

Anti-cholesterolemic Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* (L) King and Robinson (Asteraceae): Potential for the Reduction of Cardiovascular Risk.

Jude Chigozie Ikwuchi* and Catherine Chidinma Ikwuchi

Department of Biochemistry, Faculty of Science, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

E-mail: ecoli240733@yahoo.com*

ABSTRACT

The effect of aqueous extract of the leaves of *Chromolaena odorata* (L.) on the packed cell volume (PCV), daily weight gain, plasma lipid profiles and atherogenic indices of cholesterol loaded rats was investigated. The test group received daily, by intra-gastric gavages, 100 mg/kg body weight of aqueous extract and 10 g/kg of cholesterol; the test control received 10 g/kg cholesterol and water; while the control received only water. The PCV, mean daily weight gain, and plasma concentrations of triglyceride, LDL, VLDL, non-HDL and total cholesterol, as well as the atherogenic indices [cardiac risk ratio, atherogenic coefficient and atherogenic index of plasma] of the treated animals were all significantly lower ($P < 0.05$) than those of the test control and control. The plasma HDL-cholesterol level of the treated animals was significantly higher ($P < 0.05$) than that of the test control, although significantly lower than that of the control. These results suggest a possible protective role of the extract against the development of cardiovascular complications, due to dyslipidemic conditions, whether primary or secondary to diabetes mellitus, hypertension and obesity.

(Keywords: atherogenic indices, *Chromolaena odorata*, hematocrit, hypocholesterolemia, plasma triglyceride, weight loss)

INTRODUCTION

Cardiovascular disease is one of the world's leading causes of death, and has dyslipidemia as one of its major risk factors. Dyslipidemia may be primary or may accompany hypertension, diabetes mellitus and obesity (Zicha *et al.*, 1999; Franz *et al.*, 2002; Leikin and Lipsky, 2003;

American Dietetic Association, 2004; Gylling *et al.*, 2004; Rang *et al.*, 2005; Martirosyan *et al.*, 2007; Brunzell *et al.*, 2008). Usually, it involves elevated plasma levels of triglycerides, total-, LDL- and VLDL-cholesterol and a low level of HDL cholesterol (Kwiterovich, 1998; Shepherd, 1998; Zicha *et al.*, 1999; Franz *et al.*, 2002; Shen, 2007). Thus nutritional and pharmacologic interventions aiming to normalize abnormal lipid metabolism could be useful for reducing the risk of cardiovascular diseases (Zicha *et al.*, 1999; Brunzell *et al.*, 2008). In recent times, there has been a growing interest in the use of herbal products for the management of hypertension and other cardiovascular diseases. Amongst the plants used are *Tridax procumbens*, *Chromolaena odorata*, *Stachytarpheta jamaicensis* and *Acalypha wilkesiana*.

Chromolaena odorata (L) King and Robinson (family Asteraceae), native to South and Central America, is presently found throughout the tropics, Nigeria inclusive (Fosberg and Sachet, 1980; State of Queensland, 2007). It is commonly called Awolowo, independence weed, Siam weed, trifid weed, bitter bush, or Jack-in-the-bush (Okon and Amalu, 2003). The Ibo people of South Eastern Nigeria call it "ahihia eliza" or "obiara kara". In traditional medicine it is popular for its antispasmodic, anti-protozoal, antifungal, anti-trypanosomal, antibacterial and antihypertensive activities (Phan *et al.*, 2001). It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatotropic properties (Akinmoladun *et al.*, 2007).

In the southern part of Nigeria and in Vietnam, fresh leaves or a decoction of *Chromolaena odorata* are used for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection, dento-alveolitis, and to stop bleeding (Phan *et al.*, 2001). Consequent upon the use of this plant in

traditional medicine as an antihypertensive and diuretic, the present study was designed to investigate the effect of aqueous leaf extract of the leaves of *C. odorata* on plasma lipid profiles and atherogenic indices of cholesterol loaded rats; since hypertension is often accompanied by abnormal lipid metabolism.

