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Characterization of Bioactive Compounds in *Tamarindus indica* L. (Voamadilo) and Evaluation of its Anti-hyperglycaemic effects

Ralaivaon-dratsitonta Jumaël Edith Fabrice [2, 4, *], Andrianarijaona Mamy [2], Andrianjato Tartin [1], Robijaona Rahelivololona Baholy [3], Fiatoa Barthélemy [4] and Fatiany Pierre Ruphin [2]

[1] *Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo, Antananarivo 101 Madagascar.*
[2] *Geosciences, Physics, Environmental Chemistry and High Pathogenic System Doctoral School (GPCEHP), University of Toliara, Toliara 601 Madagascar.*
[3] *Polytechnic High School of Antananarivo, University of Antananarivo, Antananarivo 101 Madagascar.*
[4] *Androy Regional University Center (CURA), University of Toliara, Toliara 601 Madagascar.*

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Abstract

This study was conducted to investigate the chemical constituents of *Tamarindus indica L.* and assess its anti-hyperglycaemic activity. The chemical compounds from the hexane and ethyl acetate extracts of the leaves were separated using various chromatographic techniques. Their structures were then elucidated using spectroscopic methods, including nuclear magnetic resonance (NMR). During this investigation, two main compounds were identified: β-sitosterol and Campesterol. The anti-hyperglycemic activity of the ethanolic extracts from the *Tamarindus indica L.* leaves was assessed in glucose-loaded normal rabbits. At doses of 100, 250, and 500 mg/kg, these ethanolic extracts significantly ($p < 0.05$) lowered the rise in blood glucose compared to the control group. These findings are in line with the traditional use of this plant in folk medicine.

Keywords: Anti-Hyperglycemic; Chemical Compounds; Fabaceae; *Tamarindus indica L.* And NMR

1. Introduction

Diabetes is one of the most widespread diseases in the world. As of 2021, approximately 537 million people were living with diabetes globally. That same year, an estimated 6.7 million deaths were attributed to the disease and its complications. Over 80% of these deaths occurred in low- and middle-income countries. Projections by the WHO and the International Diabetes Federation (IDF) predict the number of people with diabetes will climb to 643 million by 2030, establishing it as a leading cause of mortality worldwide. [1,2] The resolution of this public health issue in Madagascar requires the full utilization of medicinal plants. These natural resources, exceptionally abundant within the country's local forests, have repeatedly demonstrated their remarkable efficacy. Many studies are being directed to find anti-diabetic agents from natural sources. The leaves of *Tamarindus indica L.* known as "Voamadilo" are used in traditional medicine to treat diabetes and proteinuria.[3] The plant is known to possess various bioactive compounds, including sterols, which have been linked to anti-hyperglycaemic properties in other plants. In this work, the isolation and identification of the chemical constituents from hexane and AcOEt extracts of the leaves and the acute antidiabetic activity of the ethanolic extracts of leaves of *Tamarindus indica L.* were studied.[4]

* Corresponding author: Ralaivaon-dratsitonta Jumaël Edith Fabrice

2. Materials and Methods

2.1. Plant Material

The leaves of *Tamarindus indica L*. were collected from the Menabe Region in the western part of Madagascar in 2024. The identification of the species was identified at the Department of Botany at the Botanical and Zoological Park of Tsimbazaza (Antananarivo).

A voucher specimen was deposited in the Geosciences, *Physics, Environmental Chemistry and High Pathogenic System Doctoral School (GPCEHP),* University of Toliara and Analytical Chemistry and Formulation Laboratory, Faculty of Sciences, University of Antananarivo at the for future references.

2.2. General experimental procedures

All NMR experiments were conducted on a Bruker 600 NMR spectrometer. 1D spectra (^{1}H, ^{13}C) and 2D spectra (^{1}H-^{13}C HSQC, HMBC) were acquired at 600.19 MHz for proton and 125.78 MHz for carbon. The solvent used was $CDCl_3$, with TMS serving as the internal standard. For chromatographic separation, column chromatography (CC) was performed using Merck's silica gel F_{254}. Thin layer chromatography (TLC) was conducted on precoated Merck silica 60F254 plates. The separated compounds were visualized either under UV light or by spraying the plates with vanillin in H_2SO_4.

2.3. Animals

Animals were only used once and in accordance with the ethical guidelines for the care of laboratory animals.

For the toxicity studies, adult female Swiss mice (Swiss/Webster) were used, weighing between 20 and 30 g. They were sourced from IMVAVET (Institut Malgache des Vaccins Vétérinaires) and spent one week acclimatizing to the laboratory environment prior to the experiments. These mice were fasted overnight before each test but were given free access to water.

