



Original Research Article

Antitrypanosomal and Antioxidant Activities of *Moringa Oleifera* Lam Leaf Extracts

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ABSTRACT

Commercially synthetic antioxidants are quite unsafe and their toxicity is a problem of concern. Antitrypanosomal and antioxidant activity of extracts and compounds obtained from *Moringa oleifera* leaves were evaluated using *in vitro* techniques. The crude ethyl acetate extract was subjected to different chromatographic techniques to obtain a fraction showing antitrypanosomal activity (MIC 25µg/ml). In addition, antioxidant activities of the crude extracts were established on the free radical scavenging activity of DPPH. The extracts showed strong antioxidant activity with 50% efficient concentration (EC₅₀) values of 24 and 44 µg/ml for the ethyl acetate and methanol extracts respectively. The results of this study suggest the potentials of ethyl acetate extract of *M. oleifera* in treating trypanosomiasis a disease caused by *Trypanosoma brucei*.

Keyword: Trypanosomiasis; antioxidant; *Moringa oleifera*; free radical scavenging

INTRODUCTION

Trypanosomiasis is a parasitic disease caused by fly (*Glossina* spp) [1, 2] There are two major species of trypanosomes: *Trypanosoma brucei* and transmitted by tsetse

rhodesiense prevalent in the Eastern and Southern Africa and *T. b. gambiense* responsible for trypanosomiasis (sleeping sickness) in West and Central parts of Africa. Other species, *T. congolense* and *T. vivax* cause nagana in livestock (cattle, sheep and goats). The disease affects both human and cattle with serious health and economic challenges in sub-Saharan Africa. Human African trypanosomiasis (HAT) is widely spread in over 30 African countries affecting over 60 million people and has reached epidemic proportions in some countries such as Angola, Southern Sudan, Uganda and the Democratic Republic of Congo [3]. World Health Organization estimates that HAT cases are about 300,000-500,000 annually. Currently, there is no vaccine for the disease. Treatment is based on only four approved drugs: Suramin, Pentamidine, Melarsoprol and Eflornithine. Incidences of high cost, toxicity and drug resistance have made the development of new, safe, and cheaper drugs from natural sources for the treatment of HAT a concern.

Based on traditional use, some Nigerian medicinal plants such as *Afrormosia laxiflora*, *Anogeissus leiocarpus*, *Annona senegalensis*, *Cochlospermum planchonii*, *Khaya senegalensis*, *Piliostigma reticulatum*, *Prosopis africana*, *Securidaca longipedunculata* and *Terminalia avicennioides* have been reported to exhibit antitrypanosomal activities [4].

Antioxidants are important inhibitors of oxidative species generation and scavengers of free radicals that provide protection to humans and animals against infections and degenerative diseases with oxidative stress aetiology. Reactive oxygen molecules (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxide ion (OH^-) are continuously generated inside a living body; under normal homeostatic conditions, ROS generated are detoxified by some inherent endogenous antioxidants.

Over production of ROS and/or inadequate antioxidant defence adversely affect the

equilibrium in favour of ROS upsurge that causes oxidative stress. These free radicals readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA. Consequently, this oxidative damage is responsible for several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process.

For adequate protection against the effect of oxidative stress, there is a growing interest on antioxidant constituents of plant materials due to their roles in the maintenance of human health. Plant and its products are rich sources of phytochemicals that have been found to possess a variety of biological activities including antioxidant potential [5]. The antioxidants could reduce this oxidative damage of a tissue indirectly by enhancing natural defences of cell and/or directly by scavenging the free radical species. Plant extracts and plant-derived antioxidants can elicit a number of *in vivo* effects such as promotion of increased synthesis of endogenous antioxidant defences or themselves acting directly as antioxidants.

M. oleifera is widely used in medicine. Reports on the active constituents and bioactivity of this plant are abundant. Some of the medicinal properties of this plant include: antitumor [6, 7], antipyretic, anti-inflammatory, antiulcer [8, 9], antispasmodic [10, 11], diuretic [10-12], antihypertensive [13, 14], cholesterol lowering [15], antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities [16]. The potentials of *M. oleifera* in the treatment of typhoid fever in Cameroon [17], and HIV/AIDS in Uganda [18] have been reported. In Nigeria, *M. oleifera* is used to treat various diseases. It is used to treat inflammatory diseases, asthma, fever, cough, earache, liver and pancreas diseases, venereal diseases, diarrhea [19]. Its potential for the

treatment of trypanosomiasis [20, 21], and malaria has also been reported [22].

