Centrepoint Journal (Science Edition)
Volume 20, No. 1, pages 1 – 11
http://www.unilorin.edu.ng/centrepoint

2141-3819/2014 $5.00 + 0.00

CPJ 2014002/20101

Hypotensive activity of aqueous leaf extract of *Croton zambesicus* Linn Euphorbiaceae

R.O. Ayanniyi [1*], B.B. Maiha [2], O.A. Salawu [2], Haas Jimoh Akanbi [3].

[1] Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria.
[2] Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.
[3] Department of Pharmacology and Therapeutics, College of Medicine, University of Ilorin, Nigeria.

Abstract

Croton zambesicus is a highly valued medicinal plant in Nigeria and in the West African sub-region. It is used by traditional medicine practitioners in the treatment of various ailments including hypertension. This study aims to determine the hypotensive activity of the aqueous leaf extract of *Croton zambesicus* in experimental animals. The hypotensive activity of the aqueous extract was assessed in anaesthetized normotensive cats and effect of the extract on isolated right atrium of the guinea pig and isolated perfused heart of the rabbit were also determined. The aqueous extract (20-40 mg/kg *i.v.*) produced a significant ($P<0.001$) reduction in the blood pressure of the anaesthetized normotensive cat. The extract (2.5 mg/ml) produced a significant ($P<0.05$) reduction in the tone and rate of contraction of the isolated right atrium of the guinea pig. In addition, the extract (6.4 mg/ml) inhibited isoprenaline (10 nM)-induced contraction of the isolated rabbit heart. The data obtained from this study revealed that the aqueous leaf extract of *Croton zambesicus* has hypotensive and cardiac depressant activities. These findings provide a pharmacologic basis for use of the plant in management of hypertension in traditional medicine.

Keywords*: Croton zambesicus, traditional medicine, blood pressure, hypotensive, cardiac depressant*

*Corresponding Author: Rashidat O Ayanniyi ayanrash13@yahoo.co.uk; 08033908610

Introduction

Hypertension affects about 1 billion people worldwide and is a major risk factor for stroke, myocardial infarction, vascular, and chronic kidney diseases (Madhur *et al*., 2010). The prevalence rate in sub-Saharan Africa particularly in urban societies seems to be as high as those seen in developed countries (Addo *et al*., 2007). Prevalence of hypertension among Nigerians is high (Ekwunife and Aguwa, 2011) and has been put at 38.6 % and 41.2 % respectively for adult males and females aged ≥25 years (WHO, 2012).

Awareness, treatment and control of hypertension are extremely low in developing countries where majority of health care resources are directed to HIV/AIDS, tuberculosis and malaria control and treatment (Tesfaye *et al*., 2009; Ulasi *et al*., 2011). Managing this chronic condition and its resultant complications constitutes a great financial burden on individual patients and the health systems of many countries (Ganiyu and Suleiman, 2014). For this reason, majority of people living in developing countries rely on traditional remedies (mainly herbs) for their health care needs (WHO, 2014). Pharmacological therapy is costly and is also associated with multiple side effects resulting in patient non-compliance. There is, thus, a need to explore alternative therapies particularly from herbal sources as these are easily accessible and cost effective (Siddiqi *et al*., 2012).

About 75 to 80 % of the world's population use herbal medicines for primary health care because of their better acceptability to the human body and their minimal side effects (Tabassum and Ahmad, 2012). A considerable number of bioactive compounds including flavonoids and terpenoids derived from plants, in addition to possessing cardio protective effect, have been shown to reduce the risk of cardiovascular disease (Vasanthi *et al*., 2012).

Croton zambesicus is a medicinal plant that is widely used amongst traditional medicine practitioners in Nigeria and Benin Republic. It is used in the management of chronic ailments including hypertension (Adjanohaun *et al*., 1989) but with limited scientific information on its ethnomedicinal use in the management of cardiovascular disorders.

The antidiabetic (*Okokon et al., 2006)*, anticonvulsant and neuropharmacological (Ayanniyi and Wannang 2008a; Ayanniyi and Wannang 2008b), *antiulcer* (Okokon and Nwafor, 2009), anti-inflammatory, analgesic and antipyretic (Okokon and Nwafor, 2010) and anticoagulant activities of the plant (*Robert* et al., 2010) have been reported scientifically. Vascular smooth muscle relaxant activity of a natural diterpene isolated from *Croton zambesicus* (Baccelli et al., 2007; Martinsen et al., 2010) and its antioxidant activity *(*Aderogba *et al., 2011)* of the plant were also reported.

