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Research Article

Determination of Phytochemical Constituents of the Aqueous Extracts of the Leaves, Stem Bark and Root Bark of *Vitex doniana* and its Effects on Lipid Profile of Albino Rats

[1]D.B. James, [1]O.A. Kadejo, [1]C. Nwochiri and [2]C.D. Luca
[1]Department of Biochemistry, Ahmadu Bello University, Samaru Zaria, Nigeria
[2]Department of Biochemistry, University of Jos, Nigeria

Abstract: The phytotochemical screening of Leaves, stem bark and root bark of *Vitex doniana* were carried out and some Lipid profile were assessed in normal albino rats after administration of the extracts. Twenty mixed sex Wistar rats weighing 150-200 g was divided into 4 groups. Group 1 serves as control while 2, 3, 4 were administered (100 mg/kg) aqueous extracts of leaves, stem bark and root bark of *Vitex doniana.* The phytochemical screening of the leaves, stem bark and root bark of *Vitex doniana* shows the presence of Glycosides, Cardiac glycosides, Saponins, Flavonoids and Tannins while the quantitative analysis showed a higher value in the amount of saponin present in the extracts. Daily oral administration of the extracts (100 mg/kg) for the period of 21days shows that all the extracts significantly ($p<0.05$) decreased the levels of serum total Cholesterol and LDL-cholesterol. The atherogenic risk predictor indices also showed that root bark extract significantly ($p<0.05$) increased HDL-Cholesterol/total cholesterol ratio which may possess antiatherogenic effect and therefore these findings may be of clinical importance to individuals at risk of cardiovascular disease.

Keywords: Atherogenic risk, Aqueous extract, phytotochemical, total Cholesterol and LDL-cholesterol, *Vitex doniana*

INTRODUCTION

A medicinal plant is any plant used for the extraction of pure substances either for direct medicinal use or for hemi-synthesis of medicinal compounds which can be used for the therapeutic purpose or as a precursor for the synthesis of useful drugs (Sofwora, 1982). Approximately 10% of these plants are used either as food or for medical purposes (Borris, 1996).

Medicinal plants have been found to contain bioactive compounds called phytochemicals (Fasuyi, 2006) and secondary metabolite that can protect humans against diseases (Kumar *et al*., 2009). Some important groups of these phytochemicals (secondary metabolites) are involved in many *in-vitro* studies and assessment of haematological parameters, antioxidant activities, anti-microbial effect and analgesic effect (Finar, 1986).

This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the development of different traditional systems of medicine. This exploration of biologically active natural products have played an important role in finding New Chemical Entities (NCEs) for example, approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived (Newman *et al*., 2003).

However, cardiovascular diseases present some of the main health problems across the globe today, the major ones being coronary heart diseases, stroke and hypertension (Alters and Shiff, 1997). Elevated plasma lipids are risks factors in cardiovascular problems. Hyperlipidemia and other abnormal blood lipid profile are largely of genetic origin or due to unwholesome nutritional habits. Lipids and other substances accumulates on arterial wall, forming plague, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver or kidney. Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure. The clinical consequences of these disease condition are serious and meaningful research efforts to improve the knowledge and understanding of the pathogenesis is essential, in order to provide a more rational approach to their prophylaxis and treatment. (Kritchersky, 1970; Kucera *et al*., 1972).

Despite the several studies on the different pharmacological activities of *Vitex doniana*, not much has been done on its lipid profile notwithstanding its widespread use in folk medicine. Thus this study was carried out to determine phytochemical constituents of the aqueous extracts of the leaves, stem bark and root bark of *Vitex doniana* and its effects on lipid profile of albino rats.

Corresponding Author: D.B. James, Department of Biochemistry, Ahmadu Bello University, Samaru Zaria, Nigeria

MATERIALS AND METHODS

Plant materials: The leaves, root bark and stem bark of *Vitex doniana* were collected from its natural habitat, Institute of Agricultural Research (IAR) Ahmadu Bello University (ABU), Zaria Kaduna state in the month of April 2012. The sample was identified at the herbarium in the Department of Biological Sciences, Ahmadu Bello University Zaria.

