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Phytochemical, Nutrient Composition and Serum Lipid Lowering Effect of *Xylopia aethiopica* Fruit

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Authors' contributions

This work was carried out in collaboration between all authors. Author YNO designed the study and wrote the protocol. Author SOO performed the statistical analysis, and wrote the first draft of the manuscript. Author MIE did the literature search and also wrote part of the manuscript. Authors SB, GO and ABA managed the animals, analyses of the study and collected all data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was carried out to evaluate the effects of *Xylopia aethiopica* on serum lipids in fed rats. Also the quantitative phytochemical and nutrient composition

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was investigated.
Study Design: Quantitative phytochemical, proximate analysis and *in vivo* effect on serum lipid profile.
Place and Duration of Study: Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike Abia State, between June 2013 and September 2013.
Methodology: The fruit were cut into small piece, dried and ground into powder. The quantitative phytochemical and proximate nutrient analyses of the powder sample were determined using standard methods. The serum lipid lowering effects of the powdered fruit in rats was determined by feeding different groups of rats with graded levels (5, 10, 20 and 50%) of the powdered fruit incorporated in their feed for 21 consecutive days and the effects on the total cholesterol, triglyceride, LDL-C, and HDL-C were compared with a negative control.
Results: The fruit sample produced significant ($p<0.05$) concentration-dependent decrease in the total serum cholesterol, triglyceride, LDL-C and VLDL-C levels and increase in serum HDL-C level in fed groups of rats when compared to the control group. The phytochemical analysis showed that the sample contained tannins (4.96%), flavonoids (0.81%), saponins (2.93%) and alkaloids (1.24%). The proximate analysis of the nutrient composition of powdered *Xylopia aethiopica* sample showed the presence of moisture, lipid, crude fibre, crude protein, ash and nitrogen free extracts in the following proportion 6.32, 12.54, 14.51, 0.91, 2.31 and 63.41% respectively.
Conclusion: The fruit sample demonstrated good hypolipidemic effects which may suggest that the consumption of *Xylopia aethiopica* fruit may help in the reduction of the incidence of hyperlipidemia related diseases in patients.

Keywords: Xylopia aethiopica; hypolipidemia; phytochemicals; proximate analysis.

1. INTRODUCTION

Plants and plant products have been used worldwide since ancient times for treatment of various human and animal diseases. The use of medicinal plants and its extract in traditional medicine is still common in developing countries attributable in part to poverty and illiteracy which militate against availability and accessibility to conventional medical services [1,2]. In traditional medicine, use of herbs play role in managing of hyperlipidemia and related diseases [3]. Some plants such as *Cassia tora* [4], *Hibiscus sabdariffa* [5], *Sesbania grandiflora* [6], and *Amaranthus spp* [3] have been shown to possess anti-hyperlipidemic properties. Their mechanism of action may be through inhibition of hepatic cholesterol biosynthesis and/or reduction of lipid absorption from the gut [3].

Hyperlipidemia *as a* disorder of lipid metabolism is characterized by elevated serum level of total cholesterol, low density and very low density lipoprotein cholesterol, and triglyceride with concomitant decrease in serum level of high density lipoprotein cholesterol. It is the most prevalent indicator of the susceptibility and severity of cardiovascular disease [6]. Cardiovascular diseases (CVD) have become a leading cause of mortality and morbidity in developing countries and rates are expected to rise further over the next few decades [7]. Once considered a problem only in high-income countries, the prevalence of CVD risk factors is dramatically increasing in low-and middle-income African countries (Nigeria), particularly in urban areas [8,9]. In orthodox medicine hyperlipidemia is treated with statin derivatives, niacin, bile acid sequestrant and/or fibrates either as a monotherapy or

combination therapy. The use of these hypolipidemic agents are associated with side effects such as: hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function, which often leads to low patients compliance [6]. Hence, the need to evaluate the therapeutic benefits of commonly used herbs and herbal product in traditional medicine for the management of hyperlipidemia.

