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Original paper

Antilipase, antiacetylcholinesterase and antioxidant activities of Moringa oleifera extracts

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Abstract Pancreatic lipase is critical for the catabolism of lipids in the intestine, making it a prime target for obesity management. Acetylcholinesterase is an important enzyme that hydrolyses the neurotransmitter acetylcholine. It inhibition is paramount in enhancing systemic acetylcholine level. Side effects of conventional drugs lead to continued search for alternative anti-obesity and anticholinesterase agents. The present study assessed lipase and acetylcholinesterase inhibition, as well as antioxidant activity of *Moringa oleifera* extracts. The inhibitory activities of the extracts on lipase and acetylcholinesterase were dose dependent. Aqueous leaf extract (IC_{50} = 3.26 ± 0.26 mg/ml) and hexane root extract (IC_{50} = 0.08 ± 0.00 mg/ml) exhibited the highest antilipase and antiacetylcholinesterase activity respectively. Aqueous extracts of root and leaf (IC_{50} of 1.43 ± 0.03 mg/ml and 1.86 ± 0.10 mg/ml respectively) had the highest N, N-dimethyl-p-phenylene diamine dihydrochloride radical scavenging activity, while ethyl acetate leaf extract had the highest nitrite scavenging activity (IC_{50} = 2.20 ± 0.06 mg/ml). Compared to other extracts, methanol leaf extract exhibited the highest ferric reducing power. These findings suggest that *M. oleifera* possess promising antilipase, antiacetylcholinesterase and antioxidant potentials. Therefore, may be employed as food additive for the management of obesity, Alzheimer and other degenerative diseases.

Keywords Acetylcholinesterase, Alzheimer, antioxidant, lipase, Moringa, obesity

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Introduction

Obesity is a life style disorder characterised by low body mass index. It is general associated with insulin resistance, hyperlipidaemia, coronary heart diseases, apoplexy, cancer of colon, social discrimination and depression[1]. The management of obesity and treatment of its accompanying morbidity are important 21st century public health challenge. Common regimen in the management of obesity involves suppressing food consumption, increasing energy expenditure, down regulation of lipocyte proliferation and pancreatic lipase (central for digestion of triglycerides in small intestine) inhibition. However, euphoric action, addiction, loss of appetite, risk of haemorrhage stroke and valvular heart disease, pulmonary hypertension, stimulant effect and gastrointestinal complications are common side effects associated with drugs used in this regimens[2-5]. These complications have led to the continuing search for natural anti-obesity agents, especially diet based lipase inhibitors[6].

Alzheimer is a neurological disease associated with memory loss, behavioural defects and disorientation. A generalised and progressive loss of nerve cells and brain function leading to dementia is observed among aged and young patients[7]. The most common agents used for Alzheimer management involves the inhibition of acetylcholinesterase. They prevent the hydrolysis of acetylcholine, thereby retaining higher systemic levels of acetylcholine especially at the synapses, and ultimately enhancing the transmission of nerve impulse[8]. Therefore, the continued search for acetylcholinesterase inhibitors and anti-Alzheimer agents remains indispensable.

Excessive free radical production and compromised antioxidant system may result to deleterious outcome such as mutagenesis, neurological disorders, cardiovascular dysfunction, and premature aging[9,10]. Plants are known to be excellent sources of compounds with remarkable antioxidant activities. A typical example is the ability of flavonoids and phenolic compounds to scavenge nitrites capable of oxidising haemoglobin to methaemoglobin or cause anaemia[11-13]. In addition, phytochemicals especially carotenoid, polyphenol, flavonoid, saponin, terpene and glycoside compounds are shown to be enzyme inhibitors, exhibit wound healing, regenerative, anti-inflammatory, antioxidants as well as antitumor activity. Consumption of plants rich in these compounds is positively correlated with lower degenerative diseases, low pathological defects and improved wellbeing[14,15].

