Nutraceutical potential of *Tasba*: acute oral toxicity, antioxidant activity, anti-hyperglycaemic and hypoglycaemic affects of *Cassia tora* (Fabaceae) leaves


Senior Lecturer*, Pharmacy Doctor*, 3th PhD program*, Associate Professor*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*,*Corresponding author: Koubé Juliette,
Senior Lecturer, Department of Pharmaceutical Sciences, FMSP, University of Douala, Cameroon

Abstract:

Many diseases are associated with dysfunction of glucose metabolism, specifically chronic hyperglycaemia. *Cassia tora* leaves are used to cook nutritious and tasty sauce, known as *Tasba* in fulfulde language, consumed in the northern part of Cameroon to lower blood sugar. However, antioxidant activity, hypoglycaemic and anti-hyperglycaemic properties of *Cassia tora* leaves was not well studied. This study was performed to access the safety, antioxidant activity, as well as hypoglycaemic and anti-hyperglycaemic properties of *Cassia tora* leaves. For this purpose, we prepare an aqueous extract which contained alkaloids, tannins, flavonoids, anthraquinons, phenols, sterols, coumarins, mucilage and saponins. The median lethal dose (LD₅₀) of extract was greater than 5 g/kg; *Cassia tora* leaves are devoid of acute oral toxicity. It had free scavenging, reducing and chelating powers, thus acting as type 1 and 2 antioxidants. The percentage of decrease in blood sugar was 72.17% for the positive control and 62.73% for the extract. The inhibition percentage of hyperglycaemia was 73.59% for reference and 64.47% for extract of *Cassia tora*. *Cassia tora* leaves appear to be effective in treatment of diabetes, as well as other components of metabolic syndrome associated sugar metabolism and oxidative stress. The results of our research revealed the immense nutraceutical potential of *Cassia tora* leaves. This suggests that *Cassia tora* leaves are strongly recommended in the diet of diabetics or people prone to metabolic diseases. However, to take advantage of all the beneficial effects of *Cassia tora* leaves, it is best to consume them fresh, precisely as juice.

Keywords: *Cassia tora* leaves, antioxidant, hypoglycaemia, antihyperglycaemia, nutraceutical potential

I. INTRODUCTION

Carbohydrates are the main source of energy used by living organisms. The metabolism of sugars interacts with that of proteins, lipids, vitamins and minerals. Obviously, an imbalance in carbohydrate metabolism affects tissue and organs including liver, kidneys, adipose tissue, pancreas, muscles and blood vessels. For example, excess glucose in the body is often a precursor to chronic diseases, the most common of which are diabetes, obesity and non-alcoholic fatty liver disease [1], [2], [3], [4]. Diabetes is metabolic disorder characterised by an abnormal increase in blood glucose level and oxidation of the cells. Nevertheless, this disease is responsible for about 4 million deaths worldwide per year [5]. Currently, around 425 million people worldwide suffer from diabetes. If prevalence continues to rise at this rate, the number of people with diabetes will reach 625 million by 2045, an increase of 48% [6]. Africa in general and Cameroon in particular, is paying a heavy price of the spread of this dreaded disease, for several reasons: changes in eating habits, adoption of sedentary behaviours, cultural and health limitations around treatment and screening [7].

Abnormal increase of glycaemia is the key factor in the establishment and development of inflammatory and metabolic syndromes. The associated oxidative processes make the body immunodepressive. Diabetes is a component of the metabolic syndrome, the complexity and hazardousness of which inspires a management that integrates hygienic-diетetic measures and drug treatment. Nutraceutical may well correspond to this therapeutic approach. With this in mind, natural products that serve as food and medicine have been explored for several years. In this regard, *Cassia tora* is a plant that combines nutritional and pharmacological properties [8], [9], [10], [11], [12], [13].
Cassia tora L. is a plant belonging to the Fabaceae family, genus Sena. Under favourable ecological conditions, Cassia tora can reach a height of 120 cm. It is used both for its food quality (tea, additive and vegetable) and for its medicinal properties. In traditional medicine, different parts of plant, such as leaves, fruits and roots are used [14]. The leaves and fruits are used in traditional medicine for the following effects: laxative, antiperiodic, liver- tonic, anthelmintic, cardio-tonic... In addition, they provide treatment for tapeworms, leprosy, skin diseases, flatulence, jaundice, fever, constipation, sore eyes, coughs, bronchitis, heart diseases and haemorrhoids [13], [14], [15]. Recent studies revealed an anti-diabetic and antioxidant potential of Cassia tora seeds [16], [17]. Cassia tora leaves are used to cooked nutritious and tasty sauce, known as Tasha in local language, consumed in the northern part of Cameroon to lower blood sugar, to the best of our knowledge, no research has been done on the hypoglycaemic and antihyperglycaemic effect of Cassia tora leaves, let alone their antioxidant activity and toxicity. Therefore, this study aimed to evaluate hypoglycaemic and antihyperglycaemic proprieties, as well as the acute oral toxicity and antioxidant activity of Cassia tora leaves.

II. MATERIAL AND METHODS

II.1. MATERIAL

II.1.1. PLANT MATERIAL

The plant material consisted to Cassia tora leaves collected in September 2020, in the administrative region the far north of Cameroon, Diamare division and the municipality of Maroua 1st. The identification of plant has been done by Professor Tchobsalla, Botanist at the Faculty of Sciences of the University of Maroua; a specimen was deposited at both the National Herbarium of Cameroon and Faculty of Medicine and pharmaceutical Sciences of the University of Douala. After harvesting, leaves were dried in the shade and pulverised with a mechanical grinder. The resulting powders were weighed, macerated in distilled water for 24 h and then filtered. The filtrate was concentrated, weighed and kept in the freezer pending its use.

II.1.2. ANIMAL MATERIAL

The study was conducted on male albino rats of the wistar strain, aged 8 to 12 weeks, weighing 150 ± 35 g. The rats were supplied by the animal house of the Douala Museum of Plants. They were caged and acclimated in the environmental conditions of the animal house of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala, for one week, before the start of experiment. These animals were fed a standard rodent diet (corns, groundnut, soya bean, fish and pinch of mineral), and had free access to tap water. The enclosure was exposed to 12 h of light and 12 h of darkness daily.

II.2. METHODS

II.2.1. PREPARATION OF WATER EXTRACT OF CASSIA TORO LEAVES

Powdered Cassia tora leaves (392 g) was mixed with 4 L of distilled water during 24 hours. The maceration was filtered through a Whatman filter paper no.1 and evaporated to dryness in an air oven at 40 °C to give 128 g of aqueous extract corresponding with an extraction yield of 32.6 %. The C. tora leaves extract was stored at +4 °C until analyses.

II.2.2. PHYTOCHEMICAL SCREENING

Phytochemical screening was carried out using the usual methods for revealing families of secondary metabolites [18] in aqueous extract C. tora leaves. Aqueous extract gave positive test for alkaloids, tannins, flavonoids, anthraquinons, phenols, sterols, coumarins, mucilage and saponins.

II.2.3. ACUTE ORAL TOXICITY TEST OF AQUEOUS EXTRACT CASSIA TORO LEAVES

The acute oral toxicity test was performed following modified OECD guidelines, protocol 423 [19]. The aqueous extract of C. tora leaves was administered to healthy and non-gravid female wistar