The Bioscientist Vol. 3(1): 101-109 , January 2015 Available online at
http://www.bioscientistjournal.com

Full Length Research Paper

EVALUATION OF THE HYPOTENSIVE PROPERTIES OF *MORINGA OLEIFERA*SEEDS

***Ugochukwu, Chidozie G,[1] Ogbunugafor, Henrietta A,[1] Archibong, E. J,[2] Onuorah, Samuel C.[2]**
[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.
[2]Dept. of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria.
*Corresponding author: cg.ugochukwu@unizik.edu.ng

ABSTRACT
The management of hypertension poses a challenge in both the developing and developed countries. However, the disease is more prevalent in the developing countries where poverty, illiteracy and poor nutrition combine to exacerbate the condition. The study was carried out to evaluate the *in vitro and in vivo* hypotensive properties of the ethanolic extract of *Moringa oleifera* Lam. (Moringaceae) seeds. The oscillographic technique was employed on guinea pig ileum and a live cat to evaluate the antihypertensive properties of the extract. In the *in vitro* relaxation studies, the extract induced contraction on the ileum in a dose-dependent manner; extract doses of 1, 2 and 4 mg mL^{-1} induced responses (mm) of 2.32 ± 0.46, 4.50 ± 0.46 and 7.52 ± 0.56 respectively, compared to the control value of 1.42 ± 0.42 induced by 2µg/ml of acetylcholine. In the *in vivo* studies, the extract significantly reduced the mean arterial blood pressure (mm Hg) of an anaesthesized normotensive cat from a control value of 116 ± 6.33 (when normal saline was administered) to 80 ± 3.30, 50 ± 3.70 ± 3.10 and 52 ± 6.60 at doses of 10, 20 and 40 mg ml^{-1} respectively. These findings corroborate the use of *M. oleifera* seed as an antihypertensive agent in ethno-medicine.

Keywords: *Moringa oleifera*, hypertension, guinea pig ileum, cat

INTRODUCTION
There has been an upsurge in the incidence of hypertension all over the world. Hypertension is the sustained elevation of the systemic arterial pressure. It is one of the major cardiovascular risk factors contributing to myocardial infarction, vascular diseases, renal diseases, congestive heart failure, peripheral vascular insufficiency and premature mortality (Lifton *et al*., 2001). The overall worldwide burden of hypertension in the year 2000 was estimated to be 26.4% of the world's adult population, with 34.26% occurring in developed and 65.74% in developing countries (Hajjer *et al*., 2006). Globally, high blood pressure is estimated to cause 7.1

million deaths annually (Tesfaye *et al*., 2007). The exact etiology of hypertension is unclear. Heredity is a strong predisposing factor, and environmental factors such as obesity, diabetes, high salt diet and stress can exacerbate this predisposition.

In Nigeria, hypertension is the commonest non-communicable disease, with a prevalence of about 20-25% among adult Nigerians (Alebiosu, 2011). In the study of patterns of cardiovascular diseases in many centers in Nigeria, hypertension was ranked first as the medical illness most frequently diagnosed in elderly Nigerians (Ogunniyi *et al*., 2001; Bella *et al*., 2006).

The management of the condition has been faced with problems emanating mainly from undesirable side effects of contemporary anti-hypertensive agents. This has resulted in a search for new ways to treat hypertension. The identification and validation of medicinal plants with hypotensive properties could be a lead to the production of cheaper plant-derived antihypertensive agents with fewer side effects. Hence, exploring plants in our locality that have been used in folkloric medicine for treatment of hypertension is an important strategy to the management of the condition. *Moringa oleifera* Lam. (Moringaceae) is one of such plants. *M. oleifera* is a small tree with sparse foliage, often planted in compounds or used as hedge in Northern Nigeria. The plant is commonly called Horse-radish tree in English and known as '*Okwe oyibo'* in Igbo, '*Zogale-gandi'* in Hausa, and '*Ewe-igbale'* in Yoruba (Dalziel, 1956). All parts of the plant are reported to possess medicinal properties in ethno-medicine and are used in the treatment of various ailments (Fahey, 2005). Some of the medicinal properties of the plant, and most of its nutritional benefits have been investigated (Anwar *et al*., 2007).

