9	Y = -5.052	
6.66	276.24 $\pm$ 13.3	47.6 $\pm$ 19.9	In x 37.981	
13.3	342.63 $\pm$ 14.6	34.6 $\pm$ 23.3	( $r^2 = 0.16$ )	
26.6	385.91 $\pm$ 11.4	22.8 $\pm$ 30.3		
33.3	319.26 $\pm$ 13.2	35.0 $\pm$ 31.9		
40	281.32 $\pm$ 16.0	22.0 $\pm$ 5.3		
330	358.86 $\pm$ 12.5	30.0 $\pm$ 6.9		

Results are expressed as means of three experiments in duplicate  $\pm$  standard deviation

**TABLE 2:** The inhibitory effect of ethanolic extract of *Momordica foetida* on Iron II sulphate induced lipid peroxidation in rat brain homogenate.

Concentration ( $\mu\text{g/ml}$ )	MDA (nmole/g tissue)	% Inhibition	Logarithm equation ( $r^2$ )	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
Normal	79.4 $\pm$ 11.3	- 11.9 $\pm$ 73.1		3.98 $\pm$ 2.00
Control	328.2 $\pm$ 20.1	-		

Solvent	360.7 ± 7.2	- 85.9 ± 71.8	
3.33	375.1 ± 21.9	- 41.9 ± 42.8	Y = 40.023
6.66	216.4 ± 19.5	19.6 ± 26.6	In x-11.229
13.3	209.2 ± 14.1	34.3 ± 11.8	(r <sup>2</sup> = 0.99)
26.6	156.9 ± 7.5	48.8 ± 38.5	
33.3	153.3 ± 13.3	51.7 ± 14.0	
40	140.7 ± 12.7	59.9 ± 14.0	
330	173.3 ± 12.6	63.2 ± 15.6	

Results are expressed as means of three experiments in duplicate ± standard deviation

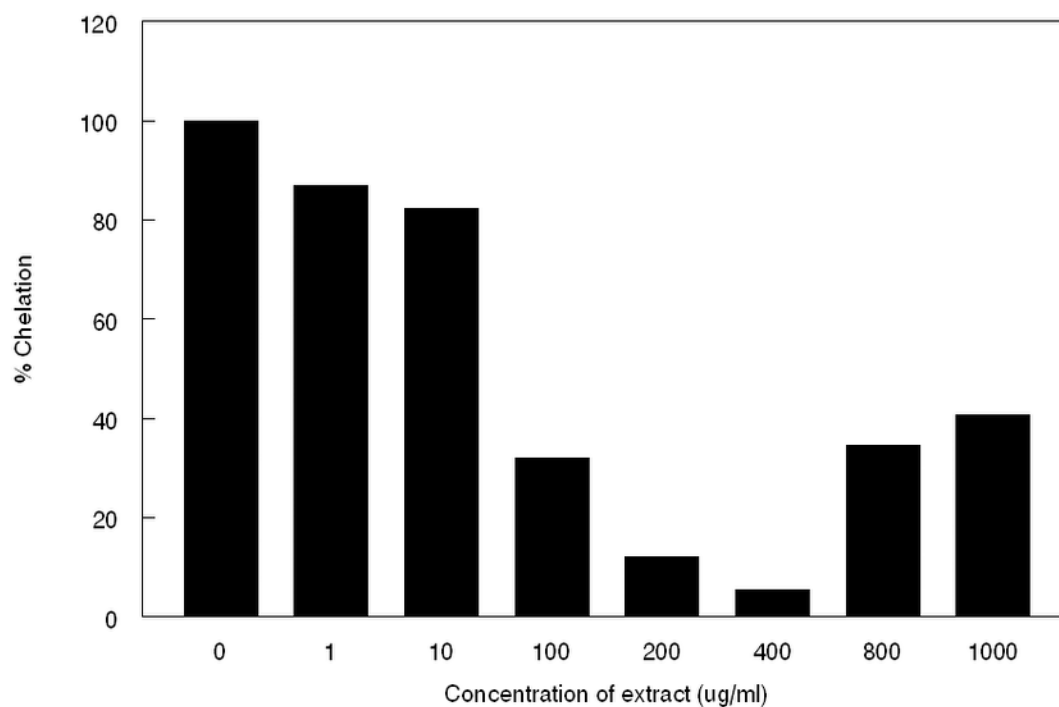


Fig 1 Iron Chelating ability of Ethanolic Extract of *M. foetida* leaf