Materials

Guinea pigs of both sexes (300-500 g), adult cats (1-1.5 kg) and rabbits (600-800 g) were obtained from the market and kept in the animal house to acclimatise. The experiments were carried out in accordance with the Guidelines for Laboratory Procedures laid down by the Ahmadu Bello University, Zaria Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria. All the animals were fasted for 16 hours, but allowed free access to water before the commencement of the experiments.

Collection and identification of plant material

The leaves of *Croton zambesicus* were collected between April and June from Iwo in Osun State, Nigeia. The plant was identified by Mr. I.A. Kareem, a taxonomist at the Federal College of Forestry, Jos, Nigeria and further authenticated at the Herbarium Section in the Department of Pharmacognosy, University of Jos, Nigeria. The plant was found to correspond with voucher specimen number UJ/PGPH/HSP/0801.

Methods

Preparation of the aqueous leaf extract of *Croton zambesicus* (AECZ)

The leaves were removed from the stem, shade dried and reduced to fine powder using mortar and pestle. The powdered leaves (300 g) were extracted by maceration with distilled water for 24 hours and filtered with a filter paper. The filtrate was evaporated to dryness on a water bath at 40 - 45 ^{o} C. The dark brownish residue obtained was weighed and stored in the refrigerator prior to use. A stock solution of the crude extract was prepared by weighing a quantity of the extract, triturating with a mortar and pestle and dissolving with deionized water. The stock solution was prepared weekly and stored in aliquot portions in the freezer until needed.

Determination of effect of aqueous leaf extract of *Croton zambesicus* on blood pressure in anaesthetized normotensive cats

An adult cat of each sex weighing 1.0 to 1.5 kg was anaesthetized by intraperitoneal thiopentone sodium (50 mg/kg). The right carotid artery and left femoral vein was cannulated for measurement of arterial blood pressure and systemic administration of drugs respectively. In order to minimize blood coagulation, heparin (100 I.U.) was administered intravenously and flushed with 0.9 % w/v sodium chloride. The arterial cannular was connected to a pressure transducer and the blood pressure (BP) readings were recorded with a micro-dynamometer. After 10 minutes stabilization period, graded doses (1-80 mg/kg)

of the AECZ were infused. Each dose was flushed with 0.1 ml normal saline and the BP was recorded. BP was allowed to return to baseline values before further doses were infused (Ojewole *et al*, 2007). One centimeter (1 cm) on the recording sheet corresponds to 10 mmHg pressure change in glass sphygmomanometer (Bako *et al*., 2012).

Determination of effect of aqueous extract of *Croton zambesicus* on isolated right atrium of guinea pig

Guinea pigs of both sexes (300-500 g) were killed by cervical dislocation. The heart was removed and the right atrium isolated. One end of the right atrium was tied to a tissue clamp and the other end was attached to the transducer connected to a microdynanometer. The tissue was mounted in Ringer-Locke solution maintained at 32 ^{o} C and continuously aerated with oxygen. The tissue was washed every 5 minutes and allowed to equilibrate for 20 minutes. After obtaining control readings, graded concentrations of the extract (0.1-2.5 mg/ml) were added cumulatively to the organ bath and responses recorded. The tone of contraction of the atrium was measured as the distance between the baseline and peak of contraction while the rate of contraction of the atrium was distance-measured between contractions.

Determination of effect of aqueous extract of *Croton zambesicus* on isolated perfused rabbit heart preparation (Langendorff)

The rabbit was weighed and heparin sodium (100 I.U.) was injected into the ear vein to prevent coagulation of blood. The rabbit was killed by cervical dislocation and the chest was opened with an incision along the left and right lateral aspects of the rib cage. The diaphragm was cut and the heart exposed. The aorta, vena cava and pulmonary vessels were cut and the heart was transferred into a petri dish containing Ringer-Locke solution. A small spring clip was attached to the ventricle and a thread was connected from spring levers to the clip to record the heart contractions. The heart was then mounted on the aorta for retrograde perfusion with Ringer-Locke solution and continuously aerated with oxygen at 37 ^{o} C and pressure of 70 mmHg. The heart was allowed to equilibrate for 10 minutes before drug administration. Drugs were added to the preparation by injection via rubber tubing into the perfusion fluid.

