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# Antidiabetic Activity of Aqueous Extracts of *Laurus nobilis*, a Spice Used by Beninese Traditional Therapists

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**Abstract** All over the world, humans developed knowledge about the use of medicinal plants to treat all kinds of diseases. Spices were included. This work aimed to evaluate the hypoglycemic property *Laurus nobilis*, a spice used by traditional therapists in Benin for diabetes management. The aqueous extracts of the leaves of *Laurus nobilis* were obtained by maceration. The phytochemical screening was carried out. Acute oral toxicity tests by single dose gavage of 2000 mg / kg body weight were performed in female Wistar rats. The weight of the animals, the serum creatinine, the ALT transaminase and the hemoglobin level were determined on day 0 and then on day 14. For the efficacy test, the extract was administered to rats at a concentration of 300 mg / Kg of body weight for 21 consecutive days. The blood tests performed were blood sugar, triglycerides and cholesterol. Histological sections were taken from the liver, kidneys and spleen. The phytochemical screening revealed the presence of tannins, mucilages and flavonoids. There were no deaths of rats and their weights, blood creatinine, ALT transaminase, and hemoglobin levels did not vary significantly during the toxicity study, indicating no toxicity. Regarding the efficacy test, *Laurus nobilis* extract significantly lowered the mean blood sugar level in rats on day 14 and day 21 ($P < 0.05$) while the mean triglyceride and cholesterol levels total did not significantly change during the experiment. *Laurus nobilis* displayed hypoglycemic properties in non-diabetic rats and was not acutely toxic. It could be used in the management of diabetes.

***Keywords:*** *Laurus nobilis, hypoglycaemic, acute toxicity, Benin*

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## 1. Introduction

Humans developed the knowledge and use of medicinal plants to cure themselves and treat all kinds of diseases [1]. Knowledge of the therapeutic values of plants, long considered to be the virtues of nature, helped the population from the most disadvantaged to upper middle class people to free themselves from pathologies and ailments [2]. Nearly 80% of the world's population used medicinal plants not because they did not have access to prescribed drugs, but because these plants demonstrated their effectiveness [3,4]. The great variation in natural resources in Benin that characterized the flora that were medicinal plants consisted of traditional leafy vegetables, spicy and aromatic plants and many other species of variable classification [5,6,7].

Despite the great scientific progress in the development of modern medicines, traditional medicine remained a recourse par excellence for rural populations because of its effectiveness and its power to relieve suffering people. In addition, the very high price of modern drugs, the side effects of synthetic drugs that could create other public health problems, forced people to turn to medicinal plants [8]. However, phytochemical screening revealed the

presence of very toxic substances in plant extracts which are likely to cause death [9]. Traditional plants can be toxic to essential human organs including the liver and kidneys. This therefore suggests a control of their harmlessness for a safe use for the treatments [10,11,12,13].

Among the natural bioactive substances that participate in the many virtues of plant drugs, were tannins, reducing compounds, coumarins, mucilages [14,15]. They were found in aromatic plants and spices which constitute the bulk of the therapeutic arsenal used by traditional healers [7]. The present study was initiated with the aim of enhancing the spices of the Beninese pharmacopoeia, in particular *Laurus nobilis*, by studying its hypoglycemic property and its safety in female rats of wistar strains.

## 2. Material and Methods

### 2.1. Plant Material and Aqueous Extraction

The leaves of *Laurus nobilis* were collected in Porto-Novo. They were dried in the laboratory at room temperature (20° - 25°C) away from sunlight and moisture for 14 days. They were then reduced to powder and stored in suitable bottles. The technique used to prepare the extracts was that of maceration. After filtration, the extracts were evaporated to dryness at 60°C using a Heidolph type rotary evaporator [16,17,18].

### 2.2. Ethics Statement

The study was approved by the National Research Ethic review Boards of Benin.

The Wistar rats used in this study were handled according to the institutional animal safety guidelines (Animal facility, National School of Applied Biosciences and Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics, Benin).