Healthy adult New Zealand White rabbits were selected for the anti-hyperglycemic tests. The male rabbits weighed approximately 2.8–4.0 kg, while the females weighed around 3.2–5.0 kg. Before testing, they were subjected to a 12-hour fast, during which they had unrestricted access to water.

2.4. Extraction

Dried leaves of *Tamarindus indica L*. were reduced to a fine powder using a mechanical grinder. The powder of leaves (500 g) was extracted by maceration with a mixture of Ethanol-water (80:20) 2000 ml) for 10 days, and concentrated to dryness under vacuum at a low pressure and low temperature of 60°C to give ethanolic extracts of leaves.

The powder of leaves (500 g) partitioned successively with ethyl acetate, methanol and hexane, by maceration to give respectively ethyl acetate, methanolic and hexane extracts.

2.5. Biological activity

2.5.1. Investigation of acute toxicity

The procedure followed the method described by Onifade et al. (2011). [6] The mice were randomly assigned to a control or treatment groups (3 animals per group). Ethanolic extracts of leaves and bark were tested separately at a dose of 250, 500 and 1000 mg/kg each. Extracts were given orally to test groups, while the control group received distilled water at the same volume (10 ml/kg b.w.). After administration, the animals were observed continuously for six hours on the first day and then monitored once daily for the next three days.

2.5.2. Anti-hyperglycaemic activity

The experiment was carried out following the method of Keti'a et *al*. (1998). Rabbits fasted overnight were randomly separated into five groups of three animals each. A single oral dose was given using a stomach tube. Group 1 was the normal control and received distilled water, while group 2 was the standard and treated with glibenclamide (10 mg/kg b.w.). Groups 3, 4, and 5 received ethanolic leaf extract of *Tamarindus indica* L. at doses of 100, 250, and 500 mg/kg, respectively. Fasting blood samples were taken from animals before treatment in groups 3 to 5. One hour after drug administration, the rabbits were orally given a glucose load of 3 g/kg. Blood samples were collected from the ear vein at 0, 30, 60, and 90 minutes after glucose intake, using an Accu-Chek glucometer (Roche Diagnostic), and serum glucose

levels were measured immediately. The results were expressed as the percentage change in blood glucose, calculated as the mean of three animals, and presented as mean/100 ml of blood ± e.s.m.

2.6. Isolation

The hexane extract (10g) was subjected to column chromatography over silica gel. A total of 110 fractions were eluted with mixtures of hexane/ethyl acetate using a gradient elution starting with 100% hexane and ending with 100% ethyl acetate. The fractions were monitored using thin-layer chromatography (TLC), viewed under UV light (254 and 365 nm), and then sprayed with a 50% H_2SO_4 reagent followed by heating at 100°C.

The fractions were combined based on their TLC profiles and purified with methanol (MeOH). Fraction 25 (8 mg), which was obtained by an isocratic elution with a 99:1 ratio of hexane to ethyl acetate, showed a single spot containing β-sitosterol. Fraction [57] (12 mg), obtained from a hexane-ethyl acetate (95:5) mixture, also exhibited a single TLC spot containing Campesterol.

2.6.1. Spectroscopic data

Campesterol : δ (ppm) ^{1}H NMR (600 MHz, $CDCl_3$,): 5.37 (1H, H-6); 3.58 (1H, 3-OH); 3.26 (1H, H-3); 2.24–1.98 (2H, H_2-4); 2.05–1.79 (2H, H_2-7); 1.82 (1H, H-20); 1.64 (2H, H-9/H-14); 1.60–1.31 (8H, ring H_2: H_2-1/H_2-2/H_2-11/H_2-12); 1.51–1.27 (2H, H_2-16); 1.47 (1H, H-8); 1.45 (1H, H-14); 1.44 (2H, H-9/H-15); 1.40 (1H, H-15); 1.38–1.13 (2H, H_2-2); 1.30 (3H, H_3-18); 1.25 (4H, H_2-22/H_2-23); 1.04 (3H, H_3-19); 0.96 (6H, H_3-26/H_3-27); 0.91 (6H, H_3-21/H_3-28). **δ (ppm) ^{13}C NMR (150 MHz, $CDCl_3$):** 121.6 (C-6), 140.8 (C-5), 71.5 (C-3), 56.4 (C-14), 56.0 (C-17), 50.7 (C-9), 42.5 (C-13), 41.7 (C-1), 39.9 (C-12), 39.5 (C-24), 39.3 (C-20), 38.7 (C-4), 37.8 (C-10), 37.7 (C-8), 37.2 (C-2), 36.1 (C-22), 33.5 (C-23), 32.4 (C-25), 32.2 (C-7), 32.0 (C-15), 31.8 (C-16), 31.7 (C-11), 26.3 (C-23'), 26.0 (C-21), 21.1 (C-11'), 20.6 (C-26, C-27), 19.4 (C-19), 15.4 (C-28), 12.0 (C-18).