Xylopia aethiopica (Dunal) belong to the family Annonaceae. The common names are: "Negro pepper" in English, "Uda" in Igbo, "sesedu" in Yoruba, and "kimba" in Hausa. It is a tropical evergreen tree growing up to 20 metres and bear aromatic seeds. Dry fruits of *X. aethiopica* contain 4-9 peppery seeds which are used as spices in the preparation of special dishes in most West African Countries especially in Nigeria, Ghana, Liberia, Benin and Cameroon, thus the name African pepper [10-12]. The fruit of *X. aethiopica* are given to women after delivery as condiment in soup and tea to enhance milk letdown [13,14]. In traditional medicine the fruit is also used in the treatment of bronchitis, asthma, male infertility, dysenteric conditions, febrile pains, stomach-aches and rheumatism [13,15]. Previous reports on the phytochemical composition of the fruit of *Xylopia aethiopica* showed that it contains alkaloids, polyphenols, terpenoids and kauranes (a class of diterpenes, namely kaurenoic and xylopic acid) [15,16]. The fruit extract of *X. aethiopica* has been shown to possess antimicrobial, antifungal, anagelsic, hypoglycemic, anthelmintic, haematopoietic, immune boosting activities, and increases lutenizing hormone and testosterone levels in rats [12,15-18]. The present study was aimed at evaluating the quantitative phytochemical and nutrient composition and the effect of incorporation of graded levels of the powdered fruit of *Xylopia aethiopica* on serum lipid profile of fed rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The freshly harvested fruit of *Xylopia aethiopica* (Uda) were bought from Ndoro market, Oboro in Ikwuano LGA of Abia State in the month of July 2013, and were authenticated by Dr. I. C. Okwulehie of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, and the voucher specimen catalogued MOUAU/COLNAS/BCM/2013/04 was kept for reference purpose in the departmental herbarium.

2.2 Preparation of Fruit Sample

The fruit *Xylopia aethiopica* was washed and air dried for about 24h. Each fruit was cut into small pieces with kitchen knife and the resulting pellets were subsequently dried in an electric oven (Gallenkamp) at 40ºC. The dry fruit pellets were ground to fine powder using manual grinder. The resulting powder was stored in an air tight container throughout the duration of the experiment.

2.3 Experimental Animals

Mature Wistar albino rats, bred in the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the experiments. They were housed in an environment of normal ambient temperature and relative humidity of 40–60%, and the lighting period was about 12h daily. The weight of the rats varied between 140 and 165g. The rats were kept in stainless steel cages, supplied with clean drinking water and fed *ad*

libitum with standard commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea [19], and the experimental protocol was approved by the institution's ethical committee.

2.4 Effect of Fruit Sample of *Xylopia aethiopica* on the Serum Lipid Profile of Fed Rats

Thirty male normal albino Wistar rats were randomly divided into five groups of six animals each. Group 1 served as the control and received plain feed without fruit sample. Group 2-5 received feed incorporated with graded levels (5, 10, 20 and 50%, respectively) of *Xylopia aethiopica* fruit sample. The animals were fed 10gram of feed per 100gram body weight daily for 21 days. Twenty-four hours after the last feed administration, the animals were anaesthetized with chloroform vapour. Overnight fasting blood sample for sera preparation were collected by direct cardiac puncture into sterile plain tubes. The serum samples were separated from the clot by centrifugation at 3000rpm for 5min using bench top centrifuge (MSE minor, England). Serum samples were separated into sterile plain tubes and stored in refrigerator for serum lipid profile analysis.

2.5 Biochemical Analysis

The serum lipid profiles of the rats were evaluated using a commercially available assay kit (Randox, UK). The serum level of total cholesterol (TC) was measured by enzymatic hydrolysis and oxidation method as described by Stein [20]. The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by Tietz [21]. The serum level of HDL-C was measured by the method of Wacnic and Albers [22]. The serum VLDLC was calculated as 1/5 of the serum triglyceride [23], while the serum LDLC was calculated using the Friedewald formula (LDL=TC–HDL–VLDL) [24].

