Trop J Nat Prod Res, September 2023; 7(9):4112-4116 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Investigations of Ameliorative Potentials Extract of *P. pellucida* on Salt - Fructose Induced Dyslipidemia in Wistar Rats

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ARTICLE INFO

Article history:
Received 20 December 2022
Revised 02 June 2023
Accepted 25 August 2023
Published online 01 October 2023

ABSTRACT

The study was undertaken to investigate the effect of extracts of *P. pellucida* on salt-fructose induced dyslipidemia in rats with a view to validating its consumption for the treatment and management of hyperlipidemia, obesity and high blood pressure. Dyslipidemia was induced in rats by oral administration of high concentrations of salt (NaCl, 18% (w/v)) and fructose (10% (w/v) for 21 days. The induced rats (30) were randomly allotted into 5 groups of 6 rats per group . Test groups were treated consistently for 21 days with either 250 mg/kg bwt, 500 mg/kg bwt of *Peperomia pellucida* methanol extract (PPME) or standard drug (Atorvastatin, 0.6 mg/kgbwt). On day 22, the rats were anesthetized with diethyl ether and blood and liver collected for biochemical analyses. Plasma lipid profile parameters (Total cholesterol, LDL, HDL and VLDL cholesterol), atherogenic indices (atherogenic index, Coronary risk index I & II) and enzymatic / non-enzymatic antioxidants (Superoxide dismutase, Peroxidase and Glutathione), were evaluated using standard procedures. Results reveal significant increase in lipid profiles and metabolites, and significant decrease in the antioxidant enzymes of test animals compared with the control as a result of induction with high salt and fructose solution. However, the altered biochemical parameters were restored in the groups treated with PPME. The study suggests that *P. pellucida* may be of good value in the prevention and management of dyslipidaemia, a risk factor for cardiovascular diseases.

***Keywords*:** *Cucumis metuliferus*, *Citrullus lanatus*, horned melon, watermelon, juice, vitamin, phytochemical, antioxidant

Introduction

Consumption of fruits and vegetables has long been recommended to combat occurrences of human diseases such as cancer, cardiovascular diseases, osteoporosis and diabetes[1] . The effectiveness of fruits and vegetables in the management and treatment of ailments has been attributed to the presence of bioactive molecules which enable them to function and exhibit antioxidant, anti-inflammatory, antibacterial and anti-microbial effects, and thus prove useful in lowering the blood cholesterol, blood sugar and blood pressure. [2,3]. While dietary and environmental factors such as smoking, high intake of saturated fats, sugars and salt, cholesterol, diabetes physical inactivity cause alteration in vascular integrity, compromised membrane integrity, increased free radical generation, reduction of endogenous antioxidants status, and dyslipidemia, [4] bioactive molecules such as xanthones, flavonoids, irridoids, secoiridoids and glycosides play significant roles in preventing and reversing these deleterious effects [5].

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Citation: Fakayode AE, Emma-Okon BO, Morakinyo AE, Fajobi AO, Akinyele CK, Oyedapo OO. Investigations of Ameliorative Potentials Extract of *P. pellucida* on Salt - Fructose Induced Dyslipidemia in Wistar Rats. Trop J Nat Prod Res. 2023; 7(9):4112-4116 http://www.doi.org/10.26538/tjnpr/v7i9.40

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Studies have revealed that phytochemicals possess and exhibit antioxidant, anti-inflammatory activities, protect vascular endothelium, prevent cardiovascular disorders, prevent or inhibit lipid oxidation, support endogenous antioxidants and are cardio protective [6] (Azemi et al. 2022). In particular, polyphenols (phenolics and flavonoids) have been widely reported to play these roles effectively [7,8,9].

Peperomia pellucida (L.) Kunth, belongs to the family Piperaceae. It is a common annual weed which is native to tropical North and South America. Locally called ´*rinrin*' in Yoruba, it is a short-lived perennial, entirely delicate, fleshy and glabrous herb usually growing to a height of about 15 - 45 cm. The stem is translucent, pale green, erect or ascending or decumbent, freely branched internodes usually 3-8 cm long and hairless *P. pellucida* is a vegetable that is consumed worldwide and has been shown to exhibit a wide range of antioxidant potentials. It has been found to be effective in the treatment and management of human diseases and disorders such as diabetes, high blood pressure and inflammatory conditions [10].