A concentration-response curve for isoprenaline (0.1 nM-0.1 µM) was obtained and the concentration that produced submaximal response was used for the study. The effect AECZ 0.64 mg/ml and 6.4 mg/ml on isoprenaline (10 nM)-induced contraction of the isolated perfused heart was recorded.

Statistical Analysis

Data obtained were represented as mean ± standard error of mean (SEM). Statistical evaluation was carried out using one-way analysis of variance

(ANOVA) followed by Bonferroni post-hoc test. Statistical significance was taken at *P < 0.05 versus Control.

Results

Effect of aqueous extract of *Croton zambesicus* on blood pressure in anaesthetized normotensive cat

The AECZ at doses of 1 and 5 mg/kg *i.v.* had no significant effect on the blood pressure of the normotensive cat. However at 10 and 20 mg/kg, the extract *i.v.* significantly (P<0.01 and P<0.001) reduced blood pressure of the anaesthetized normotensive cat compared to the control. Maximal blood pressure reduction was obtained at 20 mg/kg of the AECZ. There was no significant difference in blood pressure reduction at 20 mg/kg compared to 40 and 80 mg/kg of AECZ (Figures 1 and 2).

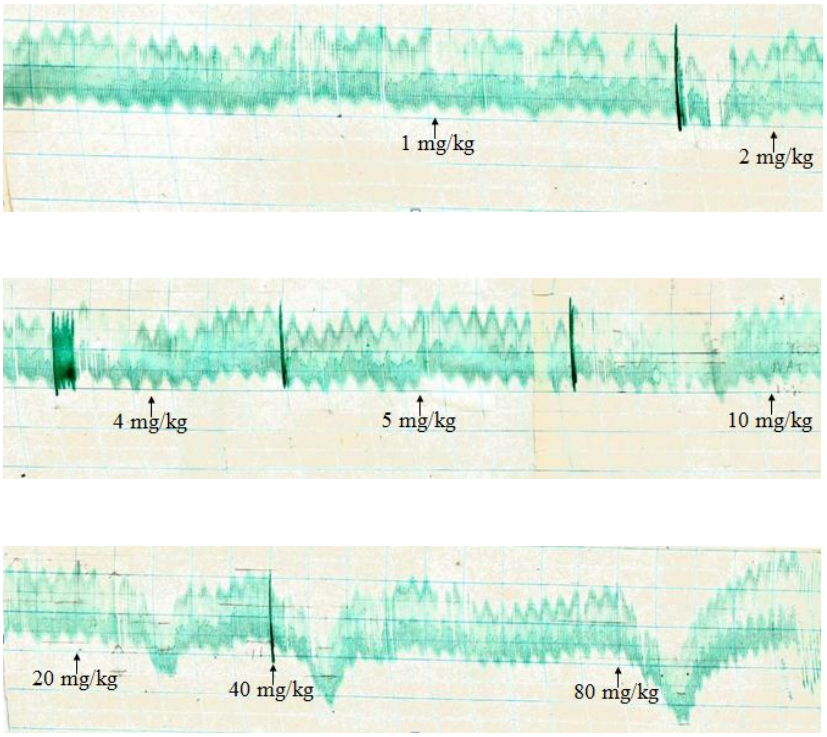

Fig1: The effect of aqueous extract of *Croton zambesicus* (1-80 mg/kg) on blood pressure of normotensive cat

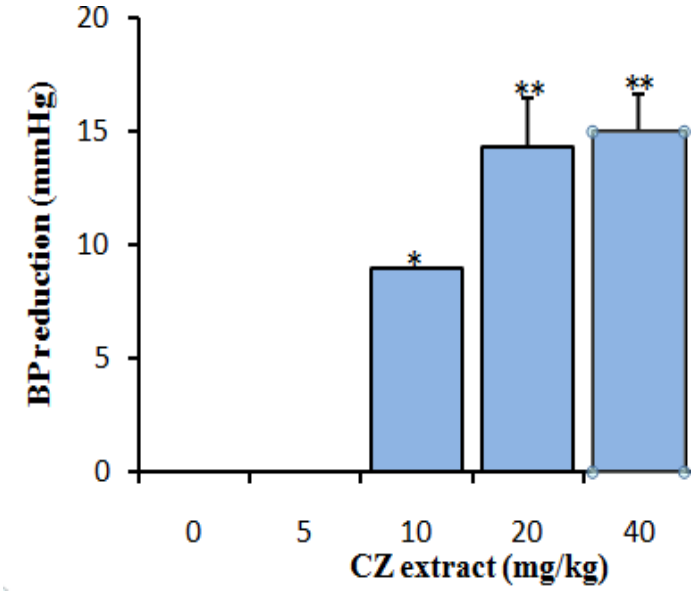

Fig. 2: Effect of aqueous extract of *Croton zambesicus* on blood pressure in anaesthetized normotensive cat. n=3, *P<0.01 and**P<0.001 vs control

