Research article

Antihyperlipidemic and antioxidant properties of hydro-alcoholic extracts from *Anogeissus leiocarpus* (Combretaceae)

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ARTICLE INFO

Keywords:
*Anogeissus leiocarpus*
Supernatant
Antihyperlipidemic
Antioxidant
Polyphenols

ABSTRACT

*Anogeissus leiocarpus* (Combretaceae) is a medicinal plant used in Togo to treat diabetes mellitus and other diseases. The present study was undertaken to evaluate the antihyperlipidemic and antioxidant activities of total extract and fractions of roots of *Anogeissus leiocarpus*.

The antihyperlipidemic activity of the total extract and the supernatant was performed *in vivo* by the fructose overload test in ICR mice. Antioxidant potential was determined *in vitro* by methods based on scavenging of DPPH*, total antioxidant capacity and reducing power. After the screening, phenolic compounds and flavonoids were evaluated by the well-known colorimetric assay using respectively Folin–Ciocalteu reagent and aluminium chloride.

The results obtained showed that the total extract and the supernatant significantly reduced the serum and liver levels of triglycerides and hence the level of VLDL-Cholesterol compared to hyperlipidemic mice. *In vitro* the total extract and fractions had the ability to scavenge free radicals, to reduce metal and possessed strong total antioxidant activity. Phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, tannins and saponosides in the extract and fractions. And the supernatant fraction contained more polyphenolic compounds than others.

From this study, it is concluded that the total extract and fraction of *Anogeissus leiocarpus* possessed strong antihyperlipidemic, antioxidant properties and were riched in polyphenols, which can be used in the treatment of diabetes mellitus' complications. Hence, the supernatant fraction was the most biologically active.

1. Introduction

Diabetes is a serious, long-term state that occurs when the body cannot produce any or enough insulin or cannot effectively use the insulin [1]. In diabetics, chronic hyperglycemia which is a source of oxidative stress, remains a very critical condition because of its nature to generate serious life-threatening health complications such as cardiovascular diseases, neuropathy, retinopathy, nephropathy [2]. In fact, wide range of cardiovascular conditions comprise the largest cause of both morbidity and mortality for people living with diabetes [3]. According to the pre-vision of International Diabetes Federation, the number of deaths resulting from diabetes and its complications in 2019 was estimated to be 4.2 million [4].

Despite the numerous antidiabetic drugs available associated to side effect, most of the population treat many diseases with plants. In diabetes, herbal alternatives have proven to provide symptomatic relief and assist in the prevention of the complications of the disease [5]. Therefore, till the last few years, different extracts from different traditional medicinal plants have been studied which helpful for management of diabetes mellitus [6].

Ethnopharmacological survey of plants carried out by [7] reported that *Anogeissus leiocarpus*, is one of the plants (Combretaceae) used traditionally in the treatment of diabetes mellitus. Our previous studies had revealed the antihyperglycemic activity of the total extract and fractions (supernatant and pellet) of roots of *Anogeissus leiocarpus*. Administered at a dose of 500 mg/kg, the hydro alcoholic extract of *A. leiocarpus* lowered hyperglycemia in ICR mice with carbohydrate overload but did not show any significant effect on basal glycemia; comparing the fractions, the supernatant fraction is the most biologically active at the dose of 100 mg/kg [8].

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https://doi.org/10.1016/j.heliyon.2021.e06648
Received 22 December 2020; Received in revised form 27 January 2021; Accepted 26 March 2021
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The present preliminary study was carried out to evaluate the *in vivo* antihyperlipidemic and antioxidant activities *in vitro* of total extract and fractions of roots of *Anogeissus leiocarpus*.

2. Material and methods

2.1. Drugs and chemicals

Commercial reagent kits for determination of Total cholesterol (Chol), HDL, LDL were purchased from Biolabo S.A. (Paris, France). 1,1-diphenyl-2-picryl hydrazyl (DPPH), aluminum chloride, gallic acid, ascorbic acid, rutin, iron chloride, polyvinylpolypyrrolidone, Folin Ciocalteu reagent, aluminum chloride, acetate Sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other unlabelled chemicals and reagents were available commercially.

2.2. Animals

ICR mice (35 ± 5 g) were housed in standard environmental conditions (temperature 24–25 °C, relative humidity and a 12h/12h light-dark cycle) and fed with standard rat diet and water *ad libitum*. The study was approved by the Ethics Committee of the University of Lome, a branch of the National Ethics Committee for control and supervision of experiments on animals (NSBM/UL/14/NS0004).

