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Evaluation of *Pteleopsis suberosa* Smith and *Nauclea latifolia* Engl & Diels effects on cholesterolemic overload in rats

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Abstract

Nauclea latifolia (*N. latifolia*) and *Pteleopsis suberosa* (*P. suberosa*) are two medicinal plants used to treat several pathologies linked to oxidative stress. This study aims to study the effects of ethanolic extracts from those two plants on the consequences of cholesterolemic overload. Oxidative stress was induced by cholesterolemic overload. *In vitro* antioxidant activity was evaluated using DPPH, total phenolic compounds and flavonoids assays; while *in vivo* FRAP test and determination of malondialdehyde (MDA) content in the liver and the kidney tissues were assayed. Lipid profile was studied by assaying triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol. Blood glucose, ALAT and ASAT content were determined. *P. suberosa* showed higher antioxidant activity *in vitro* and higher level of phenolic compounds and flavonoids as compared to *N. latifolia*. FRAP test showed that *N. latifolia* significantly increased Fe²⁺ content. Organs MDA content was significantly reduced by *P. suberosa* at 200 mg/kg. Both extracts reduced LDL-cholesterol levels and increased HDL-cholesterol significantly. Results also suggest that, *N. latifolia* and *P. suberosa* may contribute to the prevention and treatment of oxidative stress and hypercholesterolemia related diseases.

Keywords: *Pteleopsis suberosa*, *Nauclea latifolia*, antioxidant, cholesterol, lipid overload

Introduction

Oxidative stress is defined as the body's inability to defend itself against the aggression of active oxygen species (AOS), due to an imbalance related either to an increased production of AOS or a decrease in the defense capacity antioxidant [1]. AOS are part of a large group of compounds known as free radicals. Free radicals are chemical compounds (atoms or molecules) with one or more unpaired and highly reactive free electrons. Under the physiological conditions, free radicals play important roles such as the defense of the organism against bacterial and viral agents [2] or regulatory function such as the regulation of vasoconstriction [3]. Excessive production of free radicals is involved in pathological phenomena. Hence, free radicals are incriminated in the complications of several chronic diseases such as insulin resistance, hypertension, metabolic syndrome, and cardiovascular complications associated with diabetes [4]. All these diseases and their complications occur when the antioxidant defenses of the body are exceeded.

Oxidative stress can result from intoxication, exposure to extreme temperature conditions (hyperthermia, hypothermia), intense physical effort [5], and food overload. Food overload induces excessive accumulation of lipids in the body mainly cholesterol and triglycerides leading to oxidative stress which in turn induces cells proliferation, aging and the decreases of the body's reconstitution capabilities. Body's cholesterolemic overload is known as a factor that potentiates the complications of chronic diseases, particularly those of cardiovascular origin [6]. Antioxidant potentials of natural substances could be indicated in the prevention of chronic diseases resulting from oxidative stress. Thus, substances with natural antioxidant properties such as selenium, vitamins C and E, polyphenols are used as antioxidant supplements.

Nauclea latifolia (Rubiaceae) and *Pteleopsis suberosa* (Combretaceae) are widely used in the treatment of chronic diseases including arthritis, hypertension, diabetes and anemia [7]. Hence, this present investigation aimed to assess effects of hydroethanolic extracts of *Nauclea latifolia* (*N. latifolia*) and *Pteleopsis suberosa* (*P. suberosa*) on the consequences of cholesterol overload.

Materials and Methods

Plant materials

The stem barks of *P. suberosa* were collected from Sokodé (Central region, Togo) whereas roots were collected from Agou (Maritime region, Togo). The plant materials were cut into small pieces, shade dried under air conditioning and coarsely powdered. Powdered plant materials were extracted with continuous agitation in ethanol-water (80/20, v/v) for 72 hours. The resulted extracts were dried under vacuum using a rotary evaporator at 45 °C. The dried ethanolic extracts were kept at 2-8 °C for further investigations.

Animals

Wistar adult male rats weighing 300±10 g provided by the Department of Physiology/Pharmacology of “Université de Lomé” were used. All methods and protocols used in this study were observed following the established public health guidelines “Guide for Care and Use of Laboratory Animals”^[8]. The use of the animals was approved by the Ethics Committee of the University of Lomé (N° SBM/UL/15/NS0022).