Effect of Aqueous Extract of *Croton zambesicus* on Isolated Guinea pig ileum

The AECZ (0.1 and 0.25 mg/ml) had no significant effect on the tone and rate of contraction of the right atrium of the guinea pig while (2.5 mg/ml) produced a significant (P<0.05) reduction of the tone and rate of spontaneously beating isolated right atrium of the guinea pig (Figures 3 and 4).

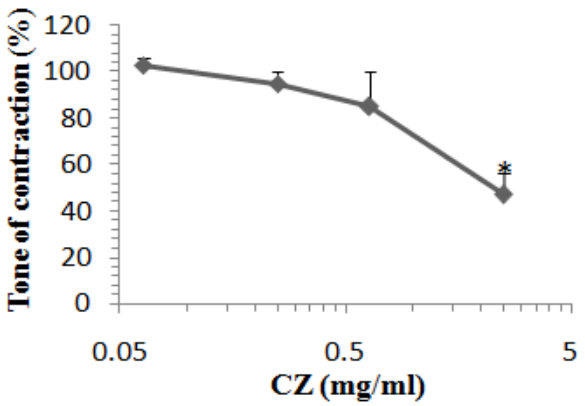

Fig 3: Effect of aqueous extract of *Croton zambesicus* (CZ) on tone of contraction of right atrium of guinea pig n=3, *P<0.05 vs control

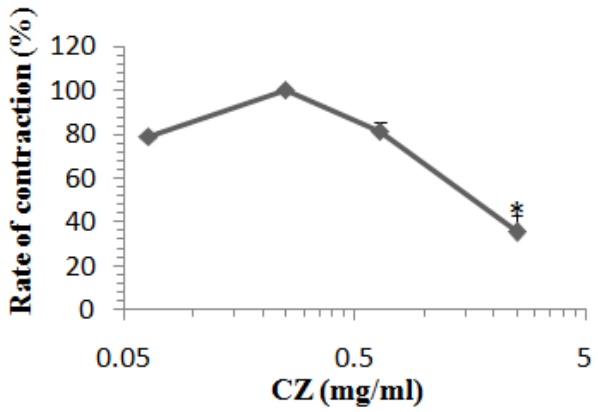

Fig. 4: Effect of *aqueous extract of Croton zambesicus* (CZ) on rate of contraction of right atrium of guinea pig n=3, *P<0.05 vs control

Effect of Aqueous extract of *Croton zambesicus* on Isolated Perfused Rabbit Heart

Aqueous extract of *Croton zambesicus* at low concentration (0.64 mg/ml) increased the tone of isoprenaline (10 nM)-induced contraction of the isolated rabbit heart. Higher concentration of the extract (6.4 mg/ml) produced a significant ($P<0.05$) inhibition of isoprenaline-induced contraction (Figure 5).

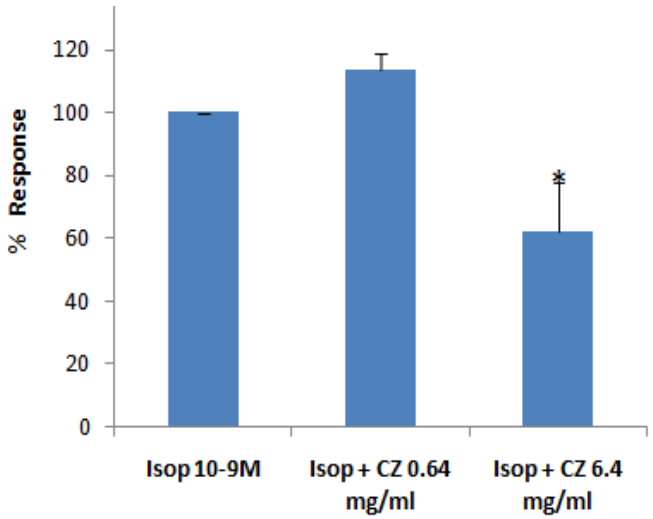

Fig. 5: Effect of aqueous extract of *Croton zambesicus* on isoprenaline-induced contraction of isolated perfused rabbit heart n=3, *P<0.05 vs control Isop: isoprenaline, CZ: *Croton zambesicus* aqueous extract