2.3. Plant material

Roots of *Anogeissus leiocarpus* (a deciduous tree) were collected from Tsévié, Zio (TOGO) in the month of July 2018. The plants have been identified in Botany and Plant Ecology Laboratory of Faculty of Science (University of Lome), where voucher specimen was deposited in the herbarium under the number TOGO 15483.

Roots of *Anogeissus leiocarpus* were cleaned out with water, cut into small pieces, dried at the Animal Physiology laboratory at 22°C and then reduced into powder with the mill Thomas Scientifique™.

2.4. Extraction and fractionation

About 400 g of Roots of *Anogeissus leiocarpus* were extracted in water/ethanol (5:5) for 72 h. The crude extract was filtered on Whatman paper and evaporated in vacuum at 45 °C using a rotary evaporator (Buchi R120). The yield of the dry extract was 5.68%.

About 30 g of hydroethanolic extract obtained was suspended in frozen ethanol 75% within 24 h. Supernatant was separated from pellet by centrifugation at 2500 rpm and evaporated in vacuum at 45 °C using a rotary evaporator. Pellet was then concentrated to dryness [8].

2.5. Effects of total extract and supernatant of roots of *Anogeissus leiocarpus* on hyperlipidemic mice

2.5.1. Experimental design

In order to evaluate the effect of the total extract on triglyceride synthesis, the fructose overload test in mice was adopted according to the method of [9] with slight modifications. Thirty normal healthy mice were kept separately in 5 random groups (n = 6) (Figure 1).

Blood samples taken in anticoagulant-free tubes were centrifuged at 3000 rpm for 10 min. The serum were separated, stored at -20 °C until usage. The serum was analyzed for Triglyceride (TG), Total Cholesterol (TG-Chol), lipoproteins (HDL, LDL, VLDL), using an automatic analyzer (Rayto Chemray-120). The animals were then euthanized by cervical dislocation and the liver of each animal was removed and kept in the freezer for triglyceride extraction.

2.5.2. Extraction of triglycerides

Triglycerides were extracted from mice liver according to the method described by [10].

0.5 g sample of liver was homogenized in 500 μL of buffer composed of: NaCl (2 mM), EDTA (20 mM) and phosphate buffer (50 mM pH 7.4). To 10 μL of homogenate, 10 μL of tert-butyl alcohol and 5 μL of a mixture of Triton X-100/methanol (1:1, v/v) were added. The triglyceride determination was performed using an assay kit (Pharmalab Innovus).

2.6. In vitro antioxidant assays

2.6.1. DPPH* radical scavenging activity

The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH*) free radical scavenging activity, was performed by the method described of [11] with slight modifications.

1.5 mL DPPH* solution (100 μmol.L⁻¹) and 0.25 mL methanol or 0.25 mL methanolic extract and fraction solution were mixed. The absorbance was determined at 517 nm, after 30 min of incubation. Ascorbic acid at different concentrations served as a standard.

The percentage inhibition activity was calculated from \[ \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \], where A0 is the absorbance of the control, and A1 is the absorbance of the extract or fraction/standard.

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**Figure 1.** Experimental design of fructose 60% overload test. H₂O d: distilled water, TE: total extract, Sup: supernatant fraction, Met: metformin, min: minutes, Fru: Fructose.
2.6.2. Reducing power

The reducing ability was determined by the method of [12]. Different concentrations of total extract and fractions (1 mL) were mixed with phosphate Buffer (2.5 mL) and potassium ferricyanide at 1% (2.5 mL). This mixture was kept at 50 °C in water bath for 20 min. After cooling, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction, The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% of ferric chloride solution (0.5 mL) after 10 min of centrifugation at 3000 rpm. Ascorbic acid served as standard. The absorbance was measured at 700 nm.

2.6.3. Total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the method of [11].

3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 0.3 mL of total extract and fractions. The tubes containing the reaction solution were incubated at 95 °C for 90 min. Ascorbic acid served as standard. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

2.7. Phytochemical study

2.7.1. Preliminary phytochemical analysis

The phytochemical analysis was performed for detection of active phyto-constituents present in the extract and fractions using standard procedure of [13] and [14].