### 2.3. Animal Specimens, Growth and Feeding Conditions

The animal material was composed of sixteen (16) strains albino Wistar female rats from the animal house of IBSA whose mean weight is from 113 to 163 g. These rats were acclimatized to ambient conditions in the animal house of the laboratory of the National School of Applied Biosciences and Biotechnologies in Benin. They had access to water and food. They were lit for 12 hours a day and have been put in spacious cages. The cage was cleaned regularly and the water was renewed very often. The behavior of the animals was observed during the two weeks of acclimatization.

### 2.4. Identification of Secondary Metabolites

The metabolites were identified by coloring and precipitation reactions specific to each metabolite family [16,19,20].

### 2.5. Acute Oral Toxicity

An acute toxicity test (AOT) was performed as recommended by the Organization for Economic Co-operation and Development guideline 423 for the testing of chemicals [18]. Two groups of rats were formed for, namely the control group and the test group. Each group consists of three female wistar rats. Each animal in the control group received by force gavage and in a single dose of distilled water and the animals in the test group received by force gavage and in a single dose 2000 mg / kg body weight of the aqueous extract of *Laurus nobilis*. Animals were observed carefully for four hours and then daily for 14 days. They were weighed and the blood was collected by orbital puncture at the start of the experiment and then after 14 days [19,22].

The following blood tests were performed. Serum creatinine for the exploration of kidney function. ALT was assayed for hepatic function. The hemoglobin level was determined [19,22,23].

### 2.6. Histology

At the end of the experiment, the animals were dissected. The liver, the kidney and the spleen were removed, fixed in 10% buffered formalin, and embedded in paraffin. The specimens sections (5 µm) were mounted on glass slides, deparaffinated, and hydrated. For histological analysis, sections were stained with hematoxylin and eosin (H&E), following a standard protocol [24]. The pictures were taken at 400X magnification.

### 2.7. Evaluation of the Effectiveness of the Extract

Five Wistar rats were force-fed the aqueous extract of *Laurus nobilis* at 300 mg / kg body weight, daily for 21 consecutive days. They were weighed and blood was taken by orbital puncture at the start of the experiment (D0), on day fourteenth (D14) and then on day twenty-one (D21). The blood tests performed are blood sugar, triglycerides and cholesterol [25].

### 2.8. Statistical Analysis

The results of the biological parameters were expressed as the mean ± 2 times the standard error on the mean (Mean ± 2 SEM). For each parameter the results of days 14 or days 21 are compared with those of Day 0 using the Dunn's multi-comparison test or Mann Whitney test. The significance level was set at 5%. The graphs were drawn using Graphpad software.

## 3. Results

### 3.1. *Laurus nobilis* Gave Good Yield in Aqueous Extraction

Table 1 showed the extraction yield. The yield was 30.55±2.93%. The plant was therefore rich in polar compounds.

**Table 1. Extraction yield**

| spice | yield |
|---|---|
| | Aqueous extract of leaves |
| *Laurus nobilis* | 30,55±2,93% |

### 3.2. *Laurus nobilis* Leaves Contained a Lot of Metabolites

Table 2 showed the phytochemical screening of *Laurus nobilis*. The screening revealed the presence in the leaves of *Laurus nobilis* of catechic tannins, flavonoids, leuco-anthocyanins, alkaloids, muscular tissues, steroids, free antracenics, C-heterosides and O-heterosides. Also the absence of gallic tannins, anthocyanins, reducing compounds, saponosides, quinone derivatives, cyanogenic derivatives, triterpenes, coumarins and cardiotonic derivatives was noted.

**Table 2. Phytochemical screening of *Laurus nobilis***

| CHEMICAL GROUPS | *Presence* |
|---|---|
| Tanins catéchiques | + |
| Tanins galliques | - |
| Flavonoïdes | + |
| Leuco-Anthocyanes | + |
| Anthocyanes | - |
| Alcaloïdes | + |
| Composés réducteurs | - |
| Mucilage | + |
| Saponoside | - |
| Dérivés cyanogéniques | - |
| Triterpènes | - |
| Stéroides | + |
| Coumarines | - |
| Dérivés quinoniques | - |
| Antracéniques libres | + |
| C-Hétérosides | + |
| O-Hétérosides | + |
| Dérivés cardiotoniques | - |

+ : presence, -: Absence.

