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Hypoglycemic Activity of Hydro-Acetonic and Hydro-Methanolic Leaf and Bark Extracts of *Bauhinia rufescens* Lam in Mice

Lahat Niang
Water, Energy, Environment and Industrial Processes Laboratory (LE3PI), at the Polytechnic School of Cheikh Anta Diop University of Dakar, Senegal
Mahamat Seid Ali
Faculty of Sciences and Techniques,
Adam Barka University (UNABA), Abeche, Tchad
Papa Guédel Faye
Nicolas Cyrille Ayessou
Mady Cisse
Water, Energy, Environment and Industrial Processes Laboratory (LE3PI), at the Polytechnic School of Cheikh Anta Diop University of Dakar, Senegal

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Abstract

The purpose of this study was to determine the hypoglycaemic effects of leaf and bark extracts of Bauhinia rufescens Lam, used in traditional medicine for diabetes in Senegal. Phytochemical screening of hydromethanolic and hydro-acetonic extracts obtained from leaves and barks was carried out according to standard analytical methods. Experiments were carried out on normoglycemic and diabetic mice. These were obtained after the injection of a single dose of glucose (4 g/kg of body weight). The results showed that polyphenolic compounds, tannins, and flavonoids were the main phytochemical constituents of the extracts. Then the oral administration of the different extracts of Bauhinia rufescens Lam at doses of 500, 700, and 1000 mg/kg of body weight led to a significant reduction in blood sugar ($p < 0.05$),

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similar to the antidiabetic action of glibenclamide (10 mg/kg of body weight). Whatever the organ and the extraction solvent, the doses of 500 and 700 mg/kg of body weight induced dose-dependent hypoglycemia, just like glibenclamide. These results show that the metabolites revealed in the extracts could be responsible for their hypoglycemic effects. Thus, supported by a complete chemical study with a view to isolating the active substances responsible for the antidiabetic effect is possible. It will be allowed to formulate from a phytomedicine with potential for diabetes which makes possible their uses by populations for treatment of diabetes.

Keywords: *Bauhinia rufescens* Lam, hypoglycemia activities, glibenclamide

Introduction

Plants are often used to fight against diseases linked to free radicals such as diabetes, cancer, and asthma (Halliwell, 2018) and degenerative diseases (Codonier-Franch *and al*, 2010). Diabetes is the most common endocrine disorder and the fastest-growing metabolic disorder in the world with an average annual growth rate of 1-2% (Wild, 2004). Therapeutic molecule uses, including insulin and oral hypoglycemic agents (biguanides, sulfonylureas), their regular administration generates adverse effects (Nissen and Wolski, 2007). The high cost of medicines, the inaccessibility of health care, and the effectiveness and availability of medicinal plants are pushing populations towards herbal medicine (Lu *and al*, 2011; Sen and Chakraborty, 2011). In Senegal, several plants used in traditional medicine for the treatment of diabetes such as *Bauhinia rufescens* have been listed (Niang, 2022), but not yet scientifically elucidated. In this context, we are interested to assess an glycemic activity of hydro-acetonic and hydro-methanolic extracts leaves and bark of *Bauhinia rufescens* Lam. This has been achieved in order to complete information and to project possible applications.

Material and Methods

Collection and Identification of samples

Leaves and trunk barks of *B. rufescens*, were collected in June 2019, in the region of Diourbel (central Senegal). The samples were identified and authenticated at the Laboratoire de Botany-Biodiversity of the Faculty of Sciences and Technology of Cheikh Anta Diop University. After washing with distilled water and oven drying at 50°C in the laboratory for two weeks, the leaves and barks were pulverized with an electric grinder (Kenwood, France). Thus, the sieved powders with a mesh size of 1mm, are then extracted with Soxhlet on a ratio of 10 g/100 mL of solvent.

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Extraction

The extraction is performed with 70% (v/v) hydromethanol, (99.98%, Scharlau Chemie S.A, SPAIN) and 70% (v/v) hydro-acetone, (99.5%, Scharlab S.L, SPAIN) for two hours. After cooling, the mixture is clarified in a centrifuge (Hittich, Universal 16A, France) at 3000 rpm for 10 minutes then filtered under vacuum on Wattman No.1 paper. In the extracts, the traces of solvent are removed using a rotary evaporator (IKA® RV10 digital, France). The extracts are stored hermetically in sealed glass bottles at 4°C.