This second in the series of studies on the biological activities of *P. pellucida* investigated the ameliorative activities of *P. pellucida* in salt-fructose induced dyslipidemia in wistar rats[11].

Materials and Methods

Collection and Identification of Plant Material

Fresh whole *Pepperomia pellucida* was collected from Obafemi Awolowo University, Ile Ife, Osun State, Nigeria in July, 2017. It was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria. The specimen sample was deposited at IFE Herbarium where specimen identification number (IFE 17461) was collected. The methanol extract of *P.*

pellucida (PPME) was prepared according to a modified method of Oyedapo and Amos [12] as reported by Fakayode Aderonke *et al* [11]

Experimental Animals (Ethical Approval Number: IPH/OAU/12/1423)

Thirty (30) wistar rats were purchased from Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The rats were acclimatized for four weeks and fed with Standard Rat Chow (Ladokun Feeds Limited, Ibadan) and water *ad libitum.*

Grouping, Induction and Treatment of Dyslipidemia-induced Rats

The rats were grouped into six groups of five rats each and acclimatized for eight weeks. The rats were fed with standard rat chow (Ladokun Feeds, Ibadan) and water *ad libitum*. The induction of hyperlipidemia was carried out according to the procedure of Mainieri *et al* [13] with slight modification. Typically, animals were given 18% (w/v) NaCl (1 mL) twice daily and given free access to 10% (w/v) fructose solution for twenty-one (21) days. The animals were grouped and treated consistently for 21 days as follows:

Group 1: Rats + distilled water; Group 2: Rats + Inducer; Group 3: Rats + Inducer + PPME (250 mg/kg bwt); Group 4: Rats + Inducer + PPME (500 mg/kg bwt); and Group 5: Rats + + Statin (0.6 mg/kg bwt)

(Inducer -(18% (w/v) NaCl + 10% (w/v) Fructose)

Collection of Tissues/Organs of Experimental Animals

The rats were subjected to 12 h. fast and then sacrificed under diethyl ether anaesthesia on day 22. The rats were dissected and the blood was collected by cardiac puncture into heparinized bottles. Liver samples were also excised, perfused in normal saline (0.85% (w/v) NaCl), blotted on tissue paper and kept frozen at -20^{o}C for analysis. Whole blood was centrifuged at 3000 rpm for 10 min. on a Bench Centrifuge (Model 90-2, Searchtech Instrument) at room temperature. The plasma was collected, stored, frozen and used for biochemical analyses.

Liver was weighed, cut into bits with clean sterile scissors and thoroughly homogenized in freshly prepared 100 mM phosphate buffer, pH 6.8. The homogenates were transferred into centrifuge tubes and centrifuged at 3000 rpm for 10 min. using Bench Centrifuge (Model 90-2, SearchTech Instrument). The supernatants were collected and stored frozen for biochemical analyses.

Biochemical Analyses

Estimation of lipid profiles; Total Cholesterol (TC), High Density Lipoprotein (HDL-c), Low Density Lipoprotein (LDL-c), Very Low Density Lipoprotein (VLDL-c), Triacylglycerol (TG) were carried out according to standard procedures using Randox Diagnostic kits [14]

The atherogenic ratios were calculated as follows: Castelli's Risk Index I (CRI-I) = $\frac{TC}{HDLc}$; Castelli's Risk Index II (CRI-II) = $\frac{LDLc}{HDLc}$; Atherogenic Coefficient = $\frac{(TC-HDLc)}{HDLc}$; Antherogenic Index of Plasma = $\frac{\log TG}{HDLc}$; Where TC (Total Cholesterol); HDL-c (High Density Lipoprotein Cholesterol); LDL-c (Low Density Lipoprotein Cholesterol); TG (Triacylglycerol).

Assay of Enzymatic Antioxidants

Assay of superoxide dismutase activity was carried out according to the method of Marklund and Marklund [15]. Typically, plasma (0.02 mL) was pipetted into test tubes and followed by the addition of 2.35 ml (0.1 M Tris-HCl buffer, pH 8.2) and 1.8 ml distilled water. The mixture was transferred into a cuvette with immediate addition of 0.15 mL of 4.5 mM pyrogallol. The absorbance was read at 420 nm for every 30 seconds for a period of 2.5 min.

$$\text{Percentage Inhibition} = \frac{Increase\ in\ Absorbance\ of\ substrate}{Increase\ of\ Absorbance\ of\ blank} \times 100$$