The use of natural products with antiprotozoal activities especially for the treatment of malaria is well documented [3], while few have addressed the effect of *M. oleifera* and its constituents against trypanosomiasis. *Moringa oleifera* leaves's widespread therapeutic use in traditional medicine is probably due to its high content of phenolics (flavonoids) which are affected by season and agroclimatic locations [23, 24]. The present study is aimed at investigating the *in vitro* antitrypanosomal and antioxidant activities of *M. oleifera* extracts and fractions.

MATERIALS AND METHODS

Plant material and chemicals

Fresh leaves of *M. oleifera* were collected in January 2012 from a cultivated garden in Gasau, Zamfara State, Nigeria. The plant material was authenticated by a botanist at the University of Lagos Nigeria. All reagents and solvents used are of analytical grade.

Sample Preparation/Extraction

Fresh *M. oleifera* leaves were dried under shade at room temperature for one week. The dried leaves were ground into powder using a warring blender and stored in a refrigerator.

Extraction

650 g of the plant material was successively extracted with 3L hexane, ethyl acetate and methanol (72h each) using a Soxhlet extractor. The extracts were filtered (Whatman filter paper) to remove any debris and concentrated using rotary evaporator under vacuum at approximately 40°C. The extracts were further kept in fume cupboard to remove residual solvents. After removal of solvents, hexane extract (dark green), ethyl acetate and methanol extracts were obtained.

Phytochemical Screening

Qualitative phytochemical screening was carried out according to standard methods [25].

Evaluation of Antioxidant Activity

The antioxidant activity of the hexane, ethyl acetate, and methanol leaf extracts of *Moringa oleifera* was determined based on the free radical scavenging activity of 2, 2- diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Brand-Williams [26] (slightly modified). Different concentrations (7.75, 15.5, 31, 62, 125, 250, µg/ml) of *M. oleifera* extracts were prepared using methanol. Solution of DPPH 0.004 % prepared in methanol was used as the standard. DPPH solution (5 ml) was mixed with 5 ml of extract solution. These solution mixtures were kept in the dark for 30 min. The degree of de-colorisation of DPPH from purple to yellow indicates the scavenging effectiveness of the extracts. The absorbance of the combination was determined at 515 nm using UV-Visible Spectrophotometer and DPPH was used as a positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity\%} = \frac{[(\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}})]}{\text{Absorbance}_{\text{Blank}}} \times 100$$

In-vitro Trypanocidal screening

The anti-trypanosomal bioassay was determined using a modified redox-activated fluorescent dye assay as described by Raz [27]. The bioassays were carried out in 96well microtitre plates using the redox indicator Alamar blue. The fluorescence values for the test plates were measured at wavelengths of 560 and 590nm respectively. Wells containing active compounds were easily identified as they remain blue in colour and have background levels of fluorescence. Positive and negative controls and a sterility checks were included in all assays.

RESULTS AND DISCUSSION

Percentage yield as presented in table 1 was calculated using formula 1.

$$\% \text{ Yield} = \frac{\text{weight of the extract}}{\text{Weight of plant material}} \times 100 \quad (1)$$

Table 1: Percentage Yield

S/N	Extract	Weight in gm	%Yield
1	MOH	52.7	8.1
2	MOE	22.6	3.5
3	MOM	81.9	12.6

MOH= *M. oleifera* hexane extract, MOE= *M. oleifera* ethyl acetate extract, MOM= *M. oleifera* methanol extract

The methanol extract gave the highest yield 12.6%, followed by the hexane extract 8.1% and ethyl acetate extract (3.5%).

Preliminary investigation of phytochemical constituents of the extracts showed the presence of Flavonoid, Terpenoids, Carbohydrates, and Phenols in ethyl acetate and methanol extracts (Table 2). There was no detection of saponins, alkaloids and anthraquinone in both extracts.

