PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ANTIHYPERCHOLESTEROLEMIC ACTIVITY OF THE CAKE OF SAFOU (DACRYODES EDULIS) IN RATS (RATTUS NORVEGICUS)

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Abstract

Safou (Dacryodes edulis) is consumed by the Ivorian population for its therapeutic virtues. The present study aims to make known the phytochemical composition and antihypercholesterolemic activity of safou cake. For this, phytochemical screening of the aqueous extract of the pulp cake of the safou was carried out. In addition, the antihypercholesterolemic activity of cake was tested in rats (Rattus norvegicus). Results obtained indicate that the aqueous extract of cake contains secondary metabolites such as polyphenols, flavonoids, saponins, polyterpenes and sterols and alkaloids. Moreover, this extract allowed, at a dose of 300 mg / kg of PC, to decrease the blood concentration of compounds such as VLDL-C, LDL-C, triglycerides, total cholesterol and total lipids. On the other hand, at a dose of 100 mg / kg of PC of this extract, the blood level of HDL-C increased significantly in hypercholesterolemic rats (P˂0.05). This study confirms the antihypercholesterolemic activity of safou cake. Therefore, safou cake could be used to prevent oxidative stress, arterosclerosis, high blood pressure, diabetes and ischemic cardiovascular disease.

Introduction:

Hypercholesterolemia is a risk factor for cardiovascular diseases which represent a third of mortality in the world (Fuant, 2003). This hypercholesterolemia is responsible according to WHO for 4.4 million deaths each year (Fournier, 2011). This risk factor for cardiovascular disease causes 23.1% of deaths in Africa (WHO, 2016). In Côte d’Ivoire, the latest WHO estimates put the prevalence of high total cholesterol levels in the population at over 19.9% (WHO, 2011). This pathology constitutes a major public health issue. It is therefore essential to manage hypercholesterolemia (Jung, 2005). The current management of hypercholesterolemia is based mainly on the use of statins because of their powerful cholesterol lowering effect (Jung, 2005). However, they are not stripped of side effects with not occurred in particular of digestive disorders, cancer, renal insufficiency and muscle pain.
(Matusiewicz et al., 2015; Duane and Blair., 2016). Currently, many studies report a possible activity of certain plants in the reduction of blood cholesterol (Jung, 2005).

Indeed, plants are known to have fewer adverse effects than drug therapy (Jung, 2005). Among these many plants, the safoutier (Dacryodes edulis), an oilseed with nutritional and therapeutic advantages (Ajibesin, 2011; Poligui et al., 2013).

In Côte d’Ivoire, the safoutier fruits are eaten by a fringe of the population because food resources are almost unknown to the population. Also, the safoutier (D. edulis) has not been the subject of enough scientific research in Côte d’Ivoire. Nevertheless, some work has focused on biochemical and phytochemical composition of the cakes of safoutier fruits (Dacryodes edulis) from Côte d’Ivoire (Ano et al., 2018), on the effect of solvent and press extractions on physicochemical properties of oil extracted from dacryodes edulis fruit (Ano et al., 2019) and effect of extraction methods on the fatty acid profile and antioxidant activity of oil extracted from Dacryodes edulis fruit (Ano et al., 2019). Work has also focused on the minerals of safou cake (Ndamba, 1989). According to Grigaris (2012), safou cake would have a positive effect on the reduction of blood cholesterol. This is why this study was carried out to show the beneficial effect of safou cake on health. This work focused on the phytochemical screening and the evaluation of the antihypercholesterolemic activity of safou cake in rats (Rattus norvegicus).

**Plant material**

The plant material studied consists of safoutier fruits (safou) of the variety edulis (Figure 1). These fruits were collected from village of Azaguie-Blida (Agboville Sub-prefecture, south of Côte d’Ivoire). The samples were sent to the Laboratory of Industrial Processes, Synthesis, Environment and New Energies (LAPISEN) of Yamoussoukro (Côte Ivoire). The safou pulp was separated from the kernels with a stainless knife. The pulp was dried during 3 days, crushed and deoiled by press. The cake obtained was collected, dried and packaged in sachets for analysis.