β-sitosterol: **^{1}H NMR** ($CDCl_3$, 600 MHz) δ (ppm): 5.24 (1H, H-6), 3.50 (1H, H-3), 1.20 (3H, s, H-19), 0.95 (3H, s, H-21), 0.86 (3H, t, H-29), 0.86 (3H, d, H-26), 0.8and (3H, d, H-27), 0.70 (3H, s, H-18). **^{13}C NMR** ($CDCl_3$, 125.78 MHz) δ (ppm): 140.4 (C-5), 121.4 (C-6), 71.6 (C-3), 57.0 (C-14), 55.9 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 42.1 (C-4), 39.8 (C-12), 37.and (C-1), 36.3 (C-20), 36.5 (C-10), 36.0 (C-22), 32.0 (C-7), 32.0 (C-2), 30.5 (C-25), 28.1 (C-16), 24.4 (C-15), 25.9 (C-23), 22.9 (C-28), 20.9 (C-11), 18.9 (C-26), 19.5 (C-19), 19.0 (C-27), 18,6 (C-21), 11.7 (C-29), 11.5 (C-18).

3. Results

3.1. Extraction

The yields obtained from the maceration with ethanol carried out on powders of the leaves of *Tamarindus indica L.* was 10.5% respectively. These extracts were used for biological activity. Maceration of leaves powder (500 g) using the solvent in increased polarity yielded 5.94g (1,19 %) of hexanic extract, 7.10 g (1.42 %) of ethyl acetate extract and 15.82 g (3,16 %) of methanolic extract.

3.2. Acute toxicity

The purpose of this experiment was to check for any immediate side effects from giving the animals the tamarind leaf extract. The results showed that no animals died, and they lived up to 3 days, even with a high dose. However, there was a temporary change in their behavior, specifically a decrease in movement, which began about 3 minutes after the dose and lasted for around 30 minutes. After that, the animals returned to normal. This means the extract isn't acutely toxic at these doses, but more testing is needed to be completely sure.

3.3. Anti-hyperglycaemic activity

The administration of the same doses (100, 250, and 500 mg/kg) of ethanolic extracts of leaves caused a marked and significant ($p < 0.05$) reduction in blood glucose levels, which were measured at 186.34 ± 9.11, 178.67 ± 5.11, and 178.33 ± 12.89 mg/100 ml respectively. When compared with the stem bark extract, the effect of the leaves showed no significant difference ($p > 0.05$).