Discussion

Traditional medicines of proven safety and efficacy contribute to the goal of ensuring majority of people have access to health care (WHO, 2014). *Croton zambesicus* was selected for this study based on its ethnomedicinal use in the management of cardiovascular disorders such as hypertension (Adjanohaun *et al*., 1989). The aqueous extract of *Croton zambesicus* produced significant reduction in the blood pressure of anaesthetized normotensive cats. The hypotensive effect of the extract was dose-dependent and transient, lasting for a few minutes after a single IV administration. The acute reduction in blood

pressure may be attributed to a reduction in heart rate, resulting in decreased stroke volume and cardiac output (Armstrong *et al.,* 2012), although a reduction in vascular tone cannot be excluded (Zeggwagh *et al.*, 2014). The mean arterial blood pressure is a product of cardiac output and systemic vascular resistance. Changes in either cardiac output or systemic vascular resistance will affect mean arterial blood pressure (Klabunde, 2012).

The AECZ produced a decrease in both the rate and force of contraction of the isolated right atrium suggesting that a reduction in heart rate contributes to its hypotensive activity (Ojewole *et al.*, 2007). In addition, a reduction in the positive chronotropic effect of isoprenaline on isolated perfused heart by AECZ seems to suggest a mechanism involving Beta-adrenoceptor blockade. Beta blockers reduce blood pressure by decreasing cardiac output via negative inotropic and chronotropic actions (Klabunde, 2012). When used for long term therapy, they decrease vasomotor tone with a consequent decrease in systemic vascular resistance (Armstrong *et al.,* 2012). In addition, they block the sympathetic nervous system stimulation of renin released by juxtaglomerular cells in the kidney (Remedica, 2014). The AECZ produced smooth muscle relaxation, mediated possibly through inhibition of both AT_1 receptors and extracellular calcium influx via voltage-dependent and calcium channels (Unpublished work). A rational way to maintain optimal arterial pressure would be to administer foods and herbs that reduce cardiac output and or systemic vascular resistance. This is important because dietary factors play a key role in the development of cardiovascular disease (Odugbemi, 2006). Isolation of bioactive compounds in the plants may enhance their development into safe, effective and affordable therapeutic agents for the management of hypertension (Tabassum and Ahmad, 2012).

Further purification, isolation and biological evaluation of fractions and isolated compounds from the AECZ will be carried out.

Conclusion

The results obtained from this study revealed that aqueous extract of *Croton zambesicus* has hypotensive and cardiac depressant activities. These findings provide a pharmacologic basis for use of the plant in management of hypertension in traditional medicine.

References

Addo, J., Smeeth, L. and Leon, D.A. (2007). Hypertension in Sub-Saharan Africa, a systemic review. *Hypertension*, 50:1012.

Aderogba, M.A., McGaw, L.J., Bezabih, M. and Abegaz, B.M, (2011). Isolation and characterisation of novel antioxidant constituents of *Croton zambesicus* leaf extract. *Asian Pac J Trop Med*, 4(12): 969-72.

Adjanohaun, E.J., Ajakidje, V. and de Souza S. (1989). Contribution to Ethnobothanical and Floristic Studies in Benin Republic Vol. 1 *Agency for Cultural and Technical Cooperation*. pp. 245.

Armstrong, E.J., Armstrong, A.W. and Rocco, T.P. (2012). Integrative cardiovascular pharmacology: Hypertension, ischemic heart disease and heart failure. *Principles of Pharmacology*, 437-463.

Ayanniyi, R.O. and Wannang, N.N. (2008 a). Anticonvulsant activity of the aqueous leaf extract of *Croton zambesicus* in mice and rats. *Iranian J. Pharmacol. Ther*. 7(1):79-82.

Ayanniyi, R.O. and Wannang, N.N. (2008 b). Neuropharmacological profile of the aqueous leaf extract of *Croton zambesicu*s (euphorbiaceae) in some laboratory animals. *Iranian. J. Pharmacol. Ther*. 7(2):161-164.

Baccelli, C., Navarro, I., Block, S., Morel, N. and Quetin-Leclercq, J. (2007). Vasorelaxant activity of diterpenes from Croton zambesicus and synthetic trachylobanes and their structure-Activity relationships. *J. Nat. Prod.* 70 (6):910-917.