2.7.2. Determination of total phenolic compounds and tannins

Total phenolic compound contents were firstly estimated using Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid) which is reduced during the oxidation of the phenol in a mixture of blue oxides of tungsten and molybdenum which can be determined spectrophotometrically at 735 nm.

In other to determine the amount of tannins, A second dosage was performed after the fixation of tannins by Polyvinylpyrrolidone (PVP). The difference between the first and the second dosage expresses the total amount of tannins according to the method of [16].

2.7.3. Total flavonoids content

Flavonoid content in the extract and fractions was determined according to the method used by [17]. Briefly, to 2 mL of extract/ rutin at different concentrations (5–100 μg mL⁻¹), 2 mL of aluminum chloride (20 mg mL⁻¹) and 6 mL of sodium acetate (50 mg mL⁻¹) were added. Absorbance was read at 440 nm after 150 min of incubation. Flavonoids values were expressed in term of rutin equivalent mg·g⁻¹ of extract and fractions.

2.7.4. Determination of polysaccharides

Firstly, samples were treated by the method of [18] in order to remove cell walls that contain insoluble fibers such as pectin, inorganic salts, and low, molecular weight compounds of <8000 Da, including proteins. According to the method of [19], 200 μL of a 5% (w/v) aqueous phenol solution and 1 mL of concentrated sulphuric acid were added to 200 μL of the samples (total extract, fractions, standard range, control).

This method is an based on sulfuric acid hydrolysis of complex polysaccharides. After homogenization, the mixture was heated in a water bath at 100 °C for 5 min and cooled in darkness for 30 min. Glucose at different concentrations (50–200 μg mL⁻¹) served as standard. The absorbance was determined at 480 nm using UV/VIS Spectrophotometer Wavelength.

2.8. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Dunnett’s test to evaluate significant differences between groups. The level of significance was set at p < 0.05 and statistical analysis were carried out using Graph Pad Prism 7.0.

3. Results

3.1. Effect of total extract and supernatant on hyperlipidemia

Fructose overload caused significant increase of serum triglycerides and VLDL cholesterol in hyperlipidemic mice compared to normals (Table 1). The administration of the total extract and supernatant significantly decreased serum triglycerides and VLDL cholesterol levels compared to positif control, respectively. The decrease was more pronounced in the liver than in the serum. Indeed, in the liver, the administration of the total extract at the dose of 100 mg.Kg⁻¹, and the supernatant fraction (100 mg.Kg⁻¹) significantly (p < 0.0001) decreased triglyceride levels compared to the hyperlipidemic controls. A similar effect was also noted with VLDL cholesterol levels. Supernatant was found to be highly potent as shown in Table 1.

3.2. In vitro antioxidant assays

3.2.1. In vitro DPPH* radical scavenging and total antioxidant activity of total extract and fractions of roots of A. leiocarpus

Table 2 shows that the total extract and fractions scavenge the DPPH* radical in dose dependent manner. Compared to ascorbic acid, the supernatant fraction has the highest DPPH* radical scavenging capacity.

While increasing the concentration of the extract and fractions, the antioxidant capacity increases and is correlated to the increase of absorbance. As shown in Table 2, The Total antioxidant capacity value represent all antioxidant compounds present in the extract as equivalence of ascorbic acid which act in hydrophilic and lipophilic part. In comparison, supernatant fractions possesses higher total antioxidant capacity.

3.2.2. Reducing power of the total extract and fractions of A. leiocarpus

The reducing power of the total extract and fractions was concentration dependent and significantly different from that of ascorbic acid (Figure 2).

Compared to ascorbic acid (positive control), the supernatant has a higher reducing power according to the following diagram: Supernatant > Total extract > Ascorbic acid > pellet.

3.3. Phytochemical study

3.3.1. Identification of the major chemical groups in the total extract and fractions of Anogeissus leiocarpus' roots

Phytochemical screening of the total extract and fractions of A. leiocarpus revealed the presence of alkaloids, saponins and polyphenolic compounds (Table 3).
Table 1. Effect of total extract and supernatant on lipid profile.