MATERIALS AND METHODS

Collection of Animals and Leaves, and Preparation of the Leaf Extract

Albino rats were collected from the animal house of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. Fresh plants were collected from behind the Ofrima Hall Complex of University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, their leaves were collected, rid of dirt, dried and ground into powder. The resultant powder was soaked in boiled distilled water for 12 hr, after which the resultant mixture was filtered and the filtrate was stored for subsequent use in a refrigerator. A known volume of this extract was evaporated to dryness, and the weight of the residue used to calculate the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract to the test animals. All reagents were of analytical grade.

Experimental Design

Studies were conducted in compliance with applicable laws and regulations. The rats were randomly sorted into three groups of five animals each, so that the average weight difference was approximately equal, and were housed in plastic cages, in the animal house of the Department of Biochemistry, University of Port Harcourt. After a one-week acclimatization period on guinea growers mash (Port Harcourt and Flour Mills, Port Harcourt, Nigeria), the treatment commenced and lasted for a week. The test group received daily by intra-gastric gavages, 100 mg/kg body weight extract and 10 g/kg body weight cholesterol; the test-control received daily by intra-gastric gavages, 10 g/kg body weight of cholesterol and distilled water; while the control group received appropriate volumes of water by the same route. They all had free access to food and water. The 1% cholesterol loading was a modification of that

reported by Al-Numair (Al-Numair, 2009). At the end of the treatment period the rats were weighed, fasted overnight and subjected to chloroform anesthesia. While under anesthesia, blood was collected from each rat via heart puncture and transferred into heparin and EDTA sample bottles.

Determination of Packed Cell Volume

Packed cell volume (PCV) was measured with micro-hematocrit, with 75x16 mm capillary tubes filled with blood and centrifuged at 3000 g for 5 min (Cheesbrough, 2004).

Determination of the Plasma Lipid Profiles and Indices

Plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride (TG) were assayed enzymatically with Randox test kits (Randox Laboratories, Crumlin, England). Plasma LDL- and VLDL-cholesterol (LDL-C and VLDL-C) were calculated using the Friedewald equation (Friedewald *et al.*, 1972) as follows:

$$i. \text{ [LDL C] (mmol/L) = [TC] - [HDL C] - } \frac{[TG]}{2.2}$$

$$ii. \text{ [VLDL cholesterol] (mmol/L) = } \frac{[Triglyceride]}{2.2}$$

While the plasma non-HDL cholesterol concentration was determined as reported by Brunzell *et al.* (2008):

$$[\text{Non-HDL cholesterol}] = [\text{Total cholesterol}] - [\text{HDL cholesterol}]$$

The atherogenic indices were calculated as earlier reported by Ikewuchi and Ikewuchi (2009a, 2009b, 2010) using the following formulae:

$$i. \text{ Cardiac Risk Ratio = } \frac{[\text{Total cholesterol}]}{[\text{HDL cholesterol}]}$$

$$ii. \text{ Atherogenic Coefficient} \\ = \frac{[\text{Total cholesterol}] - [\text{HDL cholesterol}]}{[\text{HDL cholesterol}]}$$

iii. *Atherogenic Index of Plasma*

$$= \log \frac{[\text{Triglyceride}]}{[\text{HDL cholesterol}]}$$

($P < 0.05$) than that of the test control and the control groups. The PCV of the animals in the test group was significantly lower ($P < 0.05$) than that of the control, but not significantly lower than that of the test control.

Statistical Analysis of Data

All values are quoted as the mean \pm SD. The values of the various parameters for the test, test control and control groups were analyzed for statistical significant differences, using the student's t-test, with the help of SPSS Statistics 17.0 software.