**Animal material**

A number of 40 rats of the species Rattus norvegicus of the Wistar strain, male and female, aged 8 to 12 weeks were used in the various experiments. These rats, with an average weight of 160 ± 0.02 g, were kept under favorable conditions of breeding in the vivarium of the Advanced Teachers’ Training College of Abidjan (ENS) from Cocody (Abidjan, Côte d’Ivoire). These rats were raised under standard environmental conditions (temperature 25 to 30 °C, humidity between 60 and 70% with a light cycle of 12h / 24h) giving them free access to water and food.
Methods:--
Preparation of the aqueous extract
Soluble dry matter content of the cakes was prepared according to the method described by Mir (2016). For the preparation, a mass of 50 g of cake powder obtained from the de-oiled pulp was macerated in 500 mL of distilled water during 24 hours. The macerate was filtered through cotton wool and once on Whatman filter paper. The filtrate obtained was first placed in petri dishes and then dried in an oven at 50°C for 3 days. Finally, the drying results in obtaining a crude dry extract of the pulp cake of safou from which the various dosages were carried out. Phytochemical screening of safou cake Different families of secondary metabolites such as polyphenols, flavonoids, sterols and terpenes, alkaloids, tannins, saponins were screened according to the method described by Bekro et al (2007) and Bagre et al (2007)

Test for polyphenols
A drop of 2% alcoholic ferric chloride solution was added to 2 mL of the extract. The appearance of blackish-blue or more or less dark green coloring indicates a positive reaction.

Test for flavonoids
A volume of 2 mL of the extract was dried on a sand bath. The dry residue obtained was cooled and taken up in 5 mL of hydrochloric alcohol (mixture of 10 mL of 96 ° ethanol, 10 mL of distilled water and 10 mL of concentrated hydrochloric acid). Then two to three magnesium shavings were added to it. The appearance of a pink-orange or purple color after adding 3 drops of isoamyl alcohol indicates the presence of flavonoids.

Test for sterols and polyterpenes
Liebermann's reagent was used for this demonstration. A mass of 0.1 g of dry extract of the pulp was dissolved at hot in 1 mL of acetic anhydride and collected in a test tube. Then, 0.5 mL of concentrated sulfuric acid (H₂SO₄) was added. The appearance of a purple or violet ring at the interphase, turning blue and then green, indicates the presence of polyterpenes and sterols.

Test for alkaloids
A mass of 1 g of dry extract was dissolved in 6 mL of 60 ° ethanol. 2 drops of DRAGENDORFF reagent (aqueous solution of potassium iodobismuth) were added to the alcoholic solution thus obtained. The appearance of a precipitate or an orange color indicates the presence of alkaloids.

Test for tannins
Catechetical tannins
A volume of 15 mL of STIASNY reagent (10 mL of 40% added with 5 mL of concentrated HCl) was added to 1 g of dry extract. The mixture was kept in a water bath at 80 ° C for 30 min and cooled under flowing water. The appearance of large precipitates in form of flakes indicates the presence of catechetical tannins.

Gallic tannins
The solution containing the flakes was filtered and the collected filtrate was then saturated with sodium acetate. To the mixture, 3 drops of 2ferric chloride were added to it. The appearance of an intense blue black color indicates the presence of gallic tannins.

Test for saponins
A mass of 0.1 g of dry extract was dissolved in 10 mL of distilled water. The solution obtained was stirred vigorously during 45 seconds. After stirring, the solution was allowed to stand for 15 minutes. The observation of a persistent foam, greater than 1 cm in height, indicates the presence of saponins.