2.6 Quantitative Phytochemical Constituents of *Xylopia aethiopica*

Total alkaloids, flavonoids, tannins and saponins were determined using the method described by Krishnaiah et al. [25] in triplicates.

Total alkaloids content was determined by extracting 5grams of plant sample with 10% ethanoic acid in ethanol. The filtrate was concentrated and reacted with concentrated ammonium hydroxide until precipitation was complete. The precipitate (alkaloids) was washed with dilute ammonium hydroxide, dried, weighed and percentage alkaloids was calculated.

The total flavonoids content was determined by extracting 10grams of plant sample repeatedly with 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was then calculated.

The total saponins content was determined by repeated extraction of 20grams of powdered fruit sample with 20% ethanol at about 55ºC and filtered with Whatmann No. 1 filter paper. The filtrate was concentrated and partitioned with the aid of separating funnel into aqueous and diethyl ether layer. The aqueous layer was combined with n-butanol and was twice with

5% sodium chloride. The remaining solution was concentrated to dryness in Gallenkamp oven and the percentage saponins were calculated.

The total tannins content was determined by extracting 500mg of the powdered fruit sample with distilled water with the aid of a mechanical shaker for 1h. This was filtered with Whatmann No. 1 filter paper. Then 5ml of the filterate was pipetted out into a test tube and mixed with 2ml of 0.1M ferric chloride in 0.IN hydrochloric acid and 0.008M potassium ferrocyanide. The absorbance was measured at 760nm within 10min.

2.7 Proximate Nutritional Analysis

Standard methods of the Association of Official Analytical Chemists [26] were used to determine the moisture, crude protein, crude fat, total ash and crude fibre contents of each sample. Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105ºC. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogenx6.25) was determined by the Kjeldahl method, using 2.0g samples. Crude fat (EE) was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60ºC) as the extractant. Ash was determined by the incineration of 10.0g sample placed in a muffle furnace maintained at 550ºC for 5h. Crude fibre was obtained by digesting 2.0g of sample with sulphoric acid and sodium hydroxide and incinerating the residue in a muffle furnace maintained at 550ºC for 5h. Each analysis was carried out in triplicate.

Nitrogen free extracts (NFE) which represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed were determined by mathematical calculation. It was obtained by subtracting the sum of the percentages of all the nutrients already determined from 100.

$$\%NFE=100-(\%moisture+\%CF+\%CP+\%EE+\%Ash)$$

2.8 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1Results

3.1.1 Effect of fruit sample of *Xylopia aethiopica* on the serum lipid profile of fed rats

The result of the effect of incorporation of powdered *X. aethiopica* fruit in the feed of albino Wistar rats on serum lipid profile is presented in Table 1. The powdered *X. aethiopica* fruit produced a significant ($p<0.05$) concentration-dependent decrease in the total serum cholesterol, triglyceride and VLDL-C levels in treated groups of rats when compared to the negative group. The *X. aethiopica* fruit caused a significant ($p<0.05$) non concentration-dependent decrease in serum level of LDL-C in fed group of rats when compared to the negative control. Also, *X. aethiopica* fruit (5 and 10% in fed) produced a significant ($p<0.05$)

increase in the serum level of HDL-C in fed groups of rats, but the *X. aethiopica* fruit (20 and 50% in fed) produced a significant ($p<0.05$) decrease in the serum level of HDL-C in fed groups of rats when compared to the control group.

3.1.2 Quantitative phytochemical constituents of *Xylopia aethiopica* fruit

The result of the quantitative phytochemical constituents of *Xylopia aethiopica* fruit is presented in Table 2. Quantitative phytochemical analysis showed that the *X. aethiopica* fruit contained tannins (4.96%), flavonoids (0.81%), saponins (2.93%) and alkaloids (1.24%).

3.1.3 Proximate nutritional analysis of *Xylopia aethiopica* fruit

The result of the proximate analysis of the nutrient composition of powdered *X. aethiopica* fruit is presented in Table 3. The proximate analysis showed the presence of moisture, lipid, crude fibre, crude protein, ash and nitrogen free extracts in the following proportion 7.40, 1.48, 2.94, 3.19, 4.39 and 80.58% respectively.