Moringa oleifera is the commonest specie among 13 cultivars of *Moringaceae* family, due to its phytochemical and pharmacological properties. It is commonly consumed as food or as food additive in Africa and Asia. All the plant parts are used for various therapeutic and industrial purposes. Common therapeutic benefits of the plant include wound healing, treatment of inflammations and ulcers, cardio-vascular diseases, gastrointestinal disturbances, hepatorenal complications, haematological disorders, act as an antiplasmodic, antimicrobial and cytotoxic agent[16]. In present study, leaf, seed and root extracts of *M. oleifera* (aqueous, methanol, ethyl acetate and hexane) were assessed for pancreatic lipase and acetylcholinesterase inhibition, as well as antioxidant activity ($DMPD^+$ radical scavenging activity, nitrite scavenging activity and ferric reducing power).

Materials and Methods

Sample collection and preparation

Various parts of *M. oleifera* collected from North-West Nigeria, were extracted as reported by Magaji *et al*., the lyophilised aqueous extracts and residues of organic extracts were stored in Eppendorf tubes at -20 °C until use[17].

Lipase inhibition test

Pancreatic lipase was prepared by dissolving 10 mg/ml of pig pancreatic type II lipase (Sigma, L3126) in Tris-HCl buffer (0.1 M; pH 8.5), followed by centrifugation for 10 minutes at -4 °C and 7000 × g so as to obtain a clear supernatants[18]. Lipase activity was assayed spectrophotometrically via hydrolysis of 4-nitrophenyl caprate (4-NPC) to 4-nitrophenol. Briefly, reaction solution containing 5 µl of moringa extract (in DMSO) as inhibitor, 10 µl of lipase (10 mg/ml) and 200 µl of 0.1 M Tris-HCl buffer solution of pH 8.5 was incubated at 37 °C for 25 minute, after which 5 µl 4-NPC (5 mM) was added. Absorbance was read against reagent blank at 405 nm after incubation at 37 °C for 15 minute using a microplate reader[19]. Orlistat was used as standard inhibitor of lipase in this study. The percentage lipase inhibition was calculated as follows:

$$\% \text{ Lipase inhibition} = [1 - (Absorbance\ of\ Test/Absorbance\ of\ Control)] \times 100$$

Acetylcholinesterase inhibition test

The modified method of Ingkaninan *et al*. was employed for assaying acetylcholinesterase activity[20]. Briefly, 20 µl of inhibitor, 40 µl of 0.1 M Tris-HCl (pH 8.0), 100 µl of 3 mM 5,5'-dithiobis-(2-nitro-benzoic acid) and 20 µl of 15 mM acetylthiocholine iodide were added into a microplate and mixed. Thereafter, 20 µl of 0.28 U/ml acetylcholinesterase (Sigma, C3389-2KU) was added and incubated for 2 minutes at 37 °C. Absorbance was read at 405 nm against reagent blank. Tacrine was used as standard inhibitor of the enzyme. Percentage enzyme inhibition was calculated as shown below:

$$\% \text{ Acetylcholinesterase inhibition} = [1 - (Absorbance\ of\ Test/Absorbance\ of\ Control)] \times 100$$

Antioxidant assay

$DMPD^+$ radical scavenging test

$DMPD^+$ scavenging activity was analysed by incubating 0.1 ml test solution in 2.0 ml radical solution; composed of 1 ml of 100 mM DMPD, 100 ml acetate buffer

(0.1 M, pH 5.25) and 0.2 ml ferric chloride (0.05 M). After 10 minutes incubation in a dark cupboard, optical density (OD) was measured at 505 nm against acetate buffer[21]. The standard antioxidant used was L (+) ascorbic acid. The $DMPD^+$ scavenging effect was evaluated as follows:

$$\% \text{ DMPD}^+ \text{ scavenged} = [1 - (Absorbance\ of\ Test/Absorbance\ of\ Control)] \times 100$$