RESULTS

Table 1 shows the effect of aqueous extract of the leaves of *Chromolaena odorata*, on the mean daily weight gain and packed cell volume of cholesterol loaded rats. The mean daily weight gain of the test animals was significantly lower

The effect of the aqueous extract of the leaves of *Chromolaena odorata* on plasma lipid profiles and atherogenic indices of cholesterol loaded rats are given in Tables 2 and 3. The plasma total triglyceride, LDL-, VLDL-, non HDL- and total cholesterol levels of the treated animals was significantly lower ($P < 0.05$) than those of the test control and control. The plasma HDL-cholesterol levels of the treated animals was significantly higher ($P < 0.05$) than that of the test control, although significantly lower than that of the control. The atherogenic indices: cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index of plasma (AIP), of the treated animals was significantly lower ($P < 0.05$) than those of the test control and control animals.

Table 1: The Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Packed Cell Volume and Mean Daily Body Weight of Cholesterol Loaded Rats.

Parameter	Magnitude		
	Control	Test-control	Test
Packed cell volume (%)	40.59 \pm 5.45 ^a	26.52 \pm 8.04 ^b	25.37 \pm 7.50 ^b
Mean daily weight gain (g/day)	6.07 \pm 2.22 ^a	6.57 \pm 0.12 ^a	2.86 \pm 0.58 ^b

Values are means \pm SD, n=5 per group. Entries with different superscripts are significantly different at $P < 0.05$.

Table 2: Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Plasma Lipid Profile of Cholesterol Loaded Rats.

Parameter	Concentration (mmol/L)		
	Control	Test-control	Test
Plasma triglyceride	1.81 \pm 0.01 ^a	2.04 \pm 0.03 ^b	1.40 \pm 0.04 ^c
Plasma total cholesterol	6.15 \pm 1.10 ^a	7.37 \pm 1.24 ^b	3.38 \pm 0.58 ^c
Plasma HDL cholesterol	2.49 \pm 0.03 ^a	1.40 \pm 0.04 ^b	1.62 \pm 0.05 ^c
Plasma VLDL cholesterol	0.82 \pm 0.01 ^a	0.93 \pm 0.02 ^b	0.64 \pm 0.02 ^c
Plasma LDL cholesterol	2.83 \pm 0.90 ^a	5.03 \pm 1.18 ^b	1.31 \pm 0.51 ^c
Plasma non HDL cholesterol	3.66 \pm 0.46 ^a	5.97 \pm 0.60 ^b	1.76 \pm 0.27 ^c

Values are means \pm SD, n=5 per group. Entries with different superscripts are significantly different at $P < 0.05$.

Table 3: Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* on the Atherogenic Indices of Cholesterol Loaded Rats.

Parameter	Magnitude		
	Control	Test-control	Test
Cardiac risk ratio	2.47 \pm 0.49 ^a	5.22 \pm 0.73 ^b	2.20 \pm 0.30 ^a
Atherogenic coefficient	1.47 \pm 0.49 ^a	4.22 \pm 0.73 ^b	1.20 \pm 0.30 ^a
Atherogenic index of plasma	-0.15 \pm 0.11 ^a	0.16 \pm 0.01 ^b	-0.06 \pm 0.00 ^a

Values are means \pm SD, n=5 per group. Entries with different superscripts are significantly different at $P < 0.05$.

DISCUSSION AND CONCLUSION

The reduction in weight gain, produced by the extract (Table 1) may be due to its diuretic activity. According to Freis *et al.* (1988), diuresis leads to weight loss due to volume loss, which correlates with reduction in blood pressure. Weight loss helps improve and control coronary risk incidence, diabetes mellitus, dyslipidemia, hypertension, obesity and physical functioning (Krauss *et al.*, 2006; Trussell *et al.*, 2005; Bantle *et al.*, 2006; Brunzell *et al.*, 2008) and is one of the strategies for increasing low HDL cholesterol levels (Assmann and Gotto, 2004), as well as alleviating insulin resistance (Krauss *et al.*, 2006).

The diuretic effect may have been due to the saponins present in the leaves (Igboh *et al.*, 2009); since saponins are known to produce diuresis (Soetan, 2008). Therefore, the significantly low mean daily weight gain produced by the extract, in the test animals implies that the extract may be useful in the management of hypertension, obesity and dyslipidemia. The implication of the low hematocrit in the test animals is that the plant extract could not protect the animals against the hypercholesterolemia induced lowering of PCV, and may have added to the effect, probably due to its negative effect on the hemopoietic system of the test rats or by an outright cytotoxic effect on their red blood cells.