The aim of this study therefore, was to investigate the effect of the ethanol extract of the seeds of this plant on isolated guinea pig ileum and the hypotensive effect of the extract ina live cat. In addition, an *in vitro* evaluation of the most probable mode of action of the hypotensive activity of the extract was also studied.

MATERIALS AND METHODS

Chemicals and reagent:

Adrenaline, acetylcholine, noradrenaline, phentolamine, and atropine were purchased from Sigma-Aldrich, England. All other chemicals and solvents were of analytical grade.

Collection and authentication of plant material:

Fresh *Moringa oliefera* pods were collected from Saminaka, Kaduna state, and was identified and authenticated by a taxonomist with the Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of plant material:

The pods were broken to expose the seeds, hulled and crushed using a mortar and pestle.

The seeds were groundinto a fine powder with an electronic blender (BLG-555 Binatone, India).

Extraction of plant material:

The extraction procedure of plant materials as indicated by Harborne, (1973), and Culei, (1964) were employed. Two hundred grams of M. *oleifera* seed was macerated in 500 mL of ethanol (80 %) for 72 h by shaking the mixture at regular intervals. The mixture was filtered using Whatman No. 1 filter paper in a Buchner funnel. The filtrate obtained was left to evaporate at room temperature away from direct sunlight for 48 h. This was subsequently lyophilized (Yorke, Scientific Industries PVT, India) to obtain the crude extract which was refrigerated at 4^{o}C until needed.

Quantitative phytochemical analysis:

Oxalate was estimated by the method described by Munro and Bassir, (1969). Standard phytochemical procedures as prescribed in the Association of Official Analytical Chemistry (AOAC) manual was employed to determine percentage amount of alkaloid, saponins, and tannins (AOAC,1995), cardiac glycosides determined by Keller-Killani test as described by Sofowara, (1993). Flavonoids were quantified by the method described by Boham *et al*., (1974).

Animals:

A guinea pig and a cat were used for the hypotensive studies. The guinea pig was obtained from the Animal House of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus, and the cat obtained from the Department of Veterinary Parasitology and Entomology of University of Nigeria, Nsukka. The guinea pig was fed with elephant grass (*Pennisetum purpureum*) and the cat was fed with commercial canned cat food. The animals were acclimatized for 7 days and handledaccording to guidelines stipulated by the National Health Research Committee (NHRC) of Nigeria (2010) for handling animals.

***In vitro* relaxant studies with isolated guinea pig ileum**.

A female guinea pig (500 g) was sacrificed by cervical dislocation; the small intestine was quickly removed and cut helically into strips of 3-4 cm. Each strip was mounted in a 10 mL organ bath (Harvard Apparatus, Kent, England) containing Krebs-Hanseleit solution with a composition of (mM): NaCl 118.2, NaHCO325.0, KCl 4.7, $CaCl_2$ 2.5, KH_2 PO_4 1.3, $MgSO_4$ 1.2 and Glucose 11.7; (pH 7.4), at 37^{o}C and gassed with 95 % O_2 and 5 % CO_2. The strips were placed under an initial passive tension and allowed to equilibrate for 30 min. Responses of the ileum strips to doses of 1, 2, 4, and 8 mg mL^{-1} of the extract, adrenalin (1 and 2 µg mL^{-1}) and acetylcholine (1 and 2 µg mL^{-1}) were recorded, with the use of 40 µg ml^{-1} phentolamine kept constant, via a force displacement transducer (model A-6360, Harvard Apparatus, Kent, England) coupled to an Oscillograph (model 50 – 8622, Harvard Apparatus, Kent, England) as described by Gilani *et al*., (1994).

Effect of the extract on K^+ pre-contracted tissue

The isolated tissue was pre-contracted with high concentration of K^+ (80 mM) and doses of 1, 2 and 4 mg mL^{-1} of the extract added, and responses noted.

***In vivo* studies of extract on anaesthetized cat**

A female cat (1.5 kg) was anaesthetized with a 5 ml kg^{-1} mixture of urethane (10 %) and chloralose (1 %) given intraperitoneally as described by Mak-Mensah, (2010). The cat was mounted and prepared for measurement of blood pressure and heart rate. The arterial blood pressure was monitored from the carotid via an arterial cannula connected to a pressure transducer (model A-6360;Harvard Apparatus Ltd, Kent, England) coupled to an oscillograph (model 50 - 8622, Harvard Apparatus Ltd, Kent, England). The filtered extract and drugs was injected in the form of bolus injection via a cannula. The temperature of the animal was maintained at 37^{o}C by the use of an incandescent overhead lamp for the duration of the experiment.