Nitrite scavenging assay

The method of Choi *et al*. was adapted for this assay[12]. A 0.1 ml of extract (in DMSO), 0.1 ml of 1 mM $NaNO_2$, 0.2 ml of 0.1 N HCl and 0.6 ml distilled water were vigorously mixed and kept for 3 hours at 37 °C. Then 0.5 ml of 2% acetic acid and 40 µl Griess reagent was thereafter added, shaken and kept in dark cardboard for 15 minute at 37 °C. Optical density was read spectrophotometrically at 540 nm. Quercetin was employed as standard control. The percentage nitrite scavenged was calculated as follows:

$$\% \text{ Nitrite scavenged} = [1 - (Absorbance\ of\ Test/Absorbance\ of\ Control)] \times 100$$

Ferric reducing power assay

To 0.1 ml test solution, 0.25 ml of 0.2 M phosphate buffer (pH 6.6) and 0.25 ml of 1% $K_3[Fe(CN)_6]$ were added and left to stand for 30 minutes at 50 °C. At 3000 rpm, the mixture was centrifugation for 10 minutes after adding 0.25 ml 10% TCA. To 0.25 ml of the supernatant, equal volume of distilled water and 0.05 ml of 0.1% $FeCl_3$ was added, and then incubated for 10 minutes in dark. Thereafter, absorbance was monitored at 700 nm[22]. Quercetin and ascorbic acid were employed as standard for the reducing test.

Statistical Analysis

Using regression analysis data, half maximum inhibition/scavenging concentration (IC_{50}) was calculated from % enzyme inhibition activities and % antioxidant activities (for $DMPD^+$ and nitrite scavenging). IC_{50} values are inversely correlated to inhibition/antioxidant activities. Results of ferric reducing power are presented as OD of test solution.

Results and discussion

Lipase inhibition

The inhibition activities of moringa extracts on pancreatic lipase are presented in Table 1. The inhibition activities were dose dependent. The highest lipase inhibition corresponding to IC_{50} of 3.26 ± 0.26 mg/ml was exhibited by aqueous leaf extract. This was closely followed by ethyl acetate leaf extract (IC_{50} = 4.73 ± 0.09 mg/ml), methanol seed extract (IC_{50} = 5.01 ± 0.03 mg/ml), hexane leaf extract (IC_{50} = 5.42 ± 0.33 mg/ml) and then aqueous seed extract (IC_{50} = 5.77 ± 0.24 mg/ml). The extracts with lowest lipase inhibition are: methanol leaf (IC_{50} = 7.68 ± 0.60 mg/ml) > aqueous root (IC_{50} = 11.97 ± 0.22 mg/ml) > hexane root (IC_{50} = 18.37 ± 0.47 mg/ml) > ethyl acetate root (IC_{50} = 19.11 ± 0.91 mg/ml). Contrary, both ethyl acetate and hexane seed extracts as well as methanol root extract did not exhibit lipase inhibitory effect. Orlistat which was used as standard had a remarkably high lipase inhibition of IC_{50} = 0.001 ± 6.81×10^{-5} mg/ml. Previous study indicates that red-pericarp mutant rice bran extracts inhibited lipase with IC_{50} value of between 35.95 to 35.97 mg/ml[23]. A 10 mg/ml of *Everniastrum cirrhatum* methanol extract gave an approximately 40 % inhibition of pancreatic lipase activity[24]. Also, report by Toma *et al*. showed that the IC_{50} of aqueous-ethanol *M. stenopetala* leaves extract was more than 5 mg/ml[25]. These findings are similar to the present study in which IC_{50} ranged between 3.26 mg/ml to 19.11 mg/ml. The overall lipase inhibition effect of Moringa can be credited to the rich phytochemical constituents of the plant extracts e.g. quercetin, caffeic acid, kaempferol, rutin, myricetin etc, or their various derivatives previously isolated from other plants and confirmed to exhibit pancreatic lipase inhibition[26-30].