Elevated plasma triglyceride levels is both an independent and synergistic risk factor for cardiovascular diseases (Dobiášová, 2004; Martirosyan *et al.*, 2007; McBride, 2007; Brunzell *et al.*, 2008) and is often associated with hypertension (Shepherd, 1998; Zicha *et al.*, 1999;), abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Shepherd, 1998; Krauss *et al.*, 2006; McBride, 2007; Brunzell *et al.*, 2008). The extract significantly reduced plasma levels of triglycerides. Raised plasma total cholesterol level is a recognized and well-established risk factor for developing atherosclerosis and other cardiovascular diseases (Ademuyiwa *et al.*, 2005) and is often found in hypertension (Zicha *et al.*, 1999). Therefore, a reduction in plasma total cholesterol level reduces the risk of cardiovascular diseases. Thus, the significantly lower plasma total cholesterol levels produced by the extract, connotes the ability of the extract to protect against cardiovascular diseases.

The cholesterol lowering effect may have been due to the saponin content, since saponins have hypocholesterolemic properties (Soetan, 2008; European Food and Safety Authority, 2009). High plasma levels of LDL and VLDL cholesterol is a risk factor for cardiovascular disease (Ademuyiwa *et al.*, 2005; Lichtenstein *et al.*, 2006) and often accompanies diabetes mellitus (Rang *et al.*, 2005; Shen, 2007; Brunzell *et al.*, 2008), obesity (Krauss *et al.*, 2006) and hypertension (Shepherd, 1998; Zicha *et al.*, 1999). In this study, we observed a significantly lower plasma LDL and VLDL cholesterol levels in the treated animals. Decreases in plasma LDL cholesterol have been considered to reduce risk of coronary heart disease (Rang *et al.*, 2005; Shen, 2007).

Decreased plasma HDL cholesterol is a risk factor for cardiovascular diseases (Lewis and Rader, 2005; Rang *et al.*, 2005; Lichtenstein *et al.*, 2006; Martirosyan *et al.*, 2007) and is often found in hypertension (Shepherd, 1998; Zicha *et al.*, 1999), obesity (Krauss *et al.*, 2006) and diabetes mellitus (Shepherd, 1998; Rang *et al.*, 2005; Shen, 2007; Brunzell *et al.*, 2008). In this study, the extract increased plasma HDL cholesterol levels. Clinical data show that increase in plasma HDL cholesterol concentration decreases cardiovascular risk (Assmann and Gotto, 2004; Rang *et al.*, 2005).

Increases in plasma HDL cholesterol have been considered to reduce risk in coronary heart disease (Assmann and Gotto, 2004; Rang *et al.*, 2005). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell (Marcel *et al.*, 1980) i.e. by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification through lecithin: cholesterol acyltransferase and delivering it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body (Assmann and Gotto, 2004; Ademuyiwa *et al.*, 2005); and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (Assmann and Gotto, 2004; Ademuyiwa *et al.*, 2005; Brunzell *et al.*, 2008) and anti-inflammatory property (Ademuyiwa *et al.*, 2005).

Many studies have demonstrated that non-HDL cholesterol is a better predictor of CVD risk than is LDL cholesterol (Liu *et al.*, 2005; Pischon *et al.*,

2005; Brunzell *et al.*, 2008). Therefore, the significantly lowered plasma non HDL cholesterol observed in the test animals portends ability of the extract to reduce cardiovascular risk.

Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Brehm *et al.*, 2004; Dobiášová, 2004; Usoro *et al.*, 2006; Martirosyan *et al.*, 2007). In this study, we observed that the extract produced significantly lower atherogenic indices CRR, AC and AIP. This effect is comparable to that of *Tridax procumbens* (Ikewuchi and Ikewuchi, 2009b). Low atherogenic indices are protective against coronary heart disease (Usoro *et al.*, 2006).