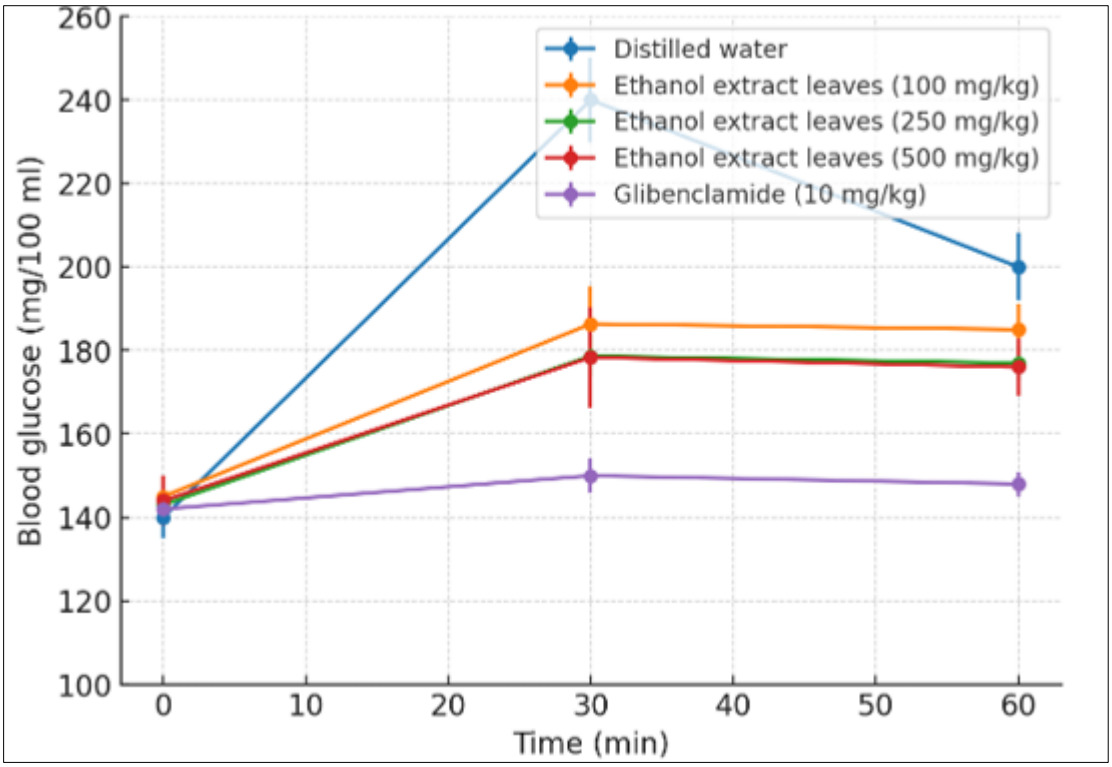


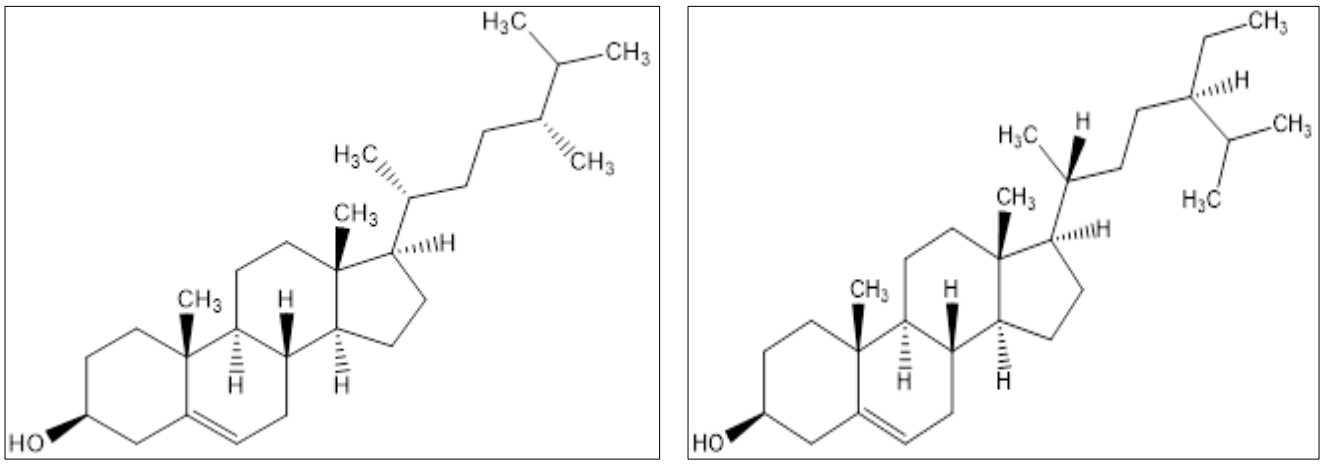
Figure 1 Anti-hyperglycaemic activity of ethanolic leaf extract

3.4. Phytochemical screening

The phytochemical screening was performed on the crude ethanolic extract by means of different chemical assays. It revealed the presence of flavonoids, tannins, steroids.

3.5. Identification

The structures of the isolates were determined through analysis of their spectroscopic data. The NMR spectra of all isolated compounds are consistent with steroids skeletons. By means of 1-D and 2-D NMR spectra and comparison with literature compounds P1 and P2are recognized as Campesterol [7,8] and β-sitosterol [7] respectively (Figure 2).



P1 P2

Figure 2 Isolated compounds chemical structures from *Tamarindus indica L.* leaves

4. Discussion

The study showed that the ethanolic extracts from *Tamarindus indica* leaves have a clear effect in lowering blood sugar, which supports why people traditionally use them against diabetes. [9] The extracts also appeared to be of low toxicity when checked using the Hodge and Sterner scale. Some compounds were found in the hexane and ethyl acetate extracts of the leaves, and these had never before been reported in the *Tamarindus* species, even if they are present in other plants. [10] No tests were done directly on these compounds, but earlier works already described their biological effects. Steroids are often the main active substances in medicinal plants with anti-diabetic action. For example, β-sitosterol can help release insulin and protect against oxidative stress thanks to its antioxidant action, while Campesterol has also been reported to have a beneficial effect on lowering blood glucose and improving insulin secretion [11].

5. Conclusion

The present study corroborated the hypothesis that *Tamarindus indica* leaves possess beneficial effects against diabetes, thereby validating their utilization in traditional treatment regimens. A considerable body of research remains to be conducted on this particular tree, though it should be acknowledged that the nature and focus of such research are contingent on the individual undertaking it.

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