Experimental design

Rats were weighed and randomly divided into six (6) group (n = 5 per group). Cholesterol is dissolved in corn oil. Rats were fasted overnight before experimentation but had free access to water. All the animals except those of the negative control group (Group B) received orally a suspension of cholesterol at 250 mg/kg. Rats were administrated with the cholesterol suspension daily for 14 consecutive days. From the 1st to the 14th day, rats were treated with extracts thirty minutes after cholesterol administration. All the experimental groups and treatments are presented as follow:

Group A (positive control): cholesterol (250 mg/kg) + distilled water

Group B (negative control): corn oil (5 ml/kg) + distilled water

Group C: cholesterol (250 mg/kg) + *P. suberosa* extract (200 mg/kg)

Group D: cholesterol (250 mg/kg) + *P. suberosa* extract (400 mg/kg)

Group E: cholesterol (250 mg/kg) + *N. latifolia* extract (200 mg/kg)

Group F: cholesterol (250 mg/kg) + *N. latifolia* extract (400 mg/kg)

The standard orogastric cannula was used for cholesterol and extracts oral administration in animals. Twenty four (24) hours after the last administration, rats were anesthetized and blood samples were collected in plain tubes. Samples were centrifuged and serums were collected for the measurement of the ferric reducing antioxidant power (FRAP) and biochemical parameters. Rats were then sacrificed by cervical dislocation and organs including the liver and kidneys were removed for the determination of malondialdehyde (MDA) content.

Determination of biochemical parameters

Total cholesterol, HDL-cholesterol, LDL-cholesterol, blood glucose, triglycerides, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) was performed on the serum using reagent purchased from Biorex diagnostics Ltd (Antrim, United Kingdom). Assays were performed using chemical analyzer. The risk of atheroma, which is the ratio

between LDL and HDL concentrations, is calculated to evaluate the plants effect against atherosclerosis.

Determination of *in vitro* antioxidant activity of extracts

DPPH radical scavenging activity

Antioxidant scavenging activity of extract was determined *in vitro* using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as previously described by Mcune and Johns^[9] and used by Dosseh *et al*^[10]; 2.5 mL of extracts (0-1000 µg/mL) was mixed with 2.5 mL of methanolic solution containing DPPH at 100 µmol/L. After 10 min, the change in the absorbance was determined at 517 nm against the blank (methanolic solution). Assays were carried out in triplicate. The inhibition percentage was calculated using the following formula:

DPPH scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$; where A_0 is the absorbance of the control without extracts and A_1 is the absorbance of sample.

Total phenolic content

Total phenolic content was measured using Folin-Ciocalteu's method according to Al-Farsi *et al*^[11]. Extract (100 µL) was mixed with 750 µL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) for 5 min at room temperature. Aqueous sodium bicarbonate (Na_2CO_3) (60 g/l) was added (750 µL), and the mixture was incubated for 90 minutes at room temperature. The absorbance was measured at 725 nm against the blank using a UV-visible spectrophotometer. Gallic acid at concentrations ranging from 0 to 450 µg/mL was used as the standard to generate the calibration curve.

Total flavonoids content

Total flavonoid content was measured by using colorimetric method described by Al-Farsi *et al*^[12]. 100 µL of ethanolic solution containing extract (1 mg/ml) were mixed with 400 µL of distilled water and 30 µL of aqueous solution of sodium nitrite (NaNO_2) at 5%. The mixture is incubated for 5 minutes. Then, 20 µL of aqueous solution of aluminum trichloride (AlCl_3) at 1M were added and the mixture was incubated for 5 minutes. Finally, 250 µL of distilled water were added and the absorbance was measured at 510 nm against a blank using UV-visible spectrophotometer. Quercetin (0-500 mg/mL) was used to generate the calibration curve. Assays were carried out in triplicate.

Ferric reducing antioxidant power (FRAP) assay

The ability of extracts to reduce ferric ions was measured using the method described by Agbonon and Gbeassor^[13]. FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM tripyridyl triazine (TPTZ) solution and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in a ratio of 10:1:1. Extracts (100, 200, 300, 400 and 500 µg/mL) were added to 3 ml of FRAP reagent and incubated at 37°C for 30 minutes. Absorbance was measured at 593 nm. The absorbance of the samples was compared to a FeSO_4 standard curve.