Phytochemical Screening

Standard phytochemical analysis methods were used to test for the presence of phytoconstituents in the extracts. Chemical tests were carried out on hydro-acetone and hydro-methanolic extracts using standard methods for the detection of tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's test according to Karumi *and al*, 2004), steroids, and triterpenoids (Liebermann-Buchard test according to Miliauskas *and al.*, 2004) alkaloïds (Valser-Mayer and Dragendorff's Reagents tests), saponins (foaming index) (WHO, 2013).

Experimental animals

Juvenile male Wistar albino mice from the Laboratory animal facility of Institut Pasteur of Dakar; weighing 18-34 g were used for *in vivo* studies of the glycemic activity of the extracts. The animals were kept in well aerated laboratory cages into groups of five mice in this same animal house and were allowed to acclimatize to the laboratory conditions for 2 weeks. The animals were maintained on standard animal feeds and drinking water *ad libitum* during the stabilization period. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally.

Treatment with plant extract and technical collection of blood

The mice are fasted for 12 hours before administration of the substances in milligrams per kilogram of body weight (b.w) orally using a loaded syringe. The sample is taken from the tail vein of the animal in order to have a drop of blood sufficient to determine the blood sugar level. The latter is measured using an Accu-Cheik Active glucometer and test strips.

Treatment of normoglycemic mice with plant extract and glibenclamide

The guinea pigs are divided into 4 groups of 6 mice and 2 groups of 2 mice respectively for the extracts and the controls. Group 1 (negative control) receives distilled water (ED) at a dose of 10 mg/kg b.w. Commercially available Glibenclamide tablets were dissolved in distilled water and

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administered orally to the mice at 10 mg/kg b.w which served as a positive control (Sharma *and al*, 2001). The hydro-methanolic extract leaf (EMF) and bark (EME), and hydro-acetonic extract of the leaf (EAF) and bark (EAE) were administered at doses of 500, 700, and 1000 mg/kg b.w.

Treatment of hyperglycemic mice with plant extract and glibenclamide

Diabetes mellitus was induced by oral administration of glucose to mice at a dose of 4 g/kg b.w (Ndoua *et al.*, 2015). Mice were divided into 4 groups of 6 mice and 3 groups of 2 mice respectively for extracts and controls. The different groups of mice received extracts, distilled water, and glibenclamide at the same doses as the normal glycemic mice. Thus, the glycaemia of the guinea pigs of each batch is measured before the administration of the substances or the distilled water then, after the treatment, at intervals of 30 minutes, for 2 hours and 30 minutes. The percentage of induction of hyperglycemia and the percentage of reduction of induced hyperglycemia of the animals are then calculated.

Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) with STATISTICA 7.1 to determine the significance. Means and standard errors (SE) of all data were calculated based on three replicates (n = 3). Comparisons among means were carried out using the Tukey HSD test at a significance level of $P < 0.05$.

Results

Phytochemical screening

Phytochemical screening of *Bauhinia rufescens* Lam extracts revealed a high presence of polyphenols, tannins, flavonoids, and traces of saponosides and Sterols-Terpenes but there is a low alkaloid content in the leaves and bark of the plant, the results of which are given in table 1.

Table 1. Results of phytochemical screening of *B. rufescens* Lam extracts

Plant species		Alkaloids RM	Alkaloids RD	Flavonoids	Tannins	Saponosides	Sterols Terpenes
B.R. Lam	**Fe**	-	+	++	++	+	+
	E.T	-	+	++	+	+	++

Hypoglycemic activity of extracts in normoglycemic mice

Glycemia results show that the administration of hydro-methanolic extracts of leaves and bark caused a significant reduction in the blood sugar levels of normoglycemic mice (Figure 2). Discount percentage in glycemia of mice varies according to the extraction solvent and the organ of the plant studied. In fact, the doses of 500 and 700 mg/kg b.w. give the best reduction

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percentage with values varying respectively from 18.56 to 42.27% (barks) and 7.22 to 45.36% (leaves) within 2.5 hours after administration. The results reveal that the lowest percentage of reduction is obtained with the dose of 1000 mg/kg b.w. Distilled water (negative control) causes a gradual decrease in blood sugar with no significant difference. However, glibenclamide (positive control) reduces 76.04% of glycemia 2.5 hours after administration and 51.04% after 30 minutes.