Preparation of plant (whole plant): The collected plant samples were rinsed in clean water and dried at room temperature for two weeks. The dried plant samples were ground into powder using a mortar and pestle, the powder obtained was then used to prepare the extracts.

Extractions: One hundred gram of each of the ground leaves, stem bark and root bark were weighed into two conical flasks and 500 mL of distilled water was poured into each of the conical flasks. The contents of the 3 conical flasks were shaken and the tops were covered with aluminium foil and kept at room temperature for 48 h (2 days) after which the extracts were obtained by filtering using a filter paper. The extracts were then concentrated by drying in a water bath maintained at a temperature of 45ºC until brownish black residues were obtained and these were kept in sealed containers and refrigerated at 2-4ºC until required (James *et al*., 2010).

Phytochemical screening: Phytochemical tests were conducted on the aqueous extracts of leaves, stem bark and root bark of *Vitex doniana* to determine the presence of alkaloids, anthraquinone, tannins, terpenoids, saponins, flavonoids, cardiac glycosides and carbohydrates using standard protocols (Sofwora, 1982; Trease and Evans, 1993).

Lethal dose 50 (LD_{50}): Lethal dose 50 test involves the administration of aqueous extracts of a substance to a group of animals at increasing doses in order to determine the dose that kills 50% of the test subjects within a set time frame. Administration of leaves, stem barks and root barks of *Vitex doniana* were orally. The animals used for LD_{50} were grouped into 3 phases. All the phases had 3 groups with 3 animals in each group.

EXPERIMENTAL ANIMALS AND GROUPING

A total of 24 Wistar healthy both sexes albino rats weighing 150-200g were purchased from University of Jos, Plateau State, Nigeria. They were housed in cages and kept at room temperature where light/dark cycle was maintained. They were allowed free access to water and feed diet (Vital Agricultural feeds Nigeria Limited) throughout the period of the experiment.

The experimental animals were randomly assigned to four experimental groups of six rats each for A, B, C and D. Group A serve as control and other groups represent the Leaves extract (B), Stem bark extract (C) and Root bark extract (D) of *Vitex doniana* respectively, in addition to water and feed. The extracts were given by oral tubation for 21 days:

Group A: Control animals given water and feed only for 21days.

Group B: Animals given water, feed and 100 mg/ kg body weight leaves extract for 21days.

Group C: Animals given water, feed and 100 mg/kg body weight stem extract for 21days

Group D: Animals given water, feed and 100 mg/kg body weight root extract for 21 days

At the end of the experimental period, the rats were reweighed, starved overnight and sacrificed under chloroform anaesthesia. Blood sample was collected from each animal by cardiac puncture using sterile needle and syringe. The blood sample was put into test tubes and allowed to clot for 30 min and serum separated using pasture pipette into cleaned and labeled sample bottle for lipid analysis.

Serum Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C) and Triacylglycerol (TG) were determined by enzymatic methods as described by Stein (1987). The Low-Density Lipoprotein Cholesterol (LDL-C) was calculated using the Friedewald *et al*. (1972). The atherogenic risk predictor indices were calculated using the formulae of Dobiasova and Frohlich (2001).

Statistical analysis: The results were analyzed using Duncan multiple range test. All data were expressed as mean±SD. Differences between groups were considered at 95% confidence limit and probability level of 0.05. Probability <0.05 was taken as significant.

RESULTS AND DISCUSSION

The results of phytochemical screening of leaves stem bark and root bark extracts of *Vitex doniana* shows the presence of glycosides, cardiac glycosides, Flavonoids, saponin and tannin while the test for alkaloids, anthraquinone and Steroid and Tritepenes showed negative results (Table 1). The quantitative analysis shows that Flavonoids had the highest value in leaves whereas tannin had the highest value in stem bark, there was no significant ($p>0.05$) difference between saponin and cardiac glycoside of leaves, stem bark and root bark of *Vitex doniana* (Table 2).