Statistical analysis

Results were statistically analyzed using One-way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Prism 5 software (Graph Pad Software Inc., La Jolla, CA, USA). All values were expressed as Mean ± SEM (standard error of the mean, n=5). Difference was considered significant when $p < 0.05$.

Results

In vitro antioxidant activity

DPPH radical scavenging activity

The IC₅₀ of *P. suberosa* and *N. latifolia* extracts were found to be respectively 17.158±0.024 µg/mL and 622.519±1.560 µg/mL as shown in Table 1.

Table 1: In vitro antioxidant activities of *P. suberosa* and *N. latifolia* extracts

Extracts/standard	IC ₅₀ (a)	Phenolic content (b)	Flavonoids (c)
<i>P. suberosa</i>	17.16±0.02	392.79±0.36	264.46±1.69
<i>N. latifolia</i>	622.52±1.56	51.60±0.23	88.82±2.18
Quercetin	8.57±0.15	--	--

a: µg / mL of extract, b: µg Galic acid equivalent / mg of dry extract (GAE µg / mg), c: µg quercetin equivalents / mg of dry extract (QE µg / mg)

Ferric Reducing Antioxidant Power (FRAP)

The concentration of Fe²⁺-TPTZ (Fe²⁺ complex tripyridyltriazine) is lower (246.48±37.045 µM) in the positive control group (cholesterol + water) as compared to the negative control group (water + oil). *P. suberosa* extract insignificantly increased, the concentration of Fe²⁺-TPTZ (397.92±34.685 µM and 378.21±87.542 µM for doses of 200

and 400 mg/kg respectively). *N. latifolia* extract significantly increased ($p < 0.001$) the concentration of Fe²⁺-TPTZ as compare to untreated rats (686.76±71.29 µM and 651.86±57.78 µM for doses 200 and 400 mg/kg respectively). In both extracts, the concentration of Fe²⁺-TPTZ complex is inversely proportional to the administered dose; however, differences between doses were not significant (Figure 1).

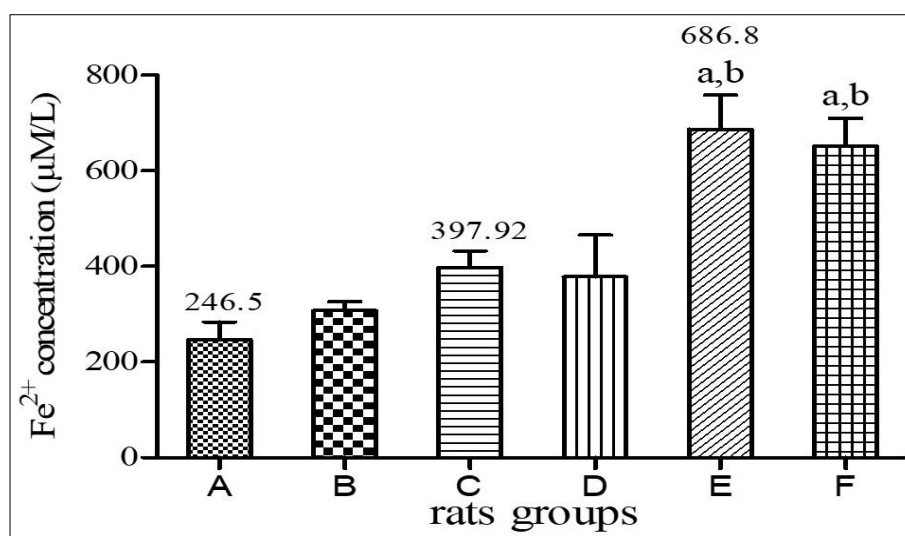


Fig 1: Effect of *P. suberosa* and *N. latifolia* extracts on serum antioxidant potential

A (positive control): cholesterol+distilled water (10mL / kg), B (negative control): corn oil (5mL / kg)+distilled water, C: cholesterol+ *P. suberosa* extract (200mg / kg), D: cholesterol + *P. suberosa* extract (400 mg / kg), E: cholesterol+*N. latifolia* extract (400 mg / kg), F: cholesterol+*N. latifolia* extract (200 mg / kg). ^a $p < 0.001$ compared to group A; ^{a,b} $p < 0.05$ compared to groups C and D.