|       | NC             | HC             | TE 500         | Sup 100        | Met 100        |
|-------|----------------|----------------|----------------|----------------|----------------|
| Serum |                |                |                |                |                |
| TG (g.L⁻¹) | 0.36 ± 0.04 | 1.08 ± 0.05**** | 0.73 ± 0.04*   | 0.41 ± 0.07**** | 0.83 ± 0.11    |
| Chol-T (g.L⁻¹) | 1.29 ± 0.06 | 1.59 ± 0.13    | 1.55 ± 0.18    | 1.17 ± 0.07    | 1.18 ± 0.09    |
| HDL-C (g.L⁻¹) | 1.01 ± 0.05 | 0.92 ± 0.06    | 1.26 ± 0.10*   | 1.10 ± 0.09    | 0.93 ± 0.03    |
| LDL-C (g.L⁻¹) | 0.28 ± 0.04 | 0.69 ± 0.11*   | 0.36 ± 0.14    | 0.14 ± 0.04**  | 0.23 ± 0.08*   |
| VLDL-C (mg.dL⁻¹) | 7.2 ± 0.9  | 21.6 ± 1.1***** | 14.8 ± 1.0*    | 8.2 ± 1.6***** | 16.6 ± 2.2     |
| Liver |                |                |                |                |                |
| TG (g.L⁻¹) | 0.43 ± 0.04 | 1.15 ± 0.11**** | 0.49 ± 0.03**** | 0.30 ± 0.03**** | 0.67 ± 0.05*** |
| VLDL-C (mg.dL⁻¹) | 9.26 ± 0.88 | 23.02 ± 2.37**** | 11.13 ± 1.28**** | 5.26 ± 0.55**** | 13.36 ± 1.05*** |

The NC and HC groups received distilled water and the groups (TE 500, Sup 100 and Met 100) were treated respectively with total extract (500 mg·Kg⁻¹), supernatant (100 mg·Kg⁻¹) and metformin (100 mg·Kg⁻¹) for 4 days. The results are treated with Anova one way and represent the mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; Compared to hyperlipidemic control (HC), ****p < 0.0001; *p < 0.05; Compared to normal control (NC).

Table 2. In vitro DPPH⁺ radical scavenging and total antioxidant activity of total extract and fractions of roots of *Anogeissus leiocarpus*.

|                  | IC 50 (µg·mL⁻¹) | Total antioxidant (mg AA·g⁻¹) |
|------------------|-----------------|------------------------------|
| Ascorbic acid    | 46.9 ± 0.15     | -                            |
| Total extract    | 48.7 ± 0.15     | 233 ± 1.5                    |
| Supernatant      | 23.3 ± 0.35     | 237 ± 1.5                    |
| Pellet           | 72.7 ± 0.70     | 141 ± 2.5                    |

The total antioxidant were expressed in mg/g of Ascorbic Acid. The results represent the means ± SEM. N = 3.

Figure 2. Reducing power of total extract and fractions of roots of *Anogeissus leiocarpus*. Ascorbic acid was used as a positive control. Each point represents the mean ± ESM (N = 3).

3.3.2. Dosage of total phenols, tannins, flavonoids and polysaccharides

Table 4 shows that the amount of phenolic compounds, tannins, flavonoids and polysaccharides were significantly high in the supernatant followed by the total extract and the pellet of *Anogeissus leiocarpus*.

4. Discussion

Several factors of hyperlipidemia include a high fat dietary, physical inactivity, obesity smoking and smoking [20]. Diabetes combined with chronic hyperlipidemia could accelerate the progress of atherosclerosis and increase the incidence of cardiovascular disease [21]. In fact, patients live with hyperlipidemia have usually a high levels of total cholesterol, triglycerides, and LDL-cholesterol; as well as a decrease in HDL-cholesterol [22]. Elevated LDL-cholesterol and triglycerides lead to diseases such as biliary obstruction, type 2 diabetes mellitus, coronary artery disease and artherosclerosis which are main cause of cardiovascular disease worldwide [20] in which elevated oxidative stress plays a pivotal role [23]. Therefore, the control of total cholesterol, lipoproteins and triglycerides, as well as reducing of oxidative stress will be a great importance in the management of the disease.

This present study was undertaken to evaluate *in vivo* the anti-hyperlipidemic property and the antioxidant activities of total extract and fractions of roots of *Anogeissus leiocarpus*.