Table 1. Effect of fruit sample of *Xylopia aethiopica* on the serum lipid profile (Mean±SEM) of fed rats

Treatment group	Cholesterol (mg/dl)	HDL-C (mg/dl)	Triglyceride (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Control (plain feed)	97.77±2.05	46.63±0.24	116.13±3.72	23.23±0.74	27.90±2.68
5% feed	66.96±1.57*	51.72±0.32*	40.86±2.15*	8.17±0.34*	7.06±1.69*
10% feed	62.81±1.65*	49.46±0.14*	30.11±1.95*	6.02±0.43*	7.32±1.19*
20% feed	53.92±0.59*	40.84±0.37*	23.65±2.02*	4.73±0.41*	8.35±0.68*
50% feed	44.44±1.02*	30.10±0.01*	21.51±2.24*	4.30±0.23*	10.03±1.11*

**=p<0.05 when compared with control group*

Table 2. Quantitative phytochemical constituents of *Xylopia aethiopica*

Phytochemicals	Mean values±SEM (%)
Tannins	4.96±0.014
Flavonoids	0.81±0.014
Alkaloids	1.24±1.11
Saponins	2.93±0.00

Table 3. Proximate nutritional analysis *Xylopia aethiopica* fruit

Proximate composition	Mean values±SEM (%)
Moisture content	6.32±0.62
Lipid	12.54±0.28
Crude fiber	14.51±4.31
Crude Protein	0.91±0.014
Ash	2.31±0.014
Nitrogen free extracts	63.41±0.68

3.2 Discussion

In this study, the quantitative phytochemical, nutrient composition and the effects of powdered fruit of *Xylopia aethiopica* on the serum lipid profile of fed rats were evaluated.

The powdered fruit of *X. aethiopica* demonstrated a significant ($p<0.05$) hypolipidemic effect which may be mediated by the phytochemical constituents of the plant material.

The *X. aethiopica* fruit produced its optimum hypolipidemic effect at 5% inclusion rate, since the increase in concentration of *X. aethiopica* did not produce either appreciable increase in the serum level of HDL-C or decrease in the serum level of LDL-C in the fed rats. The decrease in hypolipidemic activity as observed at higher concentration may be due to receptor site saturation and/or possible inhibition of activity by other component of the fruit [27]. The quantitative phytochemical analysis of the powdered fruit indicated the presence of the following compounds; tannins, saponins, alkaloids and flavonoids in descending order of percentage composition. These phenolic substances as well as the alkaloids in plants have been listed as the most important bioactive constituents of natural products which are valuable supplements used for the maintenance of human health and sometimes possessing remarkable therapeutic potentials [28-30].

The result of proximate nutrient analysis showed that the powdered fruit of *X. aethiopica* contain 14.51% crude fibre which is high when compared to other reports [31]. The dietary fibre has been reported to play a role in weight loss. Consumption of high fibre diet is encouraged in those who want to achieve weight loss because it cause increased gastric emptying time and produce reduced lipid and glucose absorption from the gut [32].

The mechanism of X. aethiopica fruit hypolipidemic effects is not known but could be by reduced cholesterol absorption from the intestinal tract, possibly mediated either by the fibre or the phytochemical content. The decrease in absorption of exogenous cholesterol and increased metabolism of endogenous cholesterol into bile acids in the liver leads to increased expression of LDL receptor on hepatocytes, and increased clearance of LDL-C from the plasma [33].

Another possible mechanism through which lipid lowering drugs (bile acid sequestrant) act is by binding to bile acid in intestine, which will impair its reabsorption from the intestine. The depletion of bile acid pool leads to up regulation of cholesterol 7-α-hydroxylase and increased conversion of cholesterol to bile acids. This causes an increased demand for cholesterol by the hepatic cells, resulting in the dual effect of increased transcription and activity of HMG-CoA reductase, and increased number of hepatic LDL receptors. These compensatory effects result in increased clearance of LDL-C from the blood, resulting in decreased serum LDL-C levels. Serum TG levels may increase or remain unchanged [34].