Bako, I.G., Mabrouk, M.A., Maje, I.M., Buraimoh, A,A. and Abubakar, M.S. (2010). Hypotensive effect of aqueous seed extract of *Hibiscus sabdariffa* on normotensive cat. *International Journal of Animal and Veterinary Advances*, 2:5-8.

Ekwunife, O.I. and Aguwa, C.N., (2011). A meta analysis of prevalence rate of hypertension in Nigerian populations. *J. Public Health Epidemiol*., 3(13):604-607.

Ganiyu, K.A. and Suleiman, I.A. (2014). Economic Burden of Drug Therapy in Hypertension Management in a Private Teaching Hospital in Nigeria. *British Journal of Pharmaceutical Research*, 4(1):70-78.

Klabunde, R.E., (2012). Beta-Adrenoceptor Antagonists (Beta-Blockers). Available at http://www.cvpharmacology.com/cardioinhibitory/betablockers. htm. Accessed on: March 18.

Madhur, M.S., Lob, H.E., McCann, L.A., Iwakura, Y., Blinder, Y., Guzik, T.J. and Harrison, D.G. (2010). Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hypertension*; 55(2):500-507.

Martinsen, A., Baccelli, C., Navarro, I., Abad, A., Quetin-Leclercq, J. and Morel, N. (2010). Vascular activity of a natural diterpene isolated from Croton

zambesicus and of a structurally similar synthetic trachylobane. *Vasc. Pharmacol*., 52(1-2):63-69.

Odugbemi, T. (2006). Outlines and Pictures of Medicinal Plants from Nigeria. *Nutrition Journal*, pp.1-85.

Ojewole, J., Kamadyaapa, G.M.M., Moodley, K. and Musabayane, C.T. (2007). Cardiovascular effects of Persea Americana Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals. *Cardiovasc. J. S. Afr*., (18)2: 69-76.

Okokon, J.E. and Nwafor, P.A. (2010). Antiinflammatory, analgesic and antipyretic activities of *Croton zambesicus*. *Pak J Pharm Sci*, (23)4:385-392,.

Okokon, J.E., Bassey, A.L. and Obot, J. (2006). Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* muell. In Alloxan diabetic rats. *Afr. J. Trad. Complement Altern. Med*., 3(2):21-26.

Okokon, J.E. and Nwafor, P.A. (2009). Antiulcer and anticonvulsant activity of *Croton* zambesicus. *Pak. J. Pharm. Sci.*, (22)4: 384-390.

Remedica. (2014). Hypertension in Cardiology. Available at http://www.ncbi.nlm.nih.gov/books/NBK2217/ . Retrieved September 2014.

Robert, S., Baccelli, C., Devel, Dogné, J.M. and Quetin-Leclercq, J. (2010). Effects of leaf extracts from *Croton zambesicus* Müell. Arg. on hemostasis. *J. Ethnopharmacol*., 128(3):641-648.

Siddiqi, H.S., Mehmood, H.M., Rehman, N.U. and Gilani, H.A, (2012). Studies on the antihypertensive and antidyslipidemic activities of *Viola odorata* leaves extract. *Lipids Health Dis*, 11:16.

Tabassum, N. and Ahmad F. (2012). Role of natural herbs in the treatment of hypertension. *Pharmacogn Rev*, 5:30-40.

Tesfaye, F., Byass, P. and Wall, S. (2009). Population based prevalence of high blood pressure among adults in Addis Ababa: Uncovering a silent epidermic. BMC Cardiovasc Disord., 9:39.

Ulasi, I, Ijoma, C., Onwubere, B., Arodiwe, E., Onodugo, O. and Okafor, C. (2011) High prevalence and low awareness of hypertension in a Market population in Enugu. *International Journal of Hypertension*, 10.4061/2011/869675.

Vasanthi HR, Shri Mal N, Das DK: Phytochemicals from plants to combat cardiovascular disease. *Current Med. Chem*., 19: 2242-2251, 2012.

WHO: Traditional medicine strategy: 2014-2023. (2014) Available at http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/. Accessed September 2014.

WHO: World Health Organization, World Health Statistics. (2012). Available at http://www.who.int/healthinfo/EN_WHS2012Full.pdf. Retrieved December 2012.

Zeggwagh, N.A., Michel, J.B. and Eddouks, M. (2014). Acute hypotensive and diuretic activities of *Artemisia herba alba* aqueous extract in normal rats. Asian. *Pac. J. Trop. Biomed.* 4(2):S644-S648.