All of these results indicate a possible protective mechanism of the extract against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterize obesity, hypertension and diabetes mellitus. It also implies that the extract may help manage the dyslipidemic conditions, which according to Katzung (2004), and Salvetti and Ghiadoni (2006), accompany the administration of thiazide diuretics.

REFERENCES

- Ademuyiwa, O., R.N. Ugbaja, F. Idumebor, and O. Adebawo. 2005. "Plasma Lipid Profiles and Risk of Cardiovascular Disease in Occupational Lead Exposure in Abeokuta, Nigeria". *Lipids in Health and Disease*. 4:19. <http://www.lipidworld.com/content/4/1/19>
- Akinmoladun, A.C., E.C. Ibukun, and I.A. Dan-Ologe. 2007. "Phytochemical Constituents and Antioxidant Properties of Extracts from the Leaves of *Chromolaena odorata*". *Scientific Research and Essays*. 2(6):191-194.
- Al-Numair, K.S. 2009. "Hypocholesteremic and Antioxidant Effects of Garlic (*Allium sativum* L.) Extract in Rats Fed High Cholesterol Diet". *Pakistan Journal of Nutrition*. 8(2):161-166.
- American Dietetic Association. 2004. "Dyslipidemia Management in Adults with Diabetes". *Diabetes Care*. 27:S68-S71.
- Assmann, G. and A.M. Gotto, Jr. 2004. "HDL Cholesterol and Protective Factors in Atherosclerosis". *Circulation*. 109 [suppl III]: III-8 – III-14. DOI:10.1161/01.CIR.0000131512.50667.46.
- Bantle, J.P., J. Wylie-Rosett, A.L. Albright, C.M. Apovian, N.G. Clark, M.J. Franz, B.J. Hoogwerf, A.H. Lichtenstein, E. Mayer-Davis, A.D. Mooradian, and M.L. Wheeler. 2006. "Nutrition Recommendations and Interventions for Diabetes—2006: A Position Statement of the American Diabetes Association". *Diabetes Care*. 9(9):2140-2157. DOI: 10.2337/dc06-9914.
- Brehm, A., G. Pfeiler, G. Pacini, H. Vierhapper, and M. Roden. 2004. "Relationship between Serum Lipoprotein Ratios and Insulin Resistance in Obesity". *Clinical Chemistry*. 50(12):2316–2322.
- Brunzell, J.D., M. Davidson, C.D. Furberg, R.B. Goldberg, B.V. Howard, J.H. Stein, and J.L. Witztum. 2008. "Lipoprotein Management in patients with Cardiometabolic Risk". *Journal of the American College of Cardiology*. 51(15):1512-1524. doi:10.1016/j.jacc.2008.02.034.
- Cheesbrough, M. 2004. *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press: U.K. ISBN 0521665469.
- Dobiášová, M. 2004. "Atherogenic Index of Plasma [log(triglyceride/HDL-Cholesterol)]: Theoretical and Practical Implications". *Clinical Chemistry*. 50(7): 1113-1115. doi:10.1373/clinchem.2004.033175.
- European Food and Safety Authority. 2009. "Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Saponins in *Madhuca Longifolia* L. as Undesirable Substances in Animal Feed". *The EFSA Journal* (2009). 979:1-36.
- Fosberg, F.R. and M.-H. Sacht. 1980. *Flora of Micronesia, 4: Caprifoliaceae-Compositae*. Smithsonian Institution Press, Washington. Smithsonian Contributions to Botany Number 46. 71p.
- Franz, M.J., J.P. Bantle, C.A. Beebe, J.D. Brunzell, J.L. Chiasson, A. Garg, L.A. Holzeister, B. Hoogwerf, E. Mayer-Davies, A.D. Mooradian, J.Q. Purnell, and M. Wheeler. 2002. "Evidence-Based Nutrition Principles and Recommendations for the Treatment and Prevention of Diabetes and Related Complications". *Diabetes Care*. 25(1):148-198.
- Freis, E.D., D.J. Reda, and B.J. Materson. 1988. "Volume (Weight) Loss and Pressure Following Thiazide Diuretics". *Hypertension*. 12:244-250.
- Friedewald, W.T., R.I. Levy, and D.S. Friedrickson. 1972. "Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without use of the Preparative Ultracentrifuge". *Clinical Chemistry*. 18(6):499-502.