Hyperlipidemia in mice was induced by a 60% fructose overload. Indeed, studies in animals have shown that a rich diet in fructose can lead to hyperglycemia and hypertriglyceridemia followed by insulin resistance ([24, 25]). The results showed a significant increase in serum triglycerides and LDL-C levels in hyperlipidemic controls. The
administration of the total extract and the supernatant caused a significant decrease of triglycerides and LDL-C levels compared to the hyperlipidemic control groups. A significant increase of triglycerides level was also observed in the hyperlipidemic mice when triglyceride were extracted. This triglycerides level was reduced significantly in the liver of groups treated with total extract and supernatant. The in vivo potential antihyperlipidemic was also found by [28] on the leaves of Anogeissus leiocarpus on diabetic model of rats. The antihyperlipidemic activity of the total extract and especially supernatant may be due to the enhancement of triglycerides catabolism. As reported by many researchers, the restoration of the catabolism of triglycerides could be due to the stimulation of the lipolytic activity of plasma lipoprotein lipase [27]. These mechanisms of action of A. leiocarpus were similar to antihyperlipidemic agents like Statins and fibrates whose enhance LDL-cholesterol clearance and decrease in hepatic VLDL-cholesterol production [28]. A significant (p < 0.05) increased in HDL-cholesterol in treated groups prove that A. leiocarpus can be used to prevent artherosclerosis and reduce oxidative stress. As the matter of fact, HDL-cholesterol inhibit the artherosclerosis formation and remains an important marker of oxidative stress in cholesterol metabolism. The reduction of oxidative stress was in accordance with antioxidant activity of A. leiocarpus.

The method of fractionation of the hydroalcoholic extract of the roots of Anogeissus leiocarpus [8] has allowed two fractions to be obtained; the supernatant fraction and the pellet fraction. With phytochemical screening, the presence of alkaloids, saponins and polyphenolic compounds were revealed in the different fractions as well as in the total extract, in accordance to the work of [29]. Quantitative tests confirmed the presence of these phenolic compounds. However, the supernatant fraction contained more phenolic compounds at concentrations of 75.5 and 70.5 mg equivalent of AG g \(^{-1}\) respectively fo for total phenol and tannins, and 57 mg equivalent of Routine/g for flavonoids. The supernatant is more richer in polysaccharides than the total extract and pellet.

Nowadays, many studies demonstrated that plants contained polyphenolic compounds and polysaccharides were able to eliminate free radicals and could therefore be categorized as natural antioxidants [30]. Thus, the antioxidant activities of the total extract and fractions were evaluated in vitro through the DPPH\(^+\) radical scavenging capacity, the reducing power and the total antioxidant capacity.

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH\(^+\) test is used to prove the capacity of the total extract and fractions of roots of Anogeissus leiocarpus to act as donors of hydrogen atoms. The results have shown the free radical DPPH\(^+\) scavenging ability of the fractions and total extract and fractions in dose dependent manner. In comparison, the supernatant has the higher capacity to scavenge the DPPH\(^+\) radical. Iron and other metals (copper, chromium, cobalt, vanadium, arsenic....) promote oxidation by acting as catalysts in the formation of free radicals through the transfer of free electrons during the reaction [31]. Likewise, iron in the free state is a powerful electron donor that reacts notably with hydrogen peroxide (H\(_2\)O\(_2\)) to generate highly reactive hydroxyl radicals (HO\(^-\), HO\(^{•}\)) according to the Fenton reaction [32]. The results showed that the total extract and fractions have reduced in concentration dependent manner the Fe\(^{3+}\) to the Fe\(^{2+}\). In other hands, the fractions and total extract have shown a dose-dependent antioxidant capacity. Furthermore Compared to ascorbic acid (reference), The supernatant showed high level of radical scavenging activity, reduced more the metals with higher antioxidant activities than the total extract and pellet fractions. Hence, the supernatant presented the most considerable total antioxidant capacity.

The antihyperlipidemic and antioxidant activities of the total extract and supernatant may be due to the polyphenolic compounds and related compounds present in them. The supernatant fraction contains more bioactive compounds than other. This could explain the most pronounced in vivo antihyperlipidemic effect and antioxidant activities of this fraction.

5. Conclusion

It concluded that the total extract and fractions of roots of Anogeissus leiocarpus have shown remarkable and strong antihyperlipidemic and antioxidant properties. Such activities may be due to the phytochemical compounds present in the total extract and fractions. It may be helpful in preventing the macrovascular complications associated with diabetes mellitus and this knowledge could be used for future investigation as novel natural antioxidiant and antihyperlipidemic drugs.

Declarations

Author contribution statement

Aku Enam Motto: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Povi Lawson-Evi; Kwashie Eklu-Gadegbeku: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Batomayena Bakoma: Analyzed and interpreted the data; Wrote the paper.

Kodjo Aklikokou: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.