Malondialdehyde (MDA) content in tissues

Malondialdehyde content in the liver homogenate increased in control group in comparison to the negative control group (8.84±0.82 nM); MDA contents were found to be relatively lower in rats treated with *N. latifolia* extract at 200 mg/kg.

Results showed that MDA content in rats treated with *P. suberosa* extract at 200 mg/kg is significantly lower (4.79±0.27 nM, $p < 0.01$) as compared to MDA content in rats treated with the same extract at 400 mg/kg (7.61±1.1 nM) (Figure 2).

In kidney homogenates, MDA content showed similar results. In comparison to negative control group, MDA value of positive control group showed the highest MDA content in kidney tissue (8.44±1.11 nM). Both extracts reduced MDA content in kidney tissue. However, the reduction is not significant ($p > 0.05$) as compared to positive control group (Figure 3).

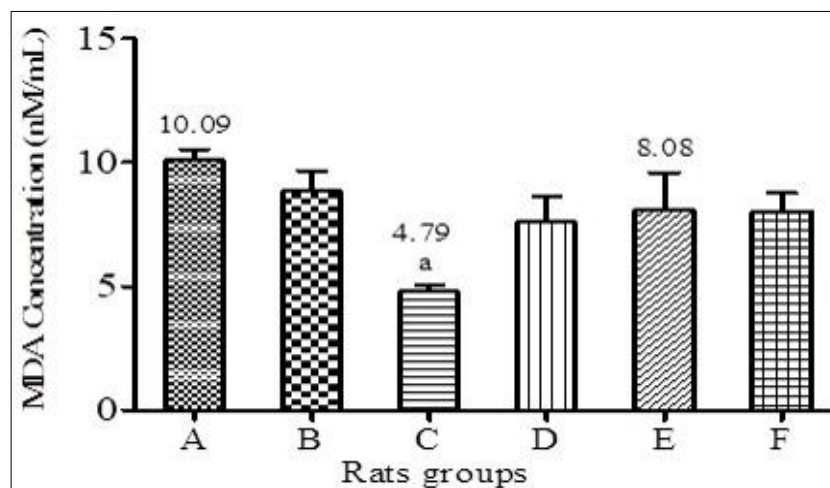


Fig 2: Effect of *N. latifolia* and *P. suberosa* extracts on MDA content in hepatic tissue

A (positive control): cholesterol+distilled water, B (negative control): corn oil (5mL / kg) +distilled water, C: cholesterol+*P. suberosa* extract (200mg / kg), D: cholesterol + *P.*

suberosa extract (400 mg / kg), E: cholesterol+*N. latifolia* extract (400 mg / kg), F: cholesterol+*N. latifolia* extract (200 mg / kg). ^a $p < 0.01$ compared to group A.

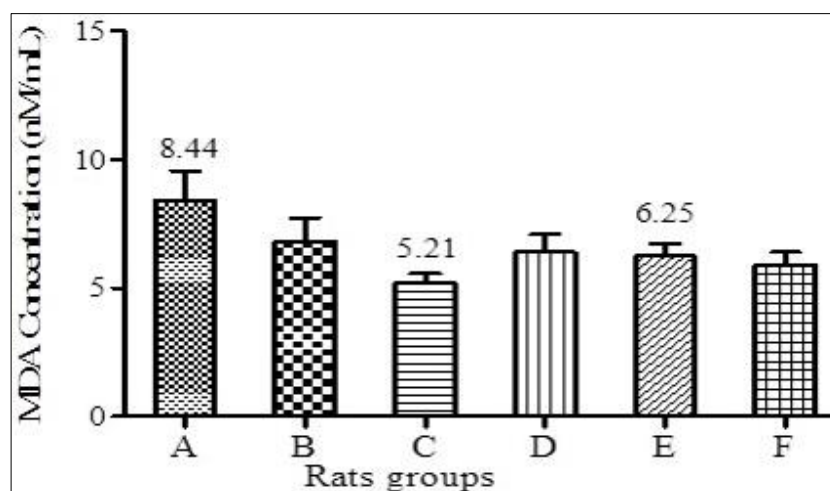


Fig 3: Effect of *N. latifolia* and *P. suberosa* extracts on MDA content in renal tissue