16. Gylling, H., J.A. Tuominen, V.A. Koivisto, and T.A. Miettinen. 2004. "Cholesterol Metabolism in Type 1Diabetes". *Diabetes Care*. 53(9):2217–2222.
17. Igboh, M.N., J.C. Ikwuchi, and C.C. Ikwuchi. 2009. "Chemical Profile of *Chromolaena odorata* L. (King and Robinson) Leaves". *Pakistan Journal Nutrition*. 8(5):521-524.
18. Ikwuchi, J.C. and C.C. Ikwuchi. 2009a. "Alteration of Plasma Lipid Profiles and Atherogenic Indices by *Stachytarpheta jamaicensis* L. (Vahl)". *Biokemistri*. 21(2):71-77.
19. Ikwuchi, J.C. and C.C. Ikwuchi. 2009b. "Alteration of Plasma Lipid profile and Atherogenic Indices of Cholesterol Loaded Rats by *Tridax procumbens* Linn: Implications for the Management of Obesity and Cardiovascular Diseases". *Biokemistri*. 21(2):95-99.
20. Ikwuchi, J.C. and C.C. Ikwuchi. 2010. "Hypocholesterolaemic Effect of Aqueous Extract of *Acalypha wilkesiana* 'Godseffiana' Muell Arg on Rats Fed Egg Yolk Supplemented Diet: Implications for Cardiovascular Risk Management". *Research Journal of Science and Technology*. 2(4):78-81.
21. Katzung, B.G. 2004. *Basic and Clinical Pharmacology*, 9th edition. (Lange Basic Science), McGraw-Hill Medical: New York. ISBN: 0-07-141092-9.
22. Krauss, R.M., P.J. Blanche, R.S. Rawlings, H.S. Fernstrom, and P.T. Williams. 2006. "Separate Effects of Reduced Carbohydrate Intake and Weight Loss on Atherogenic Dyslipidaemia". *American Journal of Clinical Nutrition*. 83(6):1025–1031.
23. Kwiterovich, P.O. Jr. 1998. "The Antiatherogenic Role of High Density Lipoprotein Cholesterol". *American Journal of Cardiology*. 82(8):13Q-21Q.
24. Leikin, J.B. and M.S. Lipsky (editors). 2003. *American Medical Association Complete Medical Encyclopedia*. Random House References: New York. pp. 690-692. ISBN: 0-8129-9100-1 (bc).
25. Lewis, G.F. and D.J. Rader. 2005. "New Insights into the Regulation of HDL Metabolism and Reverse Cholesterol Transport". *Circulation Research*. 96(12):1221-1232. doi: 10.1161/01.RES.0000170946.56981.5c.
26. Lichtennstein, A.H., L.J. Appel, M. Brands, M. Carnethon, S. Daniels, B. Franklin, P. Kris-Etherton, W.S. Harris, B. Howard, N. Karanja, M. Lefevre, L. Rudel, F. Sacks, L. van Horn, M. Winston, J. Wylie-Rosett, and H.A. Franch. 2006. "Diet and Lifestyle Recommendations Revision 2006. A Scientific Statement from the American Heart Association Nutrition Committee". *Circulation*. 114(1):82-96. DOI:10.1161/CIRCULATIONAHA.106.176158.
27. Liu, J., C. Sempos, R. Donahue, J. Dorn, M. Trevisan, and S.M. Grundy. 2005. "Joint Distribution of non-HDL and LDL Cholesterol and Coronary Heart Disease Risk Prediction among Individuals with and without Diabetes". *Diabetes Care*. 28(8):1916-1921.
28. Marcel, Y.L., C. Vezina, B. Teng, and A. Snidermann. 1980. "Transfer of Cholesterol Esters between Human High Density Lipoprotein and Triglyceride Rich Lipoproteins Controlled by Plasma Protein Factor". *Atherosclerosis*. 35(2):127-133.
29. Martirosyan, D.M., L.A. Miroshnichenko, S.N. Kulokawa, A.V. Pogojeva, and V.I. Zolodov. 2007. "Amaranth Oil Application for Heart Disease and Hypertension". *Lipids in Health and Disease*. 6: 1. doi:10.1186/1476-511X-6-1 .
30. McBride, P.E. 2007. "Triglycerides and Risk for Coronary Heart Disease". *JAMA* 298(3):336-338.
31. Okon, P.B and U.C. Amalu. 2003. *Using Weed to Fight Weed*. *Leisa Magazine*. <http://www.metafro.be/leisa/2003/194-21.pdf>
32. Phan, T.T., L. Wang, P. See, R.J. Grayer, S.Y. Chan, and S.T. Lee. 2001. "Phenolic Compounds of *Chromolaena odorata* Protect Cultured Skin Cells from Oxidative Damage: Implication for Cutaneous Wound Healing". *Biological and Pharmacology Bulletin*. 24(12):1373—1379. doi:10.1248/bpb.24.1373. JOI:JST.JSTAGE/bpb/24.1373.
33. Pischon, T., C.J. Girman, F.M. Sacks, N. Rifai, M.J. Stampfer, and E.B. Rimm. 2005. "Non-high Density Lipoprotein Cholesterol and Apolipoprotein B in the Prediction of Coronary Heart Disease in Men". *Circulation*. 112(22):3375-3383.
34. Rang, H.P., M.M. Dale, J.M. Ritter, and P.K. Moore. 2005. *Pharmacology*. 5th edition. Elsevier: India. ISBN: 81-8147-917-3.
35. Salvetti, A. and L. Ghiadoni. 2006. "Thiazide Diuretics in the Treatment of Hypertension: An Update". *Journal of the American Society of Nephrology*. 17(4):S25-S29. Doi: 10.1681/ASN.2005121329.
36. Shen, G.X. 2007. "Lipid Disorders in Diabetes Mellitus and Current Management". *Current Pharmaceutical Analysis*. 3:17-24.