Liver peroxidase was measured according to the method of Reddy *et al* [16]

Briefly, 1.5 mL (50 mM pyrogallol) and 10µL of liver homogenate were pipetted into a tube and mixed gently by inversion. The reaction was initiated by the addition of 10% (v/v) H_2O_2 (0.25 ml). The change in absorbance was monitored at 430nm at 30 sec intervals for 3 min. The enzyme activity was estimated using the expression:

$$\text{Peroxidase Activity} = \frac{\text{Abs/ min x TV x df}}{\text{Ɛ x SV}}$$

Where

Ɛ (molar extinction coefficient, 11.3), TV (total assay volume), df (dilution factor), SV (sample volume).

Liver glutathione concentration was estimated according to the method of Moron *et al* [17]

Briefly, 0.1mL of deproteinized liver homogenate was mixed with 0.9 ml (0.2 M sodium phosphate buffer, pH 8) and 2 mL freshly prepared 5, 5′-dithiobios-2-nitrobenzoic acid (DTNB). The absorbance was read at 412 nm after 10 min. against the reagent blank. Reduced glutathione (12 µg/ml) was used for the preparation of standard calibration curve. The glutathione level was extrapolated from the glutathione standard calibration expressed as µg/g wet tissue.

Data Analyses

Data was analyzed using GraphPad Prism 5.0 and expressed as Mean ± SEM, n=3 readings. Level of significance was taken as $P<0.05$

Results and Discussion

Effects of PPME on Plasma Lipid profile Parameters

Table 1 shows the effects of the plant extract on lipid profile of the rats.There was 165.77 % increase in the total cholesterol concentration of the induced group (group II) compared with the control (group I). Treatment of the rats with 250 mg/kg, (group III), 500 mg/kg bwt extract (group IV) and atorvastatin (group V) brought about significant decrease (66.9 %, 68.28 %, 68.63 % respectively). For Triacylglycerol, there was 141.39% increase in the induced, untreated group (group II) compared with the control group (group I). The groups treated with 250 mg/kg bwt extract, 500 mg/kg bwt extract and Atorvastatin (0.6 mg/kg bwt) showed a decrease of 38.74%, 39.41% and 61.57% respectively. There was 141.39% increase in the plasma VLDL-c of the induced group (group II) in contrast with the control group (group I). Groups treated with 250 mg/kg bwt extract, 500 mg/kg bwt extract and Atorvastatin 0.6 mg/kg bwt shows a decrease of 38.74%, 39.41% and 61.57% respectively when compared with the untreated group (group II). These differences were found to be statistically significant. For LDL-c, there was 226.03% increase in the level of LDL-c of the induced group (group II) when compared with the control (group I) while there was 75.84 80.69% and 74.65% decrease in the plasma level LDL-c of the treated groups (group III, IV and V) respectively when compared with the induced group (group II). Statistical analyses shows that there was a significant increase in the plasma level of LDL-c of the induced group (group II) when compared with the control (group 1) and the treated groups (group III, group IV and group V respectively) but there were no significant differences amongst the treated group (group III, IV and V) when compared with one another (Table 1). There was 70.85% decrease in the plasma level of HDL-c of the induced group (group II) when compared with the control (group I) while there was increase (166.98%, 172.43% and 164.30%) in the level of HDL-c in the treated groups (group III, IV and V respectively) when compared with the untreated group (group II).

Effects of PPME on Atherogenic Ratio

Table 2 shows that the atherogenic ratio (CRI-I, CRI-II, AIP, AC) of the induced group (group II) was significantly higher when compared with the atherogenic ratio of the treated groups (group III, group IV and group V) and the control (group I) .

Effects of PPME on Enzymatic Antioxidants Activities

Table 3 shows a decrease (53.78%) in the level of plasma SOD of the induced, untreated group when compared with the control, while levels of SOD in the plasma of the treated groups (group III, IV and V) increased by 94.19%, 82.09% and 98.29% respectively when compared with the induced group . A similar trend was observed for the peroxidase activity. For the glutathione levels, there was 70.48 % decrease in the liver GSH of the induced rats (group I) when compared with the control and a 200.48%, 179.52% and 156.43% increase in

groups treated with 250mg/kg bwt, 500mg/kg bwt and atorvastatin respectively when compared with the untreated group (group II).