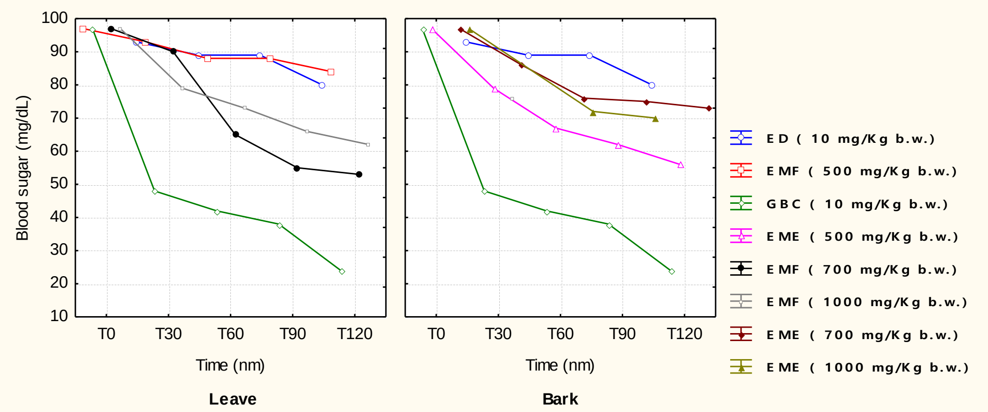

Figure 2. Evolution of glycemia in normoglycemic mice treated with hydro-methanolic extracts of *B. rufescens* Lam

As with the hydro-methanolic extract, hydro-acetone extract of leaves and bark significantly reduced the glycemia (Figure 3) like glibenclamide at 30 minutes after administration. The reduction percentage in glycaemia of mice varies according to the extraction solvent and the organ of *B. rufescens* studied. Thus, the best percentage of glycemia reduction is obtained with the dose of 700 mg/kg b.w. with leaves extract (21.43 to 40.82%) and barks's extract (21.02 to 38.06%) after administration after 30 minutes. Under the same conditions, the percentage reduction obtained with glibenclamide changes from 50 to 74.89%.

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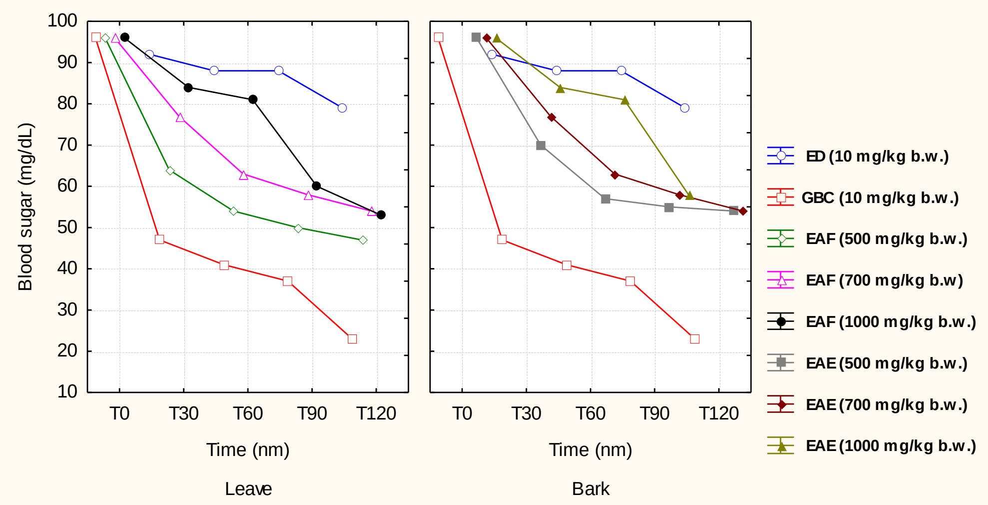

Figure 3. Evolution of glycemia in normoglycemic mice treated with hydro-acetonic extract of *B. rufescens* Lam