Induction of hypercholesterolemia in rats
Hypercholesterolemia was induced according to the experimental protocol described by Giricz et al. (2009), with some modifications. To do this, male rats of the Wistar strain (n = 35), weighing 100 to 160 g and aged 10 to 12 weeks were divided into two groups. The first group of rats (controls) (n = 5) received distilled water first. On the other hand, the second group of rats (n = 30) received extra pure cholesterol by gavage (J.P VIII, OAB, 9USPXIX, 1965 Merck, France) at a dose of 0.1 mg / kg of BW. Induction was carried out for 14 days. At the end of this period, the blood of the rats was drawn from the orbital sinus of the eye. The total cholesterol was then assayed and
the rats with a serum total cholesterol content ≥ 0.48 ± 0.03 g/L compared to controls (total cholesterol content < 0.40 ± 0.05 g/L) were considered hypercholesterolemic.

**Evaluation of the effect of safou cake in hypercholesterolemic rats**
The effect of pulp cake (TPP) on hypercholesterolemic male rats was determined according to the method of Kong et al. (2018). Following the induction phase, a group of 5 normal rats (n = 5) and 4 groups of 5 hypercholesterolemic rats (n = 20) were formed. The group of normal rats received 0.9% NaCl. On the other hand, the hypercholesterolemic rats (group 2) received orally, once a day, cholesterol (0.1 mg/kg BW). In addition, in the other groups (groups 3, 4 and 5), the hypercholesterolemic rats received once a day, by gavage, in addition to cholesterol (0.1 mg/kg BW), respectively 1.8 mg/kg of Atorvastatin, 100 and 300 mg/kg of aqueous extract of TPP. This experiment was carried out over a period of 14 days.

**Determination of triglycerides**
Triglycerides were assayed colorimetrically using a type autoanalyzer (BIOLIS24j) using the Triglyceride Reagent Kit (Buccolo and Harold, 1973). The reaction equation is as follows:

\[
\text{Glycerol} + \text{free fatty acid} \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acid}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{G3P} + \text{ADP}
\]

\[
\text{G3P} + \text{O}_2 \xrightarrow{\text{GPO}} \text{DAP} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-AF} + \text{p-chlorophenol} \xrightarrow{\text{POD}} \text{quinone} + \text{H}_2\text{O}
\]

The triglyceride level is determined at a wavelength of 500 nm. The intensity of the color formed is proportional to the triglycerides concentration present in the sample tested.

**Determination of total cholesterol**
Total cholesterol content was determined by an enzymatic colorimetric method according to Roeschlau and Allain (1974) using the Biocon Kit (Germany). To do this, cholesterol and esters were separated from lipoproteins using detergents (Triton X-100). The reaction equation is as follows:

\[
\text{Cholesterol} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxydase}} \text{Cholest-4-en-3-one + H}_2\text{O}
\]

\[
2\text{H}_3 + \text{HBA} + 4\text{-AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + 4H}_2\text{O}
\]

**HDL cholesterol assay**
LDL and VLDL cholesterol were assayed colorimetrically (Assmann et al., 1984) using a type autoanalyzer (BIOLIS24j) using the HDL Cholesterol Reagent Kit (Naito, 1984). Low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicron are precipitated by phosphotungstic acid (PTA) and magnesium chloride. After centrifugation at 4000 rev/min for 30 min at 20 °C, the recovered supernatant corresponds to HDL. The reading is taken at a wavelength of 505 nm.

**Determination of LDL cholesterol content**
LDL cholesterol content was determined by calculation using the formula of Friedwald et al. (1972).

\[
\text{LDL (mg/dL)} = \text{CT (mg/dL)} - [\text{HDL (mg/dL)} + \text{TG/5}] (\text{mg/dL})
\]

With VLDL = TG/5 and Total Fat = 2.56 x CT + 0.87 x TG

**Measurement of the atherogenicity index and coronary risk index**
- **Atherogenicity index** (Al) (Gupta et al., 2018) was determined by calculation according to the following formula:
**Coronary risk index** (CRI) (Guptha et al., 2018) was determined according to the formula below:

\[
AI = \frac{(Total \, cholesterol - HDL)}{HDL}
\]

**Statistical analysis**

Statistical tests were performed by performing a one-way analysis of variances (ANOVA) followed by a Newman-Keuls test at the 5% significance level. Statistica 7.1 software (StatSoft, Inc, USA) was used to perform the statistical analysis. Comparisons of means were performed by Newman-Keuls test at the 5% significance level. In addition, the Graph Pad Prism 5.1 software (Microsoft, USA) was used to construct the various figures of the results obtained.