Lipid profile tests are usually carried out to screen for lipid abnormalities such as high level of total cholesterol, low density lipoprotein (LDL), triacylglycerol and low levels of High density lipoprotein (HDL) which are risk factors for cardiovascular disease [18,19,20]. It is well established that high HDL level is related to lower risk of heart and blood vessel diseases while high levels of low-density lipoprotein (LDL) are linked to an increased risk of heart and blood vessel diseases, including coronary artery disease, heart attack and death. Reducing LDL levels is a major treatment target of cholesterol-lowering medications [21,22] .

In addition to the traditional lipid profile parameters, atherogenic indices of plasma have also been used as a significant predictor of atherosclerosis and a strong risk factor of cardiovascular diseases (CVD. This includes AIP, CR-I, CR-II and AC. Studies have provided evidence that these markers could serve as better predictors of cardiovascular risk than total cholesterol [23]. In particular, AI is a novel index composed of TAG and HDL-C and a strong association has been shown to exist between AI and obesity, with AI having predictive advantage compared with conventional lipid components. Significant correlation exists between AI and TC, TAG and LDL-C while HDL-C is inversely correlated with AI [24,25].

The use of high concentrations of salt and fructose solution to induce dyslipidemia is well documented [26,27] , as the hepatic metabolism of fructose has important effects on both glucose and lipid metabolism. The fructose molecule is metabolized into 2 triose phosphates that bypass the main rate-controlling step in glycolysis whereas hepatic glucose metabolism is limited by the capacity to store glucose as glycogen, by the inhibition of glycolysis and further glucose uptake resulting from the effects of citrate and ATP (inhibitors of phosphofructokinase). When large amounts of fructose are consumed, fructose continues to enter the glycolytic pathway and hepatic TAG production is facilitated. Fructose can provide carbon atoms for both the glycerol and acyl portions of acyl glycerol molecules. Thus, unlike glucose metabolism, high concentrations of fructose can serve as a relatively unregulated source of acetyl coA. Studies in human subjects have shown that fructose ingestion results in markedly increased rates of de novo lipogenesis [28]. Thus, fructose is more lipogenic than glucose, an effect that might be exacerbated in subjects with existing hyperlipidemia or insulin resistance or type 2 diabetes [29]. This is very important, especially in view of the increased consumption of food and beverages containing high levels of fructose.

Previous studies carried out on *P. Pellucida* have shown that it has antimicrobial, anti-inflammatory, analgesic, anti-sickling and antioxidant activities [30,31,32,33].

In this study, treatment of dyslipidemic rats with methanol extracts of *P. pellucida* ameliorated the derangement of TC, TG, VLDL and LDL brought about by induction with high salt and fructose concentrations. This suggests that the plant extracts are capable of preventing and ameliorating dyslipidemia. Other plants which have been shown to have lipid lowering effects include *Azadirachta indica* (flowers), *Siamensis Valeton* (flowers), *Bombax Ceiba* (pollen), *Citrus hystrix DC* (leaves), *Polygonum odoratum* (leaves) and *Solanum torvum* (fruits) [34.] It has also been suggested that *Vernonia amygdalina* reduced hepatic TAG biosynthesis and favours the redistribution of cholesterol among lipoprotein particles [35].

In addition to the traditional lipid markers, *P. Pellucida* was also found to ameliorate the increased levels of AIP, CR-I, CR-II and AC brought about by induction with the salt and fructose solution (Table 2) This further confirms that the induced group is at higher risk of CVD and that the plant extract has an ameliorative effect on dyslipidemia. *P. Pellucida* may elicit its health benefits through modulation of several physiologic functions, including the atherogenic profile [36].The observed amelioretic effects could be due to the regulatory activities of phytochemicals present in the plant extract.

Antioxidants are molecules that possess and exhibit abilities to inhibit or quench free radical reactions thereby delaying or inhibiting cellular damage.