RESULTS

Phytochemical Analysis

Table 1: Phytochemical content of *M. oleifera seeds.*

Phytochemical	Amount (g/100g)
Flavonoids	2.60
Saponins	8.02
Alkaloids	0.09
Tannin	0.33
Cardiac glycoside	0.80
Oxalates	1.40

Result of the phytochemical analysis shows the seed extract to be rich in flavonoids and saponin.

In-vitro Antihypertensive Studies

The results of the *in vitro* antihypertensive studies are presented in Tab 2 and fig 1 below: while the former shows the relaxant effect of ethanolic extract of *M. Oleifera* on strips of proximal ileum of guinea pigs, the later measures the ability of the extract to reverse the contractive effect of high concentration of K^+ on isolated ileal smooth muscles of guinea pigs.

Table 2: Dose-dependent relaxation of smooth muscles of isolated guinea pig ileum induced by the ethanolic extract of *M. oleifera* seed.

Concentration of extract ($mg.ml^{-1}$)	Mean Relaxation Response (mm)	Control (Ach) (µg/ ml)	Mean Relaxation Response (mm)
1.0	2.32± 0.46	2.0	1.42± 0.42
2.0	4.50 ± 0.46		
4.0	7.52 ± 0.56		
8.0	*AN		

The relaxant effect of graded concentration of the extract was measured by observing the dose-related inhibition of the tone and movement of rat ileum pre-contracted with phentolamine (40 µg ml^{-1}). At high concentration of extract (8mg/ml) the response observed was anomalous (AN) probably due to receptor desensitization. The extract's relaxation responses were measured against a control relaxation response induced by acetylcholine (2 µg ml^{-1}), and the difference was significant ($p<0.05$).

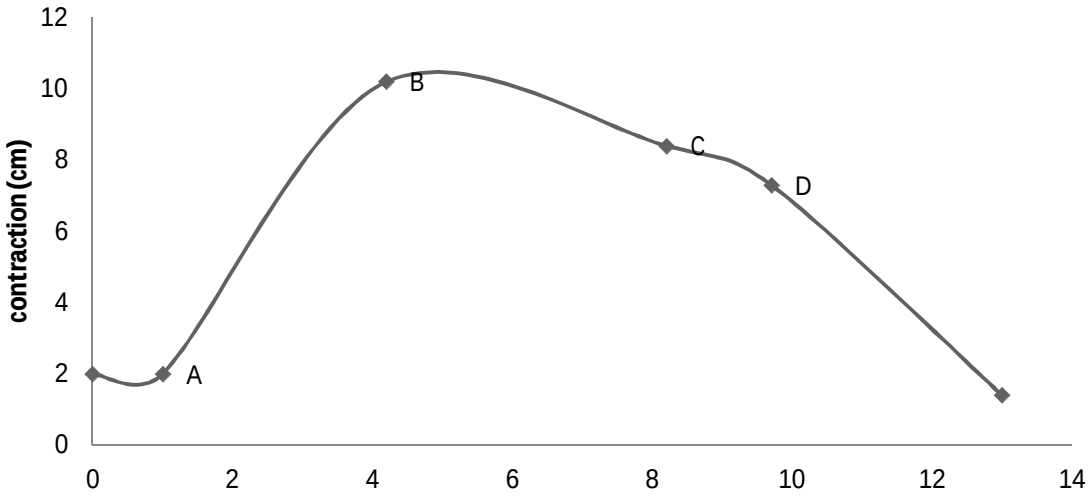

Fig 1: Plot showing the inhibition of K^+-induced contraction of isolated guinea pig ileum by ethanolic extract of *M. Oleifera.* Points A, B, C and D corresponds to the points in the tracer experiment at which K^+ (80 mM), 1, 2, and 4 $mg.ml^{-1}$ of *M. Oleifera* extractswere added at time intervals of 30s.