Hypoglycemic activity of extracts in hyperglycemic mice

The administration of glucose at 4 g/kg b.w. led to hyperglycemia in mice with a level up to 141 mg/dL. The hydro-methanolic of leaves and barks extracts administered at doses of 500, 700, and 1000 mg/kg b.w. significantly reduced the glycaemia of mice 30 minutes after induction. (Figure 4). Positive control with Glibenclamide at a dose of 10 mg/kg of body weight reduced the glycemia of mice up to a rate of 33 mg/dL, corresponding to a percentage reduction of 76.60% after 2 hours. This decrease evolves with the extracts throughout the duration of the study to give levels of 60 mg/dL (500 mg/kg b.w.), 59 mg/dL (700 mg/kg b.w.), and 105 mg/dL (1000 mg/kg b.w.). The best reduction percentage is obtained with the dose of 700 mg/kg b.w. whatever the organ 50.34% (leaves) and 72.41% (bark) within 2 hours. The latter is close to that of the positive control (75.86%), unlike distilled water whose reduction rate remains low compared to the extracts.

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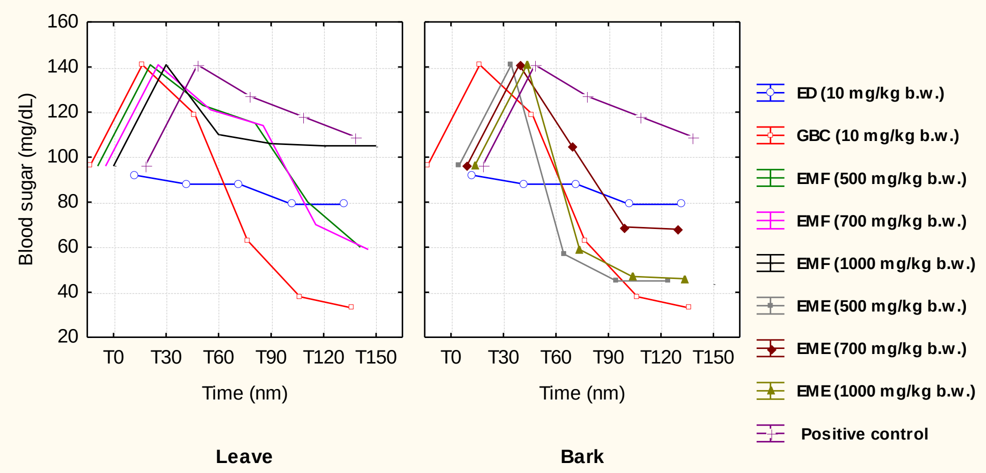

Figure 4. Evolution of glycemia in hyperglycemic mice treated with hydro-methanolic extract of *B. rufescens* Lam

The administration of different doses of acetone extracts (500, 700, and 1000 mg/kg b.w.) and of glibenclamide at 10 mg/kg body weight 30 min after hyperglycaemia of the animals (142 mg/dL), significantly modifies ($p > 0.05$) blood glucose (Figure 5). However, distilled water (control) does not cause any significant change in blood sugar levels in animals. The percentages of reduction of hyperglycemia induced by glucose with the extracts show that the doses 500 and 700 mg/kg b.w. have more effect of reduction on the blood glycemia of the hyperglycemic mice are 68.97% (24 mg/ dL; leaves) and 72.41% (14mg/dL; bark) respectively 2 hours after administration. These values are close to those of glibenclamide (reference substance) which shows a maximum reduction of 75.86% (79 mg/dL) after 2 hours.