### 3.3. The Aqueous Extract of *Lauris nobilis* is not Acutely Toxic

Table 3 showed the results of the physical, biochemical and hematological assessments of the acute oral toxicity test of the extracts of *Laurus nobilis*.

The average weight was 130 ± 27.6 g in rats on D0 and increased to 158 ± 24.8 g on D14 after treatment with 2000 mg of *Laurus nobilis* extract / kg of weight. However, this increase was not statistically significant, indicating an absence of physical disturbance to the rats.

The mean creatinine level was 7.80 ± 2.2 mg / mL in rats on D0 and 5.13 ± 1.354 mg / mL after treatment with 2000 mg of *Laurus nobilis* extract / kg of weight to D14. The mean creatinine level did not change significantly after treatment, indicating an absence of renal dysfunction in the rats.

The mean level of ALAT transaminase was 32.0 ± 3.04 U / L in rats on D0 and 37.3 ± 3.52 U / L after treatment with 2000 mg of *Laurus nobilis* extract / Kg weight at D14. The mean level of ALT did not significantly increase after treatment, suggesting an absence of hepatic cytolysis.

The hemoglobin level was 12.4 ± 0.53 g / dL in rats on D0 and 13.2 ± 0.334 g / dL in rats after treatment with 2000 mg of *Laurus nobilis* extract / Kg of weight at J14. The hemoglobin level did not drop significantly after treatment, indicating that the extract did not induce anemia.

Figure 1 showed renal, hepatic and splenic histology in the acute oral toxicity test.

**Table 3. Physical, biochemical and hematological results in the acute oral toxicity test of extracts of *Laurus nobilis***

| Parameters | Moyenne à J0 | Moyenne à J14 | P-value | Différence |
|---|---|---|---|---|
| **Weight (g)** | 130 ± 27,6 | 158 ± 24,8 | 0,40 | Non significative |
| **Creatinine (mg/L)** | 7,80 ± 2,2 | 5,13± 1,354 | 0,20 | Non significative |
| **Transaminase ALT (U / L)** | 32,0± 3,04 | 37,3 ± 3,52 | 0,20 | Non significative |
| **Hemoglobin level (g/dL)** | 12,4± 0,53 | 13,2±0,334 | 0,10 | Non significative |