Acetylcholinesterase inhibition

In Table 1, the acetylcholinesterase inhibition activity of moringa extracts are presented. Hexane root extract exhibited the highest acetylcholinesterase inhibition (IC_{50} = 0.08 ± 0.00 mg/ml), and was closely accompanied by aqueous leaf extract (0.10 ± 0.00 mg/ml), ethyl acetate leaf extract (0.10 ± 0.01 mg/ml), aqueous seed extract (0.10 ± 0.00 mg/ml), methanol leaf extract (0.10 ± 0.01 mg/ml), aqueous root extract (0.12 ± 0.01 mg/ml), methanol seed extract (0.12 ± 0.00 mg/ml) and ethyl acetate seed extract (0.13 ± 0.02 mg/ml). The least inhibition was observed in hexane extract of seed and leaf (0.21 ± 0.03 and 0.31 ± 0.01 mg/ml respectively). Methanol and ethyl acetate extract of root showed no inhibition activity. Tacrine had an exceptional inhibition with IC_{50} of 0.002 ± 6.96×10^{-5} mg/ml. Studies indicate that *Gentiana kurroo*[31] and *Areca catechu* L.[32] had neuroprotective effects and enhanced learning/memory ability. Also, *Withania somnifera* was shown to alleviate Alzheimer disease by inhibiting acetylcholinesterase with IC_{50} value of 0.00035 mM[33]. Sutalangka *et al*. demonstrated that that leaves extract of *M. oleifera* decreased malondialdehyde level and acetylcholinesterase activity of rat hippocampus tissue and increased antioxidant enzymes activities. Thus suggesting the plant had neuroprotective and memory enhancing effect[34]. Reports by Adefegha *et al*. indicates that aqueous extract of Moringa seed had inhibitory effect on acetylcholinesterase (IC_{50} =0.27 mg/ml) [35]. Similarly, Ghous *et al*. reported that methanol shoot extracts of Moringa had IC_{50} of 77.58 µg/ml on acetylcholinesterase activity[36]. These findings agree with the present studies where all parts of Moringa were found to exhibit promising antiacetylcholinesterase activity. Therefore supporting it uses as fold medicine for management and delaying the progression of Alzheimer disease in Northern Nigeria.

Table 1. Lipase and Acetylcholinesterase Inhibitory Activity of Moringa Extracts

Extract/Standard	Lipase (IC_{50}; mg/ml)*	Acetylcholinesterase (IC_{50}; mg/ml)*
Aqueous leaf extract	3.26 ± 0.26	0.10 ± 0.00
Methanol leaf extract	7.68 ± 0.60	0.11 ± 0.01
Ethyl acetate leaf extract	4.73 ± 0.09	0.10 ± 0.01
Hexane leaf extract	5.42 ± 0.33	0.31 ± 0.01
Aqueous root extract	11.97 ± 0.22	0.12 ± 0.01
Methanol root extract	ND	ND
Ethyl acetate root extract	19.11 ± 0.91	ND
Hexane troot extract	18.37 ± 0.47	0.08 ± 0.00
Aqueous seed extract	5.77 ± 0.24	0.10 ± 0.00
Methanol seed extract	5.01 ± 0.03	0.12 ± 0.00
Ethyl acetate seed extract	ND	0.13 ± 0.02
Hexane seed extract	ND	0.21 ± 0.03
Orlistat	$0.001 \pm 6.81\times10^{-5}$	-
Tacrine	-	$0.002 \pm 6.96\times10^{-5}$