Furthermore, *X. aethiopica* could have acted through the inhibition of rate limiting enzyme, HMG-CoA reductase, in the biosynthesis of cholesterol. HMG-CoA reductase catalyse the conversion of HMG-CoA to mevalonic acids [33]. The reversible and competitive inhibition of HMG-CoA reductase leads to decreased hepatic cholesterol synthesis, up regulation of LDL receptor synthesis and increased LDL-C clearance from the plasma into liver cells [33,35]. The hypolipidemic effects of the powdered fruit *Xylopia aethiopica* may have been mediated through one or a combination of the above mechanisms.

4. CONCLUSION

In conclusion, the hypolipidemic effects as demonstrated by this study suggest that the consumption of fruit of *Xylopia aethiopica* may help in the reduction of the incidence of hyperlipidemia related diseases in patients. The authors recommend that further work should be done to isolate the active principle responsible for its hypolipidemic effect and to determine the possible mechanism of action.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declarations of Helsinki and Michael Okpara University of Agriculture, Umudike, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Raji Y, Fadare OO, Adisa RA, Salami SA. Comprehensive assessment of the effect of *Sphenocentrum jollyanum* root extract on male reproductive activity in albino rats. Reproductive Medicine and Biology. 2006;5(4):283-292.
2. Sofowora A. Medicinal plants and Traditional Medicine in Africa (3rd edition). Nigeria: Spectrum Books Limited Ibadan; 2008.
3. Girija K, Lakshman K. Anti-hyperlipidemic activity of methanol extracts of three plants of *Amaranthus* in triton-WR 1339 induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine. 2011;62-65.
4. Patil UK, Saraf S, Dixit VK. Hypolipidemic activity of seeds of *Cassia tora* Linn. J. Ethnopharmacol. 2004;90:249-255.
5. Pooja CO, Priscilla D. Antioxidant and antihyperlipidemic activities of *Hibiscus sabdariffa* linn. Leaves and calyces extracts in rats. Indian Journal of Experimental Biology. 2009;47:276-282.
6. Saravanakumar A, Vanitha S, Ganesh M, Jayaprakash J, Ramaswamy NM. Hypolipidemic activity of *Sesbania grandiflora* in triton wr-1339 induced hyperlipidemic rats. International Journal of Phytomedicine. 2010;2:52-58.
7. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: Analysis of worldwide data. The Lancet. 2005;365(9455):217–223.