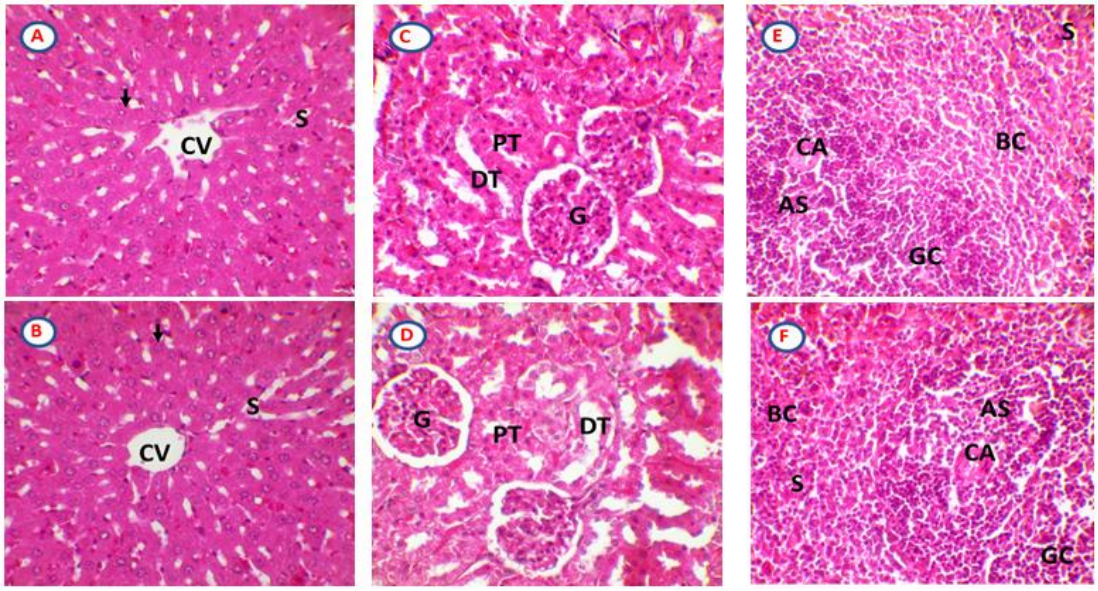


**Figure 1.** Renal, hepatic and splenic histology in the acute oral toxicity test

The liver of the rats did not show atypia on the acute oral toxicity test (Figure 1B). The hepatocytes (arrows) were neatly arranged in cords separated by the venous sinusoids (S) as in the controls (Figure 1A). Centrilobular (CV) veins were characteristic.

The renal parenchyma of the rats in the acute oral toxicity test (Figure 1D) kept the normal architecture of the controls (Figure 1C). The glomeruli (G), proximal (PT) and distal (DT) tubules did not show atypia.

The splenic architecture of the rats was not changed in the acute oral toxicity test (Figure 1F). In the white pulp, the periarteriolar sleeves (AS) around the central arterioles (CA) and the germinal centers (GC) were characteristic as in the control rats (Figure 1E). The same in the red pulp where venous sinusoids and Bilroth's cords were typical.

### 3.4. Laurus Nobilis Efficacy Test

Figure 2 showed the effect of *Laurus nobilis* extract on blood sugar.

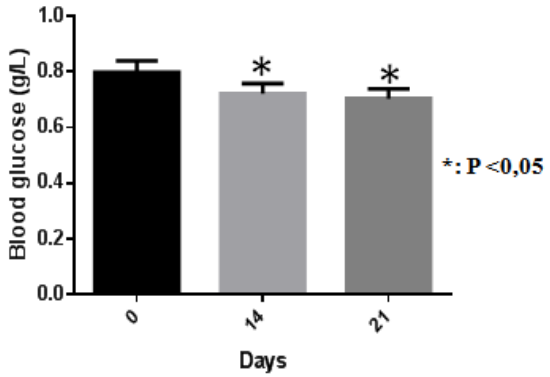


**Figure 2.** The mean blood glucose level as a function of time in rats treated with *Laurus nobilis*

The average blood sugar level is 0.80 ± 0.04 g / L on day zero (D0); 0.72 ± 0.03 on day fourteen (D14) and 0.70 ± 0.04 g / L on day twenty one (D21) in rats treated with 300 mg of *Laurus nobilis* extract / Kg / D. Compared to day 0, the mean blood glucose level decreased significantly in rats on day 14 and day 21 (P <0.05).

Figure 3 showed the effect of *Laurus nobilis* extract on triglyceridemia.

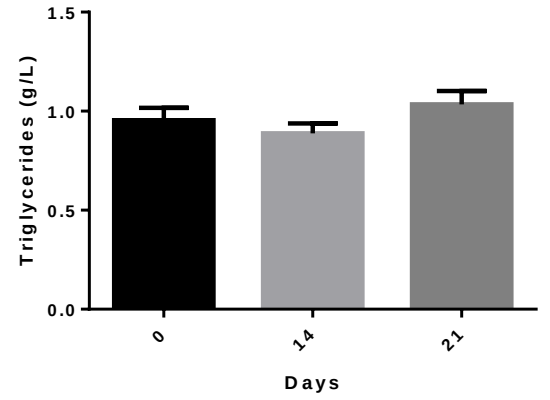


**Figure 3.** The mean triglyceride level as a function of time in rats treated with *Laurus nobilis*

The average triglyceride level was 0.95 ± 0.06 g / L on day zero (D0); 0.89 ± 0.04 on day fourteen (D14) and 1.03 ± 0.06 g / L on day twenty one (D21) in rats treated with 300 mg of *Laurus nobilis* extract / Kg / D. Compared to day 0, the mean triglyceride level did not change significantly in the rats treated on D14 and D21.