The result for acute toxicity of aqueous leaves, stem bark and root bark extracts of *Vitex doniana* shows no record of death when administered oral dose of the extracts up to 5,000 mg/kg of body weight (Table 3).

Table 1: Qualitative phytochemical constituents of aqueous extracts of leaves, stem bark and root bark of *Vitex doniana*

Phytochemical	Leaves	Stem bark	Root bark
Glycosides	Positive	Positive	Positive
Free Anthraquinone	Negative	Negative	Negative
Cardiac Glycosides	Positive	Positive	Positive
Saponins	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive
Tannins	Positive	Positive	Positive
Alkaloids	Negative	Negative	Negative
Steroid and Tritepenes	Negative	Negative	Negative

Table 2: Qualitative analysis of the phytochemical constituents of aqueous extracts of leaves, stem bark and root bark of *Vitex doniana*

Parameter	Leaves	Stem bark	Root bark
Flavonoid (mg/g)	0.32 ± 0.026^{c}	0.14 ± 0.01^{b}	0.029 ± 0.003^{a}
Saponin (mg/g)	0.656 ± 0.040^{a}	0.66 ± 0.035^{a}	0.636 ± 0.015^{a}
Glycosides (mg/g)	0.016 ± 0.002^{b}	0.016 ± 0.001^{b}	0.012 ± 0.001^{a}
Cardiac Glycosides (mg/g)	0.013 ± 0.002^{a}	0.013 ± 0.002^{a}	0.012 ± 0.001^{a}
Tannins (mg/g)	0.280 ± 0.02^{a}	0.460 ± 0.02^{b}	0.020 ± 0.002^{a}

Values are mean±SD triplicate determination; Values with different superscript in the row differ significantly ($p<0.05$)

Table 3: The effect of aqueous extracts of leaves, stem bark and root bark of *Vitex doniana* on serum lipid parameters

Parameters(mmol/l)	Control	100 mg/kg Leaves extract	100 mg/kgStem bark extract	100 mg/kg Root bark extract
Total cholesterol	205.8 ± 18.76^{b}	140.8 ± 18.76^{a}	108.3 ± 18.76^{a}	108.3 ± 18.76^{a}
Triacylglycerol	98.0 ± 51.85^{a}	111.0 ± 11.31^{a}	71.86 ± 11.31^{a}	71.86 ± 11.31^{a}
High density lipoprotein-CH	54.73 ± 5.92^{a}	54.73 ± 21.36^{a}	37.62 ± 5.92^{a}	51.31 ± 10.26^{a}
Low density lipoprotein-CH	131.5 ± 21.13^{b}	63.89 ± 25.48^{a}	56.33 ± 23.64^{a}	42.65 ± 13.18^{a}

Values are mean±SD for n = 6; Values with different superscript in the row differ significantly ($p<0.05$)

Table 4: The effect of aqueous extracts of leaves, stem bark and root bark of *Vitex doniana* on some atherogenic risk predictor indices

Parameter	Control	100 mg/kg Leaves extract	100 mg/kg Stem bark extract	100 mg/kg Root bark extract
HDL-CH/T-CH	0.31 ± 0.01^{a}	0.34 ± 0.08^{a}	0.40 ± 0.04^{ab}	0.47 ± 0.05^{b}
LDL-CH/HDL-CH	2.28 ± 0.05^{c}	1.37 ± 0.05^{b}	1.04 ± 0.05^{a}	0.96 ± 0.04^{a}
Log (TG/HDL-CH)	0.32 ± 0.023^{b}	0.21 ± 0.003^{a}	0.22 ± 0.034^{a}	0.23 ± 0.029^{a}

Values are mean ±SD for n = 6, Values with different superscript in the row differ significantly ($p<0.05$), Values of HDL-CH/T-CH ratio <0.30 are atherogenic and undesirable, values of LDL-CH/HDL-CH ratio >2.3 are atherogenic and undesirable (Ojiakor and Nwanjo, 2005)