*Mean ± SD of three replicate values; ND= Activity Not Detected

Antioxidant activity

The antioxidant activity of Moringa extracts are presented in Table 2. Amongst the extracts, aqueous extract of root and leaf had the highest DMPD radical scavenging activity with IC_{50} of 1.43 ± 0.03 mg/ml and 1.86 ± 0.10 mg/ml respectively. With an IC_{50} of 3.09 ± 0.19 mg/ml, aqueous seed extract closely followed. Extracts with comparatively moderate activity are ethyl acetate root (IC_{50} = 6.12 ± 0.34 mg/ml), methanol root (IC_{50} = 6.27 ± 0.10 mg/ml), methanol seed (IC_{50} = 7.46 ± 0.51 mg/ml) and ethyl acetate seed (IC_{50} = 13.82 ± 0.14 mg/ml). Methanol leaf extract exhibited the least scavenging activity (IC_{50} = 27.04 ± 6.18 mg/ml), while ethyl acetate extract of leaf, and the hexane extract of both leaf, root and seed did not exhibit DMPD radical mopping action. L (+) ascorbic acid had an outstanding IC_{50} value of 0.21 mg/ml. DMPD radical scavenging activity is a rapid antioxidant test based on the ability of compounds to donate hydrogen atom to $DMPD^+$, there by decolourizing the coloured radical cation formed from DMPD at acidic pH and in presence of a suitable oxidant solution. Antioxidant activity is proportional to intensity of decolouration[21]. There are no previous reports on the DMPD radical scavenging activity of *M. oleifera*. Findings of this study revealed that the Moringa extracts from polar solvents are more efficient antioxidants, with aqueous extract of all the three plant parts exhibiting higher DMPD radical scavenging activity than their methanol, ethyl acetate and hexane counterparts. This suggests that aqueous extraction was effective in abstracting antioxidant compound capable of donating hydrogen atom and possibly quenching radical/chain reaction..

Table 2. $DMPD^+$ scavenging activity, nitrite scavenging activity and ferric reducing power of moringa extracts

Extract/Standard	$DMPD^+$ Scavenging Activity (IC_{50}; mg/ml)*	Nitrite Scavenging Activity (IC_{50}; mg/ml)*	Ferric Reducing Power (O.D. at 4.0 mg/ml)
Aqueous leaf extract	1.86 ± 0.10	6.17 ± 0.01	0.47 ± 0.40
Methanol leaf extract	27.04 ± 6.18	5.06 ± 0.01	0.81 ± 0.01
Ethyl acetate leaf extract	ND	2.20 ± 0.06	0.41 ± 0.01
Hexane leaf extract	ND	7.39 ± 0.38	0.13 ± 0.00
Aqueous root extract	1.43 ± 0.03	3.19 ± 0.15	0.50 ± 0.01
Methanol root extract	6.27 ± 0.10	7.00 ± 0.75	0.48 ± 0.00
Ethyl acetate root extract	6.12 ± 0.34	7.95 ± 0.38	0.20 ± 0.01
Hexane root extract	ND	4.13 ± 0.12	0.11 ± 0.00
Aqueous seed extract	3.09 ± 0.19	34.08 ± 6.61	0.04 ± 0.01
Methanol seed extract	7.46 ± 0.51	7.63 ± 0.20	0.08 ± 0.01
Ethyl acetate seed extract	13.82 ± 0.14	ND	0.05 ± 0.00
Hexane seed extract	ND	ND	0.04 ± 0.00
L (+) Ascorbic Acid	0.21 ± 0.00	-	1.02 ± 0.00†
Quercetin	-	0.08 ± 0.00	0.93 ± 0.01†

*Mean ± SD of three replicate values; †Conc. = 0.20 mg/ml; ND= Activity Not Detected