A (positive control): cholesterol+distilled water, B (negative control): corn oil (5mL / kg) +distilled water, C: cholesterol+*P. suberosa* extract (200mg / kg), D: cholesterol + *P. suberosa* extract (400 mg / kg), E: cholesterol+*N. latifolia* extract (400 mg / kg), F: cholesterol+*N. latifolia* extract (200 mg / kg).

the negative control group (water + oil). *P. suberosa* (200 mg/kg) extract significantly reduced ($p < 0.01$) ASAT content. Blood glucose is significantly ($p < 0.01$) lowered by the same extract at 200 mg/kg and 400 mg/kg. Total cholesterol and triglycerides contents are significantly ($p < 0.01$) reduced at 200 mg/kg. HDL-cholesterol content slightly increased whereas LDL-cholesterol significantly decreased ($p < 0.01$) in treated animals causing the decrease of the artherogen risk ($p < 0.001$) as shown in Table 2.

Biochemical parameters

Effects of *P. suberosa* extract on biochemical parameters

Aspartate aminotransferase (ASAT) content is higher in the positive control group (cholesterol + water) in comparison to

Table 2: Effects of *P. suberosa* extract on biochemical parameters

Parameters	Groups			
	Cholesterol + water	Oil +water	Cholesterol +PS (200mg/kg)	Cholesterol +PS (400mg/kg)
ASAT (a)	124±20.39	73±12.83	89±8.50**	115±13.70
ALAT (a)	37±4.32	29±3.15	38±6.44	50±6.51*
Blood glucose (b)	1.33±0.05	1.28±0.10	1.10±0.07***	1.07±0.04***
Triglycerides (b)	0.37±0.06	0.33±0.07	0.12±0.04***	0.39±0.02
Total cholesterol (b)	0.68±0.04	0.62±0.04	0.56±0.02*	0.63±0.06
HDL (b)	0.21±0.06	0.30±0.07	0.29±0.05	0.30±0.05
LDL (b)	0.43±0.03	0.26±0.06	0.25±0.03**	0.24±0.04**
Atherogen risk	2.35±0.54	1.05±0.31	1.05±0.34***	0.87±0.17***

a: UI/L; b: g/L; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to the positive control group (cholesterol + water). PS: *P. suberosa*.

Effects of *N. Latifolia* extract on biochemical parameters

Treatment with *N. latifolia*, at 200 mg/kg and 400 mg/kg, significantly ($p < 0.01$) decreased serum ASAT content in treated rats as compared to untreated ones. Blood glucose is lowered significantly ($p < 0.05$) at the dose 200 mg/kg. Total

cholesterol content is not affected. HDL-cholesterol content increased while LDL-cholesterol decreased in treated rats. The atherogen risk is significantly reduced ($p < 0.01$) in treated rats as compared to control rats (Table 3).

Table 3: Effects of *N. latifolia* extract on biochemical parameters

Parameters	Groups			
	Cholesterol + water	Oil +water	Cholesterol +NL (200mg/kg)	Cholesterol + NL (400mg/kg)
ASAT (a)	124±20.39	73±12.83	85±5.15**	84±2.87**
ALAT (a)	37±4.32	29±3.15	40±3.12	35±5.02
Blood glucose (b)	1.33±0.05	1.28±0.10	1.20±0.03*	1.22±0.06
Triglycerides (b)	0.37±0.06	0.33±0.07	0.38±0.06	0.43±0.09
Total cholesterol (b)	0.68±0.04	0.62±0.04	0.66±0.03	0.69±0.08
HDL (b)	0.21±0.06	0.30±0.07	0.50±0.03***	0.28±0.06
LDL (b)	0.43±0.03	0.26±0.06	0.10±0.02***	0.32±0.09
Atherogen risk	2.35±0.54	1.04±0.31	0.17±0.03***	1.38±0.38**

a: UI/L; b: g/L; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to the positive control group (cholesterol + water). NL: *N. latifolia*