**Results and Discussion:-**

**Results**

**Phytochemical composition of safou cake**

The results of Table 1 indicate the presence of secondary metabolites in the cakes of the pulp of the safou. These are polyphenols, flavonoids, alkaloids, sterols and terpenes, and saponins.

**Table 1:- Phytochemicals in the cake extract from the pulp of safou.**

| Phytochemicals            | Cake pulp |
|---------------------------|-----------|
| Polyterpènes et Stérols   | +         |
| Polyphenols               | +         |
| Flavonoïdes               | +         |
| Saponines                 | +         |
| Tanin catéchiques         | -         |
| Tanin galliques           | -         |
| Alcaloïdes                | +         |

-: absence of metabolites  
+: presence of metabolites

**Serum total cholesterol content**

Figure 3 shows the total serum cholesterol content of the rats. After gavage the rats with extracts of safou cake at doses of 100 and 300 mg / kg, for 14 days of treatment, the serum cholesterol content of the latter remained similar to that of normal rats. On the other hand, the serum cholesterol content of hypercholesterolemic rats not treated with safou extracts increased considerably compared to that of healthy rats.

**Serum triglyceride content**

The serum triglyceride content of the rats used for this study is shown in Figure 4. We note that the serum triglyceride content in the hypercholesterolemic rats, treated with the TPP extract at a dose of 100 mg / kg, is comparable than that of normal rats (p > 0.05). When the dose of reaches 300 mg / kg, we observe a significant decrease in serum triglyceride content of about -23% compared to Atorvastatin (-25%).

**Serum HDL-cholesterol content**

Figure 5 shows the serum HDL-cholesterol content. The results obtained show a significant increase in the plasma concentration of HDL-cholesterol of +27, +33, + 41% (p <0.05) respectively in the hypercholesterolemic rats treated with TPP (100 and 300 mg / kg). and Atorvastatin (1.8 mg / kg) after 14th day of treatment compared to positive controls (untreated rats).
Serum LDL cholesterol content
The LDL-cholesterol content is shown in Figure 6. After 14 days of treatment with atorvastatin in hypercholesterolemic rats, we observe a considerable decrease in serum LDL content of -60% compared to untreated hypercholesterolemic rats.

Serum VLDL-cholesterol content
Figure 7 indicates the serum VLDL-cholesterol content. The results showed that on day 14, the VLDL-cholesterol content was not significantly reduced in hypercholesterolemic rats treated with TPP extract at a dose of 100 mg / kg compared to negative controls. On the other hand, we note a significant reduction in the VLDL content compared to the positive controls (p <0.05). Likewise, atorvastatin and the aqueous extract of TPP taken at a dose of 300 mg / kg BW significantly decrease the serum VLDL content (-26% for Atorvastatin and -23% for the aqueous extract. TPP) compared to positive controls (p <0.05).

Serum total lipids content
The content of total lipids is presented by figure 8. At the 14th day of the treatment, the content of total lipids did not know any significant variation (p > 0.05) in the rats treated with the extract of TPP (100 and 300 mg/kg) compared to the negative witnesses (normal rats). On the other hand, the content of total lipids of the untreated rats significantly decreased (p< 0.05).