Table 1: Effects of PPME on Lipid Profile**.**

Group	Total Cholesterol (mg/dL)	Triacylglycerols (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
I	223.89 ± 10.97	141.59 ± 12.65	36.69 ± 1.76	157.08 ± 13.25	28.32 ± 2.53
II	595.04 ± 24.68**	341.79 ± 3.61**	11.57 ± 4.05**	512.13 ±22.14**	68.36 ± 0.72**
	(165.77% ↑)^{a}	(141.39 % ↑)^{a}	(70.85 %↓)^{a}	(226.03 % ↑)^{a}	(141.39% ↑)^{a}
III	196.47 ± 10.27$^{\#\#}$	209.39± 19.84$^{\#\#}$	30.89 ± 3.61$^{\#\#}$	123.70 ±12.91$^{\#\#}$	41.88 ± 3.97$^{\#\#}$
	(66.98% ↓)	(38.74% ↓)^{b}	(166.98 %↑)^{b}	(75.84% ↓)^{b}	(38.74% ↓)^{b}
IV	188.73 ± 12.81$^{\#\#}$	207.08± 26.80$^{\#\#}$	31.52 ± 1.57$^{\#\#}$	98.88 ± 8.44$^{\#\#}$	41.42 ± 5.36$^{\#\#}$
	(68.28% ↓)^{b}	(39.41%↓)^{b}	(172.43 %↑)^{b}	(80.69 % ↓)^{b}	(39.41 % ↓)^{b}
V	186.66 ± 18.25$^{\#\#}$	131.35 ±10.49$^{\#\#}$	30.58 ± 3.75$^{\#\#}$	129.81 ±17.38$^{\#\#}$	26.27 ± 2.09$^{\#\#}$
	(68.63% ↓)^{b}	(61.57% ↓)^{b}	(164.30 %↑)^{b}	(74.65 % ↓)^{b}	(61.57 % ↓)^{b}

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. induced; (**) represent significant difference from control, (##) represent significant difference from the induced (group II)**.**

Group I: Rats + distilled water (Control); Group II: Rats + inducer only; Group III: Rats + Inducer + PPME (250 mg/kg bwt); Group IV. Rats + Inducer + PPME (500 mg/kg bwt); Group V. Rats + Inducer + Atorvastatin (0.6 mg/kg bwt);[Inducer 18% (w/v) NaCl + 10% (w/v) Fructose).

Table 2: Effects of PPME on Atherogenic Ratio

Group	Castelli's Risk Index (CRI-I)	Castelli's Risk Index (CRI-II)	Antherogenic Index of Plasma (AIP)	Atherogenic Coefficient (Ac)
I	5.79 ± 0.46	0.06 ± 0.00	0.06 ± 0.00	4.79 ± 0.46
II	38.07 ± 3.87*	0.17 ± 0.02*	0.17 ± 0.02*	37.07 ± 3.87*
III	6.92 ± 1.29**	0.07 ± 0.01**	0.07 ± 0.01**	5.92 ± 1.29**
IV	5.68 ± 0.44**	0.07 ± 0.00**	0.07 ± 0.00**	4.68 ± 0.44**
V	6.31 ± 0.69**	0.07 ± 0.00**	0.07 ± 0.00**	5.31 ± 0.69**

Table 3: Effects of PPME on SOD, Liver Peroxidase and Liver Glutathione

	Superoxide dismutase (SOD) (U/min./mg protein)	Liver Peroxidase (U/min./mg protein)	Liver Glutathione (GSH) (µg/g)
I	54.72 ± 3.52	2.01 ± 0.16	16.87 ± 0.82
II	25.29 ± 1.92**	0.33 ± 0.09**	4.98 ± 1.52
	(53.78% ↓)[a]	(83.58 % ↓)[a]	(70.48 % ↑)
III	49.11 ± 6.26##	0.64 ± 0.09##	14.38 ± 1.53
	(94.19% ↑)[b]	(93.9 % ↑)[b]	(200.48 % ↑)
IV	46.05 ± 1.23##	0.86 ± 0.11##	13.92 ± 1.46
	(82.09 % ↑)[b]	(160.60% ↑)[b]	(179.52 % ↑)
V	50.15 ± 4.10##	1.24 ± 0.13##	12.77 ± 1.04
	(98.29% ↑)[b]	(275.76 % ↑)[b]	(156.43 % ↑)

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. diseased; (**) represent significant difference from control, (##) represent significant difference from the induced (group II).
Group I: Rats + distilled water (Control); Group II: Rats + salt and fructose solution; Group III: Rats + PPME (250mg/kg bwt); Group IV. Rats + PPME (500mg/kg bwt); Group V. Rats + Atorvastatin.