In comparison to other extracts, ethyl acetate leaf extract, aqueous root extract and hexane root extract (with IC_{50} of 2.20 ± 0.06, 3.19 ± 0.15 and 4.13 ± 0.12 mg/ml respectively) exhibited the highest nitrite scavenging activities. Moderate scavenging activities were exhibited by methanol leaf extract (IC_{50} = 5.06 ± 0.01 mg/ml), aqueous leaf extract (IC_{50} = 6.17 ± 0.01 mg/ml), methanol root extract (IC_{50} = 7.00 ± 0.75 mg/ml), hexane leaf extract (IC_{50} = 7.39 ± 0.38 mg/ml), methanol seed extract (IC_{50} = 7.63 ± 0.20 mg/ml) and ethyl acetate root extract (IC_{50} = 7.95 ± 0.38 mg/ml). The least scavenging activity was found in aqueous seed extract (IC_{50} = 34.08 ± 6.61 mg/ml), while ethyl acetate and hexane extract of the seed had no nitrite scavenging activity at the tested extract concentration. Quercetin had superior scavenging activity with an IC_{50} of 0.08 mg/ml. Nitric oxide is an important intermediate produced by nitric oxide synthases. In human, it is an indispensable pleiotropic molecule with signalling function. It helps modulate secretion of insulin, neural and blood vessels development, peristalsis and airway tone. It also has neurotransmitter function and immune defence function[37]. As a free radical, nitric oxide reacts with superoxide anion forming peroxynitrite (a potentially cytotoxic molecule). More so, the overproduction of nitric oxide is associated with autoimmune diseases, inflammation, arthritis, diabetes, hypertension, stroke, carcinomas as well as septic shock[38-41]. Therefore regulation of tissue nitric oxide level and scavenging it is an important target in attenuation of some disease conditions[42]. Report by Aju *et al*. indicated that methanol extract of Moringa leaves had the higher scavenging action (IC_{50} = 0.32 mg/ml) when compared to hydro alcohol extract (IC_{50} = 1.46 mg/ml), while water extract had a lower activity than the former (IC_{50} = 1.50 mg/ml) [43]. However, these IC_{50} values were below those found in the present study, indicating higher nitrite scavenging activity of Moringa from Kerala compared to that used in present study.

As shown in Table 2, methanol leaf extract (at 4.00 mg/ml) exhibited the highest ferric reducing power with an OD of 0.81 ± 0.01. However, the leaf extracts reducing power was lower than that of both ascorbic acid (OD = 1.02 ± 0.00) and quercetin (OD = 0.93 ± 0.01) at 0.20 mg/ml. Other extracts with appreciably high reducing power at 4.00 mg/ml are aqueous root extract, methanol root extract, aqueous leaf extract and ethyl acetate leaf extract. Those with least scavenging activities are: ethyl acetate root extract > hexane leaf extract > hexane root extract > methanol seed extract > ethyl acetate seed extract > aqueous seed extract > hexane seed extract. These findings suggest that polar solvent had better extraction power for antioxidants present in leaves and roots. Ferric reducing power is based on capability of compounds to donate electrons, there by facilitating the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The intensity of the blue colour formed is positively correlated to reducing power of test solution. A study by Pakade *et al*. indicates that *M. oleifera* cultivated in South Africa had higher ferric reducing power than common vegetables such as peas, cabbage, spinach, broccoli and cauliflower[44]. Hydro alcohol and then aqueous extract of Moringa leaves were reported to have more potent ferric reducing power than methanol extract[43]. In a separate report, Luqman *et al*. disclosed ethanol and aqueous extract of Moringa seed had higher ferric reducing power than their corresponding leaf extracts[45]. These are in contrast to the findings of this study where methanol leaf extract displayed better reducing power compared to aqueous leaf extract, and both of these leaf extracts had higher reducing power than their counterpart seed extracts.

Conclusion

The findings of the present study suggest that *M. oleifera* can serve as a dietary source of anti-obesity and acetylcholinesterase inhibitors. Coupled with the antioxidant property, the plant can serve as an alternative herb for attenuating or managing obesity, Alzheimer and other degenerative diseases if properly exploited. More so, the strong antioxidant properties of the leaf can help lessen the impact of oxidative stress and progression of degenerative diseases when employed as food additives.

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Conflict of interest

The authors declare no conflict of interest.

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