37. Shepherd, J. 1998. "Identifying Patients at Risk for Coronary Heart Disease: Treatment Implications". *European Heart Journal*. 19:1776–1783. Article No. hj981122.
38. Soetan, K.O. 2008. "Pharmacological and Other Beneficial Effects of Antinutritional Factors in Plants - A Review". *African Journal of Biotechnology*. 7(25):4713-4721.
39. The State of Queensland (Department of Natural Resources and Water) 2007. Pest series: Siam weed (*Chromolaena odorata*), Declared Class 1. PP49.
<http://www.nrm.qld.gov.au/factsheets/pdf/pest/pp49.pdf>
40. Trussell, K.C., D. Hinnen, P. Gray, S.A. Drake-Nisly, K.M. Bratcher, H. Ramsey, and J. Early. 2005. "Case Study: Weight Loss Leads to Cost Savings and Improvement in Metabolic Syndrome". *Diabetes Spectrum*. 18(2):77-79.
41. Usoro, C.A.O., C.C. Adikwuru, I.N. Usoro, and A.C. Nsonwu. 2006. "Lipid Profile of Postmenopausal Women in Calabar, Nigeria". *Pakistan Journal of Nutrition*. 5(1):79-82.
42. Zicha, J., J. Kunes, and M.A. Devynck. 1999. "Abnormalities of Membrane Function and Lipid Metabolism in Hypertension: A Review". *American Journal of Hypertension*. 12(3):315-331.

SUGGESTED CITATION

Ikwuchi, J.C. and C.C. Ikwuchi. 2011. "Anti-cholesterolemic Effect of Aqueous Extract of the Leaves of *Chromolaena odorata* (L) King and Robinson (Asteraceae): Potential for the Reduction of Cardiovascular Risk". *Pacific Journal of Science and Technology*. 12(2):385-391.