Figure 3: Serum total cholesterol content in control rats and hypercholesterolemic rats

Figure 4: Serum triglyceride content in control rats and hypercholesterolemic rats

Figure 5: Serum HDL-cholesterol content in control rats and hypercholesterolemic rats

Figure 6: Serum LDL-cholesterol content in control rats and hypercholesterolemic rats
Figure 7: Serum VLDL-Cholesterol content in control rats and hypercholesterolemic rats

The means not followed by the same lowercase letter are statistically different at a threshold of 5% (p < 0.05); (*): Significant difference between the rats rendered hypercholesterolemic treated or not and the normal rats on the 14th day; TN: Negative control, group of non-hypercholesterolemic rats (0.9% NaCl); TP: Positive control, group of untreated hypercholesterolemic rats (0.1 mg / kg of cholesterol BW); ATOR: Group of hypercholesterolemic rats treated with Atorvastatin (1.8 mg / kg BW); TPP100: Group of hypercholesterolemic rats treated with the aqueous extract of the pulp cake at a dose of 100 mg / kg BW. TPP300: Group of hypercholesterolemic rats treated with the aqueous extract of the pulp cake at a dose of 300 mg / kg BW.

Atherogenicity index

Figure 9 shows the evolution of the atherogenicity index in the treated and untreated rats. The results revealed that in the groups of hypercholesterolemic treated and untreated rats, the atherogenicity index was not significantly reduced (p > 0.05) compared to normal rats after administration of the extracts.

Coronary risk index

Evolution of the coronary risk index is shown in Figure 10. The results obtained showed that the coronary risk index was significantly reduced in hypercholesterolemic rats treated with the aqueous extract of TPP at a dose of 300 of 41% mg / kg versus -35% at 100 mg / kg compared to normal rats.

Figure 9: Evolution of the atherogenicity index in control rats and hypercholesterolemic rats

The means not followed by the same lowercase letter are statistically different at a threshold of 5% (p < 0.05); (*): Significant difference between the rats rendered hypercholesterolemic treated or not and the normal rats on the 14th day; TN: Negative control, group of non-hypercholesterolemic rats (0.9% NaCl); TP: Positive control, group of untreated hypercholesterolemic rats (0.1 mg / kg of cholesterol BW); ATOR: Group of hypercholesterolemic rats treated with Atorvastatin (1.8 mg / kg BW); TPP100: Group of hypercholesterolemic rats treated with the aqueous extract of the pulp cake at a dose of 100 mg / kg BW. TPP300: Group of hypercholesterolemic rats treated with the aqueous extract of the pulp cake at a dose of 300 mg / kg BW.
extract of the pulp cake at a dose of 100 mg / kg BW. TPP300: Group of hypercholesterolemic rats treated with the aqueous extract of the pulp cake at a dose of 300 mg / kg BW. AI: atherogenicity index; CRI: coronary risk index.

**Discussion:**

Phytochemical screening revealed the presence of secondary metabolites such as polyphenols, flavonoids, tannins, alkaloids, saponins, polyterpenes and sterols in the pulp cake. The presence of these phytonutrients in the pulp cake of safou could justify its therapeutic use. They are recognized as having antioxidant properties, which allows them to play a role in the inflammatory treatment, cardiovascular or neurodegenerative diseases (Pandey and Rizvi, 2009; Tsao, 2010). Most of these diseases find part of their cause in the effects produced by oxidative stress (Tsao, 2010; Nguene et al., 2016). For this, the pulp cake could intervene in the fight against this stress. As for saponins, they act like anti-hyperlipidemia, hypotensive and have cardiodepressive properties (Ogboru et al., 2015). In addition to the role of antimicrobial (Faizi et al., 2003), alkaloids play a detoxifying and antihypertensive role (Awoyinka et al, 2007).

The effect pulp cake of safou (TPP) was evaluated in hypercholesterolemic rats. Analysis of the results shows that the effect of the aqueous extract of TPP on blood lipid parameters is dose-dependent. As a result, the levels of VLDL-cholesterol, LDL-cholesterol, triglyceride (TG) and total serum lipids decrease with increasing dose, while those of HDL-cholesterol increase with increasing dose. Moreover, following the increase in the dose to 300 mg / kg of BW, the aqueous extract of TPP induced a significant decrease in the content of VLDL-cholesterol, LDL-cholesterol and of triglyceride while increasing the content of HDL-cholesterol (p < 0.05). The results obtained are comparable to those of work carried out by other authors on the hypcholesterolemic effect of palm kernel and olive cake. To this end, the studies carried out by Loh et al. (2002) showed that the administration of palm kernel cake extract reduced the content of triglyceride, total cholesterol and VLDL-cholesterol. According to Bouderbala et al. (2014; 2015), olive cake, compared to that of safou, would reduce on the one hand atherogenic lipoproteins (LDL, VLDL), total cholesterol and on the other hand would significantly increase HDL-cholesterol (p < 0.05). This cholesterol-lowering effect of TPP could be justified by the presence of bioactive compounds such as polyphenols, flavonoids, alkaloids, saponins, terpenes and sterols (Nguene et al., 2016).