Oxidation reactions produce free radicals that are capable of initiating multiple chain reactions which eventually cause damage or death to cells. Antioxidants neutralize free-radical intermediates by giving up some of their own electrons, thus terminating the harmful chain reactions. Antioxidants can be classified into first line of defense, second line of defense and third line of defense based on their mode of action [37].
Levels of glutathione and the enzymes superoxide dismutase and glutathione peroxidase are used as indices of antioxidant potentials [37]. While treatment of rats with the inducer brought about significant decrease in the levels of these metabolites, administration of the *P. Pellucida* extract resulted in significant elevation of their levels, which was comparable with controls. This could be as a result of phenolic compounds present in the extract of *P. pellucida* [11] Several studies have suggested that phenolic antioxidants are capable of inhibiting free radical formations and interrupt propagation of autoxidation [38] .Previous studies carried out on *P. Pellucida* have shown that it has antimicrobial, anti-inflammatory, analgesic, anti-sickling and antioxidant activities [30,33]. The present study has added to this body of knowledge by demonstrating its lipid lowering effects.

Conclusion

P. pellucida leaves have been demonstrated to have ameliorative effects on dyslipidemia induced by high concentrations of salt and fructose . The plant also has antioxidant effects, which could be due to its phenolic content.Further studies are desirable to determine the exact component in the plant responsible for its lipid lowering effect. Such may be a candidate for use as a prophylactic agent against hypercholesterolemia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Rebecca LJ, Sharmila S, Das MP, Seshiah C. Extraction and purification of carotenoids from vegetables. J Chem & Pharm Res 2014; 6(4): 594-598.
2. Georgakouli K, Mpesios A, Kouretas D, Petrotos K, Mitsagga C, Giavasis I, Jamurtas AZ. The effects of an olive fruit polyphenol-enriched yogurt on body composition, blood redox status, physiological and metabolic parameters and yogurt microflora. Nutr 2016; 8(6): 344.
3. Khan N, Mukhtar H. Dietary agents for prevention and treatment of lung cancer. Cancer letters 2015; 359(2): 155-164.
4. Stapleton PA, Goodwill AG, James ME, d'Audiffret AC, Frisbee JC. Differential impact of familial hypercholesterolemia and combined hyperlipidemia on vascular wall and network remodeling in mice. Microcirculation 2010; 17(1): 47-58.
5. Patil PD, Patil SP, Kelkar RK, Patil NP, Pise PV, Nadar SS. Enzyme-assisted supercritical fluid extraction: An integral approach to extract bioactive compounds. Trends in Food Sci & Techn 2021; 16: 357-369.
6. Azemi AK, Nordin ML, Hambali KA, Noralidin NA, Mokhtar SS, Rasool AH. Phytochemical contents and pharmacological potential of Parkia speciosa Hassk. for Diabetic Vasculopathy: A Review. Antioxidants 2022; 11(2): 431.
7. Adegbola P, Aderibigbe I, Hammed W, Omotayo T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. Am J Cardiovasc Disease 2017; 7(2): 19.
8. Kumar Singh A, Cabral C, Kumar R, Ganguly R, Kumar Rana H, Gupta A, Pandey AK. Beneficial effects of dietary polyphenols on gut microbiota and strategies to improve delivery efficiency. Nutr 2019; 11(9): 2216.
9. Viswanatha GL, Vaidya SK, Ramesh C, Krishnadas N, Rangappa S. Antioxidant and antimutagenic activities of bark extract of *Terminalia arjuna*. Asian Pac J Trop Med 2010; 3(12): 965-970.
10. Riglar DT, Giessen TW, Baym M, Kerns SJ, Niederhuber MJ, Bronson RT, Silver PA. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. Nature Biotech 2017; 35(7): 653-658.
11. Fakayode AE, Imaghodor FI, Fajobi AO, Emma-Okon BO, Oyedapo OO. Phytonutrients, antioxidants and anti-inflammatory analysis of P*eperomia pellucida*. J Med Pharma & Appl Sci 202; 10(15): 3517-3523.
12. Oyedapo O, Amos S. Further investigations into the bioactivity of root extract of *Plumbago zeylanica*. Phyt Res 1997: 11(1): 62-63.