The daily oral administration of aqueous leaves, stem bark and root bark of *Vitex doniana* extracts for 21 days shows significant ($p<0.05$) reduction in the serum Total Cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) and there was no significant ($p>0.05$) difference between the triacylglycerol (TAG) and High Density Lipoprotein Cholesterol (HDL-c) of all the extracts treated groups and control. The mean values of atherogenic risk predictor indices [HDL-cholesterol/total cholesterol, LDL-cholesterol/HDL-cholesterol and log (triacylglycerol/HDL-cholesterol)] observed in the Table 4 shows that there was a significant ($p<0.05$) increase in HDL-cholesterol/total cholesterol ratio for the group treated with root bark extract while LDL-cholesterol/HDL-cholesterol and log (triacylglycerol/HDL-cholesterol) shows significant ($p>0.05$) decrease for all the extracts treated groups when compared with the animals in the control group.

It is well known that plants generally contain secondary metabolites and some of these secondary metabolites have been shown to be highly biologically active (Zenk, 1991) and as well as exhibiting physiological activity (Sofwora, 1982). Saponins cause hypocholesterolemia by binding cholesterol, making it unavailable for absorption (Price *et al*., 1987).

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent anti-oxidant activity against the superoxide radical (Hertog *et al*., 1993). Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of Low Density Lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Tannins hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The presence of these photochemical thus supports the medicinal uses of *Vitex doniana*.

The lowering level of serum cholesterol using diet or drugs decreases the incidence of coronary heart disease (Treasure *et al*., 1995; Steiner and Li, 2001). Increased LDL cholesterol with decreased HDL cholesterol usually increases the serum total cholesterol. This is because the plasma clearance of cholesterol is often impaired in the presence of low HDL-C. Triacylglycerol levels have also been found to increase with increase in plasma cholesterol. Atherogenicity therefore develops when LDL cholesterol, triacylglycerols and total cholesterol are elevated relative to plasma HDL-C. Elevated HDL-cholesterol improves the transportation of cholesterol from the plasma to the liver for biotransformation and

excretion, thereby preventing atheroma formation and blood vessel occlusion (Ojiakor and Nwanjo, 2005).

Daily oral administration of the aqueous leaves, stem bark and root bark extracts of *Vitex doniana* has significant ($p<0.05$) serum lipid lowering effect on the level of total cholesterol and LDL-cholesterol. The presence of saponins in the phytochemical screening carried out in the aqueous leaves, stem bark and root bark extracts of *Vitex doniana* may explain the antilipidemic effect observed in this study.

Significantly ($p<0.05$) lowered cholesterol may have contributed to the observed significant ($p<0.05$) decrease in serum LDL-cholesterol in the animals, LDL-cholesterol transport cholesterol to the arteries where they can be retained in artheria proteoglycans starting the formation of plaques, LDL-cholesterol possess a risk of cardiovascular disease when it invades endothelium and becomes oxidized since the oxidized form is more easily retained by the proteoglycan, thus increase of LDL-cholesterol is associated with atherosclerosis, heart-attack, stroke, peripheral vascular disease (Crowwell and Otvos, 2004). The importance of this LDL-cholesterol lowering effect is that the extracts may aid in the prevention or reduction of cardiovascular diseases.

The values of LDL-cholesterol/HDL-cholesterol ratio are less than 2.3 while the values of HDL-cholesterol/Total cholesterol are greater than 0.3 for all the extract treated groups. These values are desirable and they are non atherogenic (Ojiakor and Nwanjo, 2005).

CONCLUSION

The aqueous leaves, stem bark and root bark extracts of *Vitex doniana* produced hypocholesterolemic and antiatherogenic effects after 21 days of treatment with 100 mg/kg. This may imply that the plant extracts could be used to manage hyperlipidemic and atherogenic conditions. These findings agree with the current use of the plant extract by folk medicine practitioners as antihypertensive agent.

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