Table 2: Phytochemical assay

S/no	Phytochemicals	Results
1	Saponin	-ve
2	Flavonoid	+ve
3	Alkaloids	-ve
4	Terpenoids	+ve
5	Anthraquinone	-ve
6	Carbohydrate	+ve
7	Phenols	+ve

Quantification of antioxidant activity using DPPH free radical scavenging showed a dose dependant antioxidant activity for the three extracts (table 3a & 3b, fig. 1). From the results obtained, the highest antioxidant activity of 89.1% was exhibited by the methanol extract after 30 min incubation at a concentration of 125 µg/ml (table 3b). Also, ethyl acetate

extract showed good scavenging activity. The extracts showed strong antioxidant activities with 50% efficient concentration (EC₅₀) values

Table 3 a: Antioxidant activity of *M. oleifera* extracts

S/no	Conc (µg/ml)	Absorbance (nm)		
		MOE	MOM	MOH
1	250	0.197	0.138	0.412
2	125	0.103	0.094	0.602
3	62	0.135	0.303	0.656
4	31	0.317	0.534	0.707
5	15.5	0.512	0.623	0.737
6	7.75	0.610	0.708	0.754

NB: MOH= *M. oleifera* hexane extract, MOE= *M. oleifera* ethyl acetate extract, MOM= *M. oleifera* methanol extract

Table 3 b: Antioxidant activity of *M. oleifera* Extracts

S/no	Conc. (µg/ml)	% Activity		
		MOE	MOM	MOH
1	250	77.17	84.01	52.26
2	125	88.06	89.10	30.24
3	62	84.36	64.89	23.99
4	31	63.27	38.12	18.08
5	15.5	40.67	27.81	14.60
6	7.75	29.32	17.96	12.63

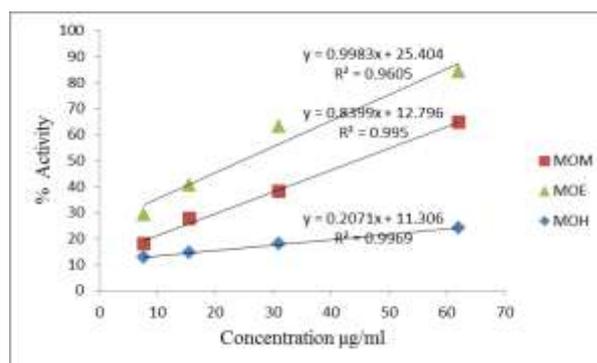


Fig 1. Antioxidant activity of *M. oleifera* extracts

of 24 and 44 µg/ml for ethyl acetate and methanol extracts respectively. The EC₅₀ is calculated by plotting the %DPPH radical activity at steady state (30 min) against various concentrations of each extract (Fig. 1). The EC₅₀

(efficient concentration in $\mu\text{g/ml}$) is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

The hexane extract showed little antioxidant activity. The strong antioxidant activity exhibited by ethyl acetate and methanol extracts is as a result of detection of flavonoids in both extracts. Flavonoids have important dietary significance due to their antioxidant activity; foods rich in flavonoids have been said to be important in ameliorating disease conditions such as cancer and heart disease.

The tested extracts showed lower absorbance values with increasing concentration. Comparatively, ethyl acetate exhibited the least absorbance values indicating a higher antiradical activity. The results are depicted in table 3b.

Two compounds pyropheophorbide a (0.9 %) and monoglycerol acetate (0.9 %) were isolated from 100% ethyl acetate fraction (vacuum liquid chromatography of ethyl acetate extract). The structures of these compounds were established using a combination of ^1H , 2D NMR and mass spectroscopy

In-vitro screening of the crude extracts and isolated compounds showed little or no antitrypanosomal activity against *trypanosoma b. brucei* S427. However, the 100% ethyl acetate fraction from vacuum liquid chromatography of the ethyl acetate extract showed antitrypanosomal activity with MIC value of $25\mu\text{g/ml}$.

CONCLUSION

The result of present investigation indicates that leaf extracts of *M. oleifera* is rich in flavonoids and their antioxidant activity could be explored in food industry and even in drug formulation for ameliorating disease conditions such as cancer and heart disease. The result of this investigation is also important in the search for new drug against *trypanosoma b. brucei*. However, it is important to carry out further

studies to determine the effect of mixing the isolated pure compounds and determine if there is any improvement on the bioactivity due to synergistic effects. Consequently, the results obtained suggest that *M. oleifera* leaf extracts can provide protection against effects of oxidative stress responsible for several chronic diseases.

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