13. Mainieri F, La Bella S, Chiarelli F. Hyperlipidemia and Cardiovascular Risk in Children and Adolescents. Biomed 2023; 11(3): 809.
14. Expert Panel on Detection E. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Jama. 2001 May 16; 285(19): 2486-97.
15. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47(3): 469-474.
16. Reddy KP, Subbani SM., Khan PA, Kumar KB. Effect of light and benzyl adenine in dark trated growing leaves, changes in the peroxidase activity. Cell Physiol 1995; 29: 984.
17. Moron MS, Depierre JW, Manneryik B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biophys Acta 1979; 582: 67-78.
18. Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, Chen J. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nature Genetics 2013; 45(11): 1345-1352.
19. Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. A colorimetric method for the determination of sugars. Nature 1951; 168: 167-167.
20. Ye J, DeBose-Boyd RA. Regulation of cholesterol and fatty acid synthesis. Cold Spring Harbor Perspectives in Biology 2011; 3(7): a004754.
21. Dashty M, Motazacker MM, Levels J, de Vries M, Mahmoudi M, Peppelenbosch MP, Rezaee F. Proteome of human plasma very low-density lipoprotein and low-density lipoprotein exhibits a link with coagulation and lipid metabolism. Throm and Haem 2014; 112(03): 518-530.
22. Ivanova EA, Myasoedova VA, Melnichenko AA, Grechko AV, Orekhov AN. Small dense low-density lipoprotein as biomarker for atherosclerotic diseases. Oxid Med Cell Longev 2017; 2017: 1273042.
23. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Vogel A. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. The Lancet Oncology 2018; 19(7): 940-952.
24. Akbas EM, Timuroglu A, Ozcicek A, Ozcicek F, Demirtas L, Gungor A, Akbas N. Association of uric acid, atherogenic index of plasma and albuminuria in diabetes mellitus. Inter J Clin Exp Med 2014; 7(12): 5737.
25. Dobiášová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FERHDL). Clin Biochem 2001; 34(7): 583-588.
26. Egli L, Lecoultre V, Cros J, Rosset R, Marques AS, Schneiter P, Tappy L. Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage. The Am J clin Nutr 2016; 103(2): 348-355.
27. Yokozawa T, Yamabe N, Kim HY, Kang KS, Hur JM, Park CH, Tanaka T. Protective effects of morroniside isolated from Corni Fructus against renal damage in streptozotocin-induced diabetic rats. Biol Pharm Bull 2008; 31(7): 1422-1428.
28. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. The Am J Clin Nutr 2002; 76(5): 911-922.
29. Geidl-Flueck B, Hochuli M, Németh Á, Eberl A, Derron N, Köfeler HC, Gerber PA. Fructose-and sucrose-but not glucose-sweetened beverages promote hepatic de novo lipogenesis: A randomized controlled trial. J Hep 2021; 75(1): 46-54.
30. Abere T, Okpalaonyagu S. Pharmacognostic evaluation and antisickling activity of the leaves of Peperomia pellucida (L.) HBK (Piperaceae). Afr J Pharm Pharm 2015; 9(21): 561-566.
31. Oloyede GK, Onocha PA, Olaniran BB. Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of Peperomia pellucida from Nigeria. Adv Env Biol 2011; 5(12): 3700-3709.
32. Raghavendra H, Kekuda P, Chethan D. Phytochemical analysis and in vitro antioxidant activity of Rubus apetalus Poir.(Rosaceae). Pharm 2018; 1: 187-195.
33. Zubair K, Samiya J, Jalal U, Mostafizur R. In vitro investigation of antdiarrhoeal, antimicrobial and thrombolytic activities of aerial parts of Peperomia pellucida. Pharm 2015; 3: 5-13.
34. Adaramoye OA, Akintayo O, Achem J, Fafunso MA. Lipid-lowering effects of methanolic extract of Vernonia amygdalina leaves in rats fed on high cholesterol diet. Vasc Health Risk Manag 2008; 4(1): 235-241.
35. Duangjai A, Limpeanchob N, Trisat K, Ounaroon A. Effects of extraction solvents of dietary plants on lipid lowering activity. J Med Plants Res 2016; 10(32): 538-545.
36. Imam MU, Ishaka A, Ooi DJ, Zamri NDM, Sarega N, Ismail M, Esa NM. Germinated brown rice regulates hepatic cholesterol metabolism and cardiovascular disease risk in hypercholesterolaemic rats. J Functional Foods 2014; 8: 193-203.
37. Grossman S, Dovrat S, Bergman M. Natural antioxidants: just free radical scavengers or much more? Trends in Cancer Res 2011; 7: 57-73.
38. Goiris K, Muylaert K, Fraeye I, Foubert I, De Brabanter J, De Cooman L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Phycol 2012; 24: 1477-1486.