***In vivo* antihypertensive study.**

Table 3: The effect of IV infusion of *M.Oleifera*extract on systolic, diastolic and mean arterial blood pressures of anaesthetized normotensive cat.

Extract dose administered (mg/kg)	**SBP (mmHg)**	**DBP (mmHg)**	**MAP (mmHg)**
5	148 ± 6.32	86 ± 11.60	106 ± 9.46
10	110 ± 321	65 ± 3.40	80 ± 3.30
20	69 ± 3.10	40 ± 3.20	50 ± 3.70
40	64 ± 7.60	46 ± 6.10	52 ± 6.60
Control (0.9% w/v NaCl)	**162 ± 6.32**	**94 ± 6.34**	**116 ± 6.33**

The effect of the graded doses of the extract on the blood pressure of normotensive cat was observed and compared to the effect induced by IV infusion of normal saline (0.90% w/v of NaCl) which served as the control. At extract doses of 10, 20, and 40 mg/kg, there was a significant ($p<0.05$) decrease in the blood mean arterial blood pressure (MAP)

DISCUSSION

The antihypertensive properties inherent in numerous plant parts are being studied by several research efforts to help formulate newer and safer antihypertensive drugs. Some of these studies have led to the

development of some efficacious anti-hypertensives.

This research was carried out to determine the phytochemicals and antihypertensive properties of the ethanolic extract of *M. oleifera* seed. The seeds are used traditionally for the treatment of a range of diseases – including hypertension.

The seeds were found to be particularly rich in saponin, oxalates and flavonoids. This result is consistent with result of phytochemical screening of the powdered seeds of *M. oleifera* obtained from Tofa Local Government Area of Kano state, Nigeria by Kawo *et al*., (2009). As the seeds used for this study were obtained from Saminaka, Kaduna State, which is in the same geographical location as Tofa, the similarity in the results of both studies show a consistency in the phytochemical content of the seeds.

The presence of pharmacologically useful substances such as tannins, flavonoids, and saponins among other pharmacologically active elements in the seed of M.*oleifera* as revealed by the phytochemistry confirms the diverse claims and application of the plant's seed in the treatment of diverse ailments.

Various scientific articles have shown that some of the secondary metabolites which were observed in the present phytochemical analysis are therapeutically active hypotensive agents. For instance, alkaloids obtained by the fractionation of the aqueous extract of the seeds of *M. oleifera* were found to have a negative inotropic effect on the heart of frogs Mekonnen, (2001). This activity was further characterized by testing it on isolated guinea pig ileum Dangi *et al*., (2002). It has been observed that flavonoids, which are contained in a relatively high amount inthe seed, significantly lowers blood pressure in spontaneously hypertensive rats (Mak-Mensah *et al*., 2010), whereas cardiac glycosides, which were present in the seed in small amount, are characterized by their specific and powerful cardiotonic action on cardiac muscles as noted by Dabella, (2002). Furthermore, the relatively high saponin content of the seed may indirectly regulate blood pressure by lowering cholesterol levels. Malinow, (1997) reported that saponin lower cholesterol levels by binding to it, thereby preventing its reabsorption and thus enhancing its excretion. Related to this, studies by Rhiouani *et al*., (1999) showed that saponins from *Herniaria glaba* significantly lowered blood pressure in spontaneously hypertensive rats when administered orally for 30 days. Thus, alkaloids, flavonoids and, saponins may be important secondary metabolites for the hypotensive effects of crude seed extract of *M. oleifera* observed in the *in vitro andin vivo*study.

The *in vitro* antihypertensive study indicates that the extract exerts a relaxant effect on isolated proximal ileum of guinea pig. The extract exhibited a dose-dependent relaxation of the tissue. The result is in consonance with the relaxation of guinea pig aorta produced by the aqueous extract of *M .oleifera* pods observed by Faizi *et al.*, (1998). More so, Gilani, *et al*., (2010) also reported a similar dose-dependent relaxant property with the leaves of *M. oleifera* on guinea pig aorta. This suggests that the principle exerting the relaxant effect may be widely distributed in the plant.