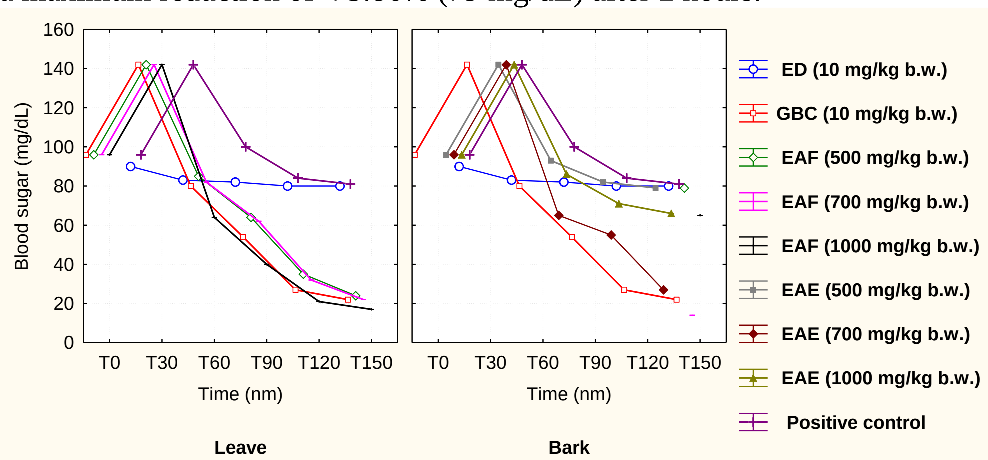

Figure 5. Evolution of glycemia in hyperglycemic mice treated with hydro-acetonic extract of *B. rufescens* Lam

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Discussion

Phytochemical screening of various extracts from leaf and bark revealed the presence of secondary metabolites such as tannins, flavonoids, and alkaloids. These metabolites were known to show medicinal activities as well as exhibiting physiological activities (Sofowora, 1993). The presence of these phytochemical compounds in the plant materials suggests the potential medicinal value of their extracts in the prevention and/or cure of specific diseases (Razyid *et al.*, 2020). The presence of these metabolites is revealed in the ethanolic extracts bark with the presence of quinones (Chaibou et *al*., 2020) and also in the methanol stem bark extract of *Bauhinia rufescens* Lam (Usman et *al*., 2009; Agush et al., 2013). These metabolites are also revealed in *Sclerocarya birrea* (Niang et *al*., 2021), *Sebastiania chamaelea* (L.) (Mamadou et *al*., 2014), and *Emilia sonchifolia* (L) (Essien et *al*., 2020). These compounds are frequently encountered in plant extracts such as *Ziziphus mauritiana* Lam, (Folié et *al*., 2017) *Bauhinia purpurea* L. and *Bauhinia variegata* L. (Pallavi et *al*., 2020). These metabolites, particularly phenols, and flavonoids, are the main and most common components of plants with antioxidant activity (Khajapeer et *al.*, 2018).

Oral administration of glucose (4 g/kg b.w.) leads to hyperglycaemia in mice, then a gradual return to the initial value. Under the same experimental condition, methanolic and acetonic extracts at doses of 500, 700, and 1000 mg/kg b.w. of *B. rufescens* Lam reduce in a dose-dependent the glycemia of normal mice and the hyperglycemia induced by glucose in the same range with the positive control. In this study, it was observed in the methanolic extracts that the leaves have greater hypoglycemic activity than the bark in normoglycemic mice. However, the barks have a more reducing effect than the leaves with the acetonic extracts on the glycemia of the animals. Under the same experimental conditions, methanolic and acetonic extracts of *B. rufescens* Lam also leads to a dose-dependent reduction in the glycemia of normal mice and glucose-induced hyperglycemia. This hypoglycemic activity was observed with methanolic leaf extracts at a dose of 400 mg/kg b.w. (Aguh et *al.*, 2013). These results show that *B. rufescens* Lam has antidiabetic activity. Thus, this hypoglycemic effect could be explained by a stimulation of insulin secretion by the pancreas (Jackson and Bressler, 1981) and/or, probably, by an increase in peripheral glucose utilization (Yasodha et *al*., 2008). In addition, the use of the leaves of *B.* rufescens Lam can be done in the traditional way with moderation by dietary habits to avoid any toxic effects from excess. In fact, the doses administered (500, 700, and 5000 mg/kg CP) during these experiences did not cause any mortality in the treated mice.

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Conclusion

Exploiting the biological potential of plant species is of major interest. The present study has demonstrated the hypoglycemic activity of hydro-acetonic and hydro-methanolic extracts leaves and bark of *Bauhinia rufescens* Lam. Results confirm that the two organs of *Bauhinia rufescens* Lam possess bioactive molecules endowed with therapeutic properties such as antioxidant and antidiabetic. Thus, *Bauhinia rufescens* Lam could therefore contribute to the management of diabetes. These results support, indeed, the motivation for the recourse to traditional medicine. From this perspective, a complete chemical study might be carried out in order to isolate the active substances and to elucidate their chemical structures and mechanism of action.

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