Discussion

The aim of this study is to evaluate the effects of hydroethanolic extracts of *N. latifolia* and *P. suberosa* on the consequences of cholesterol overload in rats. The antioxidant properties of the two plants were studied. The IC₅₀ of *P. suberosa* and that of *N. latifolia* extracts are respectively 17.158±0.024 µg/mL and 622.519±1.560 µg/mL. Total phenolic content is 392.79 µgGAE/mg for *P. suberosa* and 51.602 µgGAE/mg extract for *N. latifolia* (Table 1). *N. latifolia* extract significantly increased ($p < 0.001$) the concentration Fe²⁺-TPTZ as compare to untreated rats (686.76±71.29 µM and 651.86±57.78 µM for doses 200 and 400 mg/kg respectively). In both extracts, the concentration Fe²⁺-TPTZ complex is inversely proportional to the administered dose; however, differences between doses were not significant (Figure 1). Extracts decrease the content of MDA in renal and hepatic tissues as compared to the control group (Figures 2 and 3). The both extracts decrease the LDL-cholesterol and reduce significantly the calculated atherogen risk (Tables 2 and 3).

The consequences of lipid overload leading to chronic diseases often go through oxidative stress. Antioxidant activity of a natural substance could be indicated in the prevention of chronic diseases resulting from oxidative stress. Previous studies showed that phenolic compounds are, quantitatively, the main antioxidants of plant origin and have a high antioxidant activity [14]. Hence, antiradical activity and compounds with antioxidant activity of *N. latifolia* and *P. suberosa* have been quantified. Antiradical activity is proportional to the concentration of phenolic compounds in both extracts. It has been reported that hypercholesterolemia induces oxidative stress, accelerates cellular aging, alters cell membrane integrity, decreases cell reconstitution capacity and the overall organism antioxidant capacities [6]. In this investigation, results showed that *latifolia* and *P. suberosa* exhibited *in vivo* antioxidant potential while increasing serum FRAP and considerably decreased MDA and liver enzymes (ASAT and ALAT) contents in the liver and kidney tissues. Precisely it has been shown that *N. latifolia* and *P. suberosa* contain antioxidants such as carotenoids and inorganic substances [15]. Thus, these present results might be attributed to the antioxidant substances that are present in both extracts. *N. latifolia* and *P. suberosa* showed beneficial effect on the cholesterol profile by lowering the LDL-cholesterol and blood glucose levels and increasing the HDL-cholesterol (Tables 2 and 3). The calculated atheroma risk is reduced ($p < 0.01$) by

both extracts. These results suggest that *N. latifolia* and *P. suberosa* extracts might have beneficial effect via the reduction of atherosclerosis for LDL-cholesterol is involved in the formation of atheroma plaque [6] while HDL-cholesterol is involved in the transportation of lipids from peripheral tissues to the liver, reducing the fat deposits in the tissues. Vogiatzi *et al.* (2009) found that substances with antioxidant activity are of particular therapeutic importance in cardiovascular diseases, especially atherosclerosis, by scavenging active oxygen species (AOS) involved in the pathophysiology of these diseases [16]. Therefore, *N. latifolia* and *P. suberosa* might be used as protective natural products against cardiovascular diseases.

Conclusion

Oxidative stress is involved in several pathologies and is a major risk factor in the occurrence of their complications. However, populations traditionally use various parts of plants in the treatment of diseases in which oxidative stress is incriminated. Among these plants, Togolese populations commonly use *N. latifolia* and *P. suberosa*. This study showed that extracts of *N. latifolia* and *P. suberosa* have antioxidant properties *in vitro* and especially *in vivo* in rats. The extracts reduced the level of LDL and triglycerides, increased the HDL level. Results confirm the traditional use of these herbals in the management of diabetes and cardiovascular diseases. Results also suggest that, *N. latifolia* and *P. suberosa* may contribute to the prevention and treatment of oxidative stress and hypercholesterolemia related diseases.

Authors' contributions

The first draft was prepared by Abalo Pazimna Wiyao and Idoh Kokou. All authors read the final version and confirmed it for publication.

Conflicts of interest

The authors declare no conflicts of interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been carefully observed by the authors.

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