The *in vitro* hypotensive property of the ethanolic extract of *M. oleifera* seedis substantiated by *in vivo*investigation on anesthetized normotensive cats. Intravenousadministration of ethanolic extract of *M .oleifera* showed significant fall in systolic, diastolic and mean arterial blood pressures in a dose-dependent manner in anaesthetized normotensive cat ($p > 0.05$). The result of this study is in agreement with previous *in vivo* studies on *M. oleifera* leaves by Faizi *et al.* (1994) where the

ethanolic extract of the leavesreduced mean arterial blood pressure by as much as 35-40% at a dose of 3 mg kg^{-1} in anaesthesized normotensive Wistar rats. It was also noted by Gilani *et al*., (1994) that pure compounds isolated from *M. oleifera* leavescaused dose dependent fall in systolic blood pressure, diastolic blood pressure, and heart rate in anaesthetized normotensive Wistar rats. In this study, the maximum *in vivo* hypotensive effect of the extract was exhibited at a dose of 20mg kg^{-1}, as there was no further decrease in blood pressure at 40 mg kg^{-1}. In a similar study, it was observed that the aqueous extract of stem bark from *M. oliefera* produced a dose-dependent hypotensive effect in anesthetized mongrel dogs (7-12 kg) with a maximum effect at 20 mg kg^{-1} (Limaye *et al*., 1995).

On the investigation of the probable mechanism of action, co-administration of the extract and adrenaline did not show any synergistic effect. This suggests that the extract may not act by binding to adrenergic receptors. This is consistent with the observation of Mengistu (2007, unpublished article) on the aqueous extract of *M. stenopetala* leaves on aortic tissue preparation, where phentolamime, a non-specific alpha adrenergic blocker, inhibited the relaxation induced by adrenaline but did not affect that of the extract.

Addition of Verapamil, a calcium channel blocker, also induced a dose-dependent relaxation response on the tissue isolate. This relaxation response is consistent with the result of Franganillo *et al*., (1986) who noted that verapamil relaxed acetylcholine-induced contraction on isolated ileum of new born guinea pigs. In the present study, the relaxation response induced by verapamil was potentiated by the extract in a synergistic manner, suggesting that the extract, like verapamil, may act as calcium channel-blockers.

To further support the calcium channel-blocking potential of the extract, the extract was able to relax ileum tissue pre-contracted with high concentration of K^+ (80mM) in a dose-dependent manner. As noted by Gilani *et al.,* (1994), K^+ at high doses (> 30mM) is known to cause smooth muscle contraction by opening voltage- dependent slow calcium channels.

CONCLUSION

The results of this study show that the intravenous administration of ethanolic extract of *M. oleifera* seed exhibited blood pressure-lowering effect in normotensive anaesthetized cat.

In the *in vitro* studies, the extract of *M. oleifera* seeds exhibited relaxant effect on guinea pig ileum, inhibitory effect on high K^+-induced contraction of ileum tissue, and potentiated relaxation response by verapamil on isolated ileum. This may be by acting as a calcium channel blocker.

As shown in both the *in vivo* and *in vitro* studies, the blood pressure lowering potential of the ethanolic extract of *M. oleifera* seed provides partial scientific justification for the traditional use of the seeds as an antihypertensive agent.

REFERENCES

Alebiosu, C.O. What is Hypertension? (http:// tribune.com.ng/sat/index.php. hypertension. html) Accessed on the 13th of May, 2011.

AOAC. (1995). Official methods of analysis: Association of Official Analytic Chemists. USA: Washington; 15th Edition, p 807-928.

Anwar, F, Latif, S, Ashraf, M, Gilani A.H. (2007). *Moringa oleifera:* A food Plant with Multiple Medicinal Uses. Phytother Research, 21:17-25

Bella, A.F, Baiyewu, O, Bamigboye, A, Adeyemi, J.D, Ikuesan, B.A, Jegede, R.O. (2006). The pattern of medical illness in a community of

elderly Nigerians. Center of Afr J of Med, 39: 112-26.

Boham, B.A, Kocipal-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum and V. calicynium*. Pacific Science, 48: 458-63.

Culei, I. (1964). Practical manual on the industrial utilization of medicinal plants. Romania: University of Bucharest, p 56-71.

Dalziel, J.A. (1956). Useful plants of West Africa. London: Crown Agents for oversea Government and Administration, p 23.

Dangi, S.Y, Jolly, C.I, Norayanan, S. (2002). Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera.* Pharm Biol, 40: 144-8.