8. Sobngwi E, Mbanya JCN, Unwin NC, Kengne AP, Fezeu L, Minkoulou EM, et al. Physical activity and its relationship with obesity, hypertension and diabetes in urban and rural Cameroon. International Journal of Obesity. 2002;26(7):1009–1016.
9. Ejim EC, Okafor CI, Emehel A, Mbah AU, Onyia U, Egwuonwu T, et al. Prevalence of cardiovascular risk factors in the middle-aged and elderly population of a Nigerian rural community. Journal of Tropical Medicine. 2011; Article ID 308687. Available: http://dx.doi.org/10.1155/2011/308687.
10. Adaramoye OA, Sarkar J, Singh N, Meena S, Changkija B, Yadav K, et al. Antiproliferative action of *Xylopia aethiopica* fruit extract on human cervical cancer cells. Phytotherapy Research. 2011;25(10):1558-1563.
11. Tairu AO, Hofmann T, Schieberle P. Characterization of the key aroma compounds in dried fruits of the West African peppertree *Xylopia aethiopica* (Dunal) A. Rich (*Annonaceae*) using aroma extract dilution analysis. J. Agric Food Chem. 1999;47(8):3285-3287.
12. Taiwo IA, Bola OO, Francis-Garuba PN. Haematological properties of aqueous extracts of *Phyllantus amarus* (Schum and Thonn.) and *Xylopia aethiopica* (Dunal) A. Rich in Albino Rats. Ethno-Med. 2009;3(2):99-103.
13. Burkill HM. The useful Plants of West Tropical Africa. edn, vol. 1. Royal Botanical Garden: Kew; 1985.
14. Tatsadjieu LN, Essia Ngang JJ, Ngassoum MB, Etoa FX. Antibacterial and antifungal activity of *Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. Fitoterapia. 2003;74(5):469-472.
15. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (*Apocynaceae*) bark and *Xylopia aethiopica* (Dunal) A. Rich (*Annonaceae*) fruits mixtures used in the treatment of diabetes. African Journal of Biotechnology. 2008;7(6):701-705.
16. Woode E, Ameyaw EO, Boakye-Gyasi E, Abotsi WKM. Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich (*Annonaceae*) and the major constituent, xylopic acid in murine models. Journal of Pharmacy and Bioallied Sci. 2012;4(4):291–301.
17. Woode E, Alhassan A, Abaidoo CS. Effect of ethanolic fruit extract of *Xylopia aethiopica* on reproductive function of male rats. Int. J. Pharm. Biomed. Res. 2011;2(3):161-165.
18. Suleiman MM, Mamman M, Aliu YO, Ajanusi JO. Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. Vet. Arhiv. 2005;75:487-495.
19. Ward JW, Elsea JR. Animal case and use in drug fate and metabolism. In: Edward RG, Jean LH (eds) Methods and techniques, 1st edn., New York: Markel Dekker; 1997.
20. Stein EA. Lipids, lipoproteins and Apolipoproteins. In: Tietz, N. W. (Ed). Fundamentals of Clinical Chemistry. 3rd Edn. Philadelphia: W. B Saunders; 1987.
21. Tietz NW. Clinical Guide to Laboratory Test, Second Edition, Philadelphia: W.B. Saunders Company; 1990.
22. Wacnic RG, Alber JJ. A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholestsrol. Journal of Lipid Research. 1978;19:65-76.
23. Rifai N, Warmick GR, Remaley AT. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. In: Burtis, C. A., Ashwood, E. R. and Bruns, D. E. (Eds.), Tietz Fundamentals of Clinical Chemistry, 6th ed. Missouri: Saunders Elsevier. 2008;402–430.

24. Friedewald VT, Levy RI, Fredrickson DS. Estimation of low density lipoprotein cholesterol in plasma, without use of preparative centrifuge. Clin. Chem. 1972;18:499-502.
25. Krishnaiah D, Devi T, Bano A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Medicinal Pl. Research. 2009;3(2):67-72.
26. Association of Official Analytical Chemist. Official Methods of Analysis, 15th Ed., Arlington, VA; 1990.
27. Trease GE and Evans WC. "Pharmacognosy," 15th ed. W. B. saunders, London. 2002;58-302.
28. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 2005;4(7):685-688.
29. Kumar RS, Sivakuma T, Sunderem RS, Gupta M, Murugesh K, Rajeshwa Y, et al. Antioxidant and antimicrobial activities of *Bauhinia recemosa* L. stem bark. Braz. J. Med. Biol. Res. 2005;38:1015-1024.
30. Ezekwesili CN, Nwodo OFC, Eneh FU, Ogbunugafor HA. Investigation of the chemical composition and biological activity of *Xylopia aethiopica* Dunal (*Annonacae*). African Journal of Biotechnology. 2010;9(43):7352-7356.
31. Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGM. Nutritional and Phytochemical Screening of *Garcinia kola*. Advance Journal of Food Science and Technology. 2012;4(1):9-14.
32. Salmerón J, Stampfer MJ, Colditz GA, Manson JE, Wing AL, Willet WC. Dietary fiber, glycemic load and risk of non-insulin-dependent diabetes mellitus in women. Journal of the American Medical Association. 1997;277:472-477.
33. Rang HP, Dale MM, Ritter JM, Moore RK. Pharmacology 6th edition, Edinburgh: Churchill livingstone; 2007.
34. Fonseca VA, Rosenstock J, Wang AC, Truitt KE Jones MR. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. Diabetes Care. 2008;31(8):1479–1484.
35. Khanna AK, Rizvi F, Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipidemic rats. J. Ethnopharmacol. 2002;82(1):19-22.

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