Figure 4 showed the effect of *Laurus nobilis* extract on total cholesterol

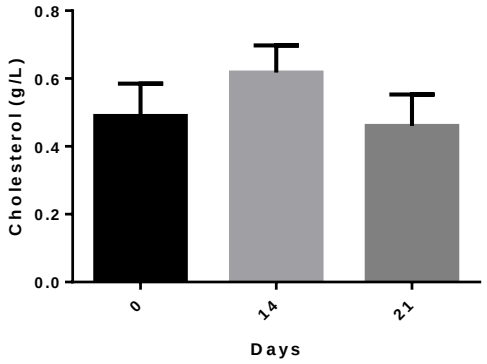


**Figure 4.** The mean total cholesterol level over time in rats treated with *Laurus nobilis*

The mean total cholesterol level was 0.49 ± 0.08 g / L on day zero (D0); 0.62 ± 0.07 on day fourteen (D14) and 0.46 ± 0.08 g / L on day twenty one (D21) in rats treated with 300 mg of *Laurus nobilis* extract / Kg / D. Compared to day 0, the mean total cholesterol level increased insignificantly on day 14 in rats treated with *Laurus nobilis.*

## 4. Discussion

The phytochemical screening of *Laurus nobilis* revealed the presence of alkaloid, flavonoid, tannin. These results are similar to those [26] except for the presence of leucoanthocyanins, mucilage, steroids, free and complex anthracene noted in our work. The presence of these numerous metabolites would justify its use in traditional medicine.

With respect to toxicity, there were no deaths in acute oral toxicity testing with *Laurus nobilis* extract. In addition, serum creatinine, serum ALT transaminases, and hemoglobin did not significantly change from start to end of the experience with acute oral toxicity. Therefore, *Laurus nobilis* extract was not toxic to the liver and kidneys. These results were confirmed by histological sections showing the absence of hepatic, renal lesions and splenic activation. This would be due to the presence of secondary metabolites which offers protection to these different organs. Similar results were reported by [26] who established the safety of *Laurus nobilis*. Moreover, similar observations have been made by other authors when studying the oral toxicity of other plants including *Psorospermum febrifugum* [22] and *Cocos nucifera* [19] which were plants used against anemia.

Regarding efficacy, *Laurus nobilis* extract significantly lowered blood sugar levels throughout the experiment. This decrease in blood sugar levels proved that *Laurus nobilis* displayed hypoglycaemic activity. These results were similar to those reported [27] who evaluated the antidiabetic activity of *Laurus nobilis* leaves in streptozotocin-induced diabetic rats. It should be noted that this plant displayed a hypoglycemic effect even on

non-diabetic rats, indicating that it showed a powerful hypoglycemic activity.

In addition, it was noted that the triglyceride and cholesterol level of the rats treated with *Laurus nobilis* did not vary. The action of this spice did not appear to interfere with these biochemical parameters. These results were contrary to those [27] who reported a decrease in triglyceride and cholesterol levels in rats treated with *Laurus nobilis*. However, he used diabetic rats which differs from our model.

## 5. Conclusion

Plants were a great chemical factory which should be taken advantage of in the treatment of disease. The presence of secondary metabolites with therapeutic effect was noted in the leaves of *Laurus nobilis*. The work revealed that the aqueous extract of the leaves of *Laurus nobilis* was not toxic to the liver and the kidneys and it displayed a hypoglycemic property. This spice could therefore be considered in the treatment of diabetes without risk to health. However, additional work, particularly chronic toxicity, should be done to confirm this harmlessness.

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