Debella, A. (2002). Manual for phytochemical screening of medicinal plants, Department of Drug Research, Ethiopian Nutrition and Research Institute. Addis Ababa, Ethiopia, p 45- 71.

Duarte, J, Perez-Palencia, R, Vargas, F, Ocete, M.A, Perez-Vizciano, F, Zarzuelo, A, Tarmargo, J. (2001). Effects of the flavonoid quercetin in spontaneously hypertensive rats. Brit. J. Pharmacology, 133(1): 117-24.

Fahey, J. (2005). *Moringa oleifera*: An Overview of the medicinal evidence for its nutritional, therapeutic and prophylactic properties. Tree of Life Journal, 1:5.

Faizi, S, Siddiqui, B.S, Saleem, R, Siddiqui, S, Aftab, K, Saleem, F, Gilani, A. (1994). Novel Hypotensive agents, Niazimin A, Niazimin B, Niazicin A and Niazicin B from *Moringa stenopetala*: Isolation of first occurring carbamates. J Chem Soc. Perkin Trans, 1: 3035-40.

Faizi, S, Siddiqui, B.S, Saleem R, Aftab, K, Saleem, F, & Gilani, A. (1998). Hypotensive constituents from pods of *Moringa oleifera*. Planta Med, 64: 225-8.

Franganillo, A.M, Carvajal, A, Alvarez F.J. (1986). Effects of verapamil on acetylcholine and field stimulation induced contraction in post natal developments of guinea pigs. *Am. Journal of pharmacology*, 56 (8) 321-329.

Gilani, H.A, Aftab, K, Suria A, Siddiqui, S, Saleem, R, Siddiqui, B.S. (1994) Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from *Moring oleifera*. Phytoesther Research, 8:87-91.

Hajjer, I, Kotchem, J.M, Kotchen, T.A.(2006). Hypertension: Trends in prevalence, incidence and control. Annu Rev Publ Health, 27:465-90

Harborne, J.B. (1973). Phytochemistry. London: Academic press, p 89-131.

Kawo, A.H, Abdulahi, B.A, Gaiya, Z.A, Halilu, A, Dabai M, Dakare, M.A. (2009). Preliminary phytochemical screening, proximate and elemental composition of *Moringa oleifera* seed powder. Bayero J Pure Appl Sci, 2: 96-100.

Lifton, R.P, Gharavi, A.G, Geller, D.S. (2001). Molecular Mechanisms of human hypertension. Cell, 104 (4): 545-556.

Limaye, A.D, Limbkar, Y.A, Jain, R, Ahmad, M. (1995). Cardiovascular effects of the aqueous extract of *Moringa ptergosperma.* Phytother Res; 9: 37-40.

Mak-Mensah, E.E, Komalaga, G, Terlabi, E.O. (2010). Antihypertensive action of ethanolic extract of *Imperata*

cylindrical leaves in animal models. Journal of Medicinal Plant Res, 4(14): 1486-91

Malinow, M.R, Mc Laughlin, P, Kohler G.O, Livingstone, A.L. (1997). Alfalfa saponins: a family of substances potentially useful for treatment of hypercholesterolemia. *J* Clin Res, 25: 974-79.

Mekonnen, Y. (2001). Effect of ethanol extract of *Moringa stenopetala* leaves on guinea-pigs and mouse smooth muscle. Phytother Res, 13: 442-4.

Munro A, Bassir, D. (1969). Oxalates in Nigerian vegetables. West Afr J Biol Applied Chemistry, 12: 14-18.

Ogunniyi, A, Baiyewu O, Gureje O, Hall K.S, Unverzagt F.W, Oluwole, S. A. (2001). Morbidity pattern in a sample of elderly Nigerians in Idikan community, Ibadan. West Afr J of Med, 20: 227-31

Rhiouani, H, Settaf, A, Lyoussi, B, Cherrah, Y, Lacaille-Dubois, A. (1999). Effects of saponins from *Herniaria glaba* on blood pressure and renal function in spontaneously hypertensive rats. Therapie, 54, 735 - 9.

Sofoworo, A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum books, p 220-4.

Tesfaye, F, Nawi N.G, Minh H.V. (2007). Association between body mass index and blood pressure in three populations in Africa and Asia. J human Hypertens, 21: 28-37.