Indeed, polyphenols are molecules which contribute to the reduction of cardiovascular conditions by reducing the absorption of cholesterol (Zern and Fernandez, 2005). This reduction in cholesterol absorption will result in decreased delivery of cholesterol to the liver by chylomicron. These, in turn, increase the expression of hepatic LDL receptor mRNA in order to compensate for substrate availability and induce reductions in plasma cholesterol (Zern and Fernandez, 2005). Indeed, polyphenols are molecules which contribute to the reduction of cardiovascular conditions by reducing the absorption of cholesterol (Zern and Fernandez, 2005). This reduction in cholesterol absorption will result in decreased delivery of cholesterol to the liver by chylomicron. These, in turn, increase the expression of hepatic LDL receptor mRNA in order to compensate for substrate availability and induce reductions in plasma cholesterol (Zern and Fernandez, 2005). In addition, saponins have a hypocholesterolemic power according to the mechanism according to which their actions are based on the decrease in the intestinal absorption of cholesterol by formation of micelles in the intestine, or they play a role in the absorption of fat (Wichtl and Anton, 2003). Regarding phytosterols, they have a structure very similar to that of cholesterol, which therefore competes with the latter for intestinal absorption (Serfaty-Lacrosniere et al., 2001). This absorption inhibition reduces the cholesterol content of intestinal chylomicron, modifies its transport to the liver via residual chylomicron, leads to an increase in hepatic LDL receptor activity and LDL clearance; which contributes to the drop in circulating LDL-Cholesterol levels (Cohn, 2010). Thus, a significant increase in the concentration of phytosterols leads to a decrease in the solubility of cholesterol and causes an increase in its precipitation and fecal excretion (Lecerf, 2006). Therefore, the administration of phytosterols increases the elimination of neutral sterols in the stool (Serfaty-Lacrosniere et al., 2001).

This study also found that administration of the TPP extract reduced the coronary risk index (p < 0.05). A significant decrease in the atherogenicity index compared to positive controls was also observed (p < 0.05). Some authors have reported that the atherogenicity index is a marker that helps prevent the risk of atherosclerosis and the coronary risk index, cardiovascular disease (Guptha et al., 2018). This protective effect is due to the polyphenol, flavonoid, phytosterol and saponin in the TPP extract (Abdelhali et al., 2018; Ganesan and Xu, 2017). In short, the bioactive compounds are believed to be the source of the cholesterol-lowering activity of safou cake. To do this, the cake could be recommended in the food and medicinal fields.
Conclusion:-
This study has shown that in addition to the presence of bioactive compounds, the cake and safou have cholesterol-lowering activity. The results showed that the safflower meal contains polyphenols, flavonoids, saponins, alkaloids, polyterpenes and sterols. It also emerges from the analyzes that the treatment of hypercholesterolemic rats showed that safou cake significantly decreases the content of VLDL-cholesterol, LDL-cholesterol, triglyceride (TG) and total serum lipids while increasing that of HDL-cholesterol. In combination and at useful doses, the constituents of the pulp meal could provide an alternative to synthetic molecules and have their place in the prevention and treatment of hypercholesterolemia.

Conflict of interests:
The authors declare that there is no conflict of interests

Contributions of the authors:
AARRA ensured the collection, handling experimental, the analysis of the data and the drafting of the manuscript. ENK, LOAA, and KJMD took part in the data analysis and the article correction. All the authors read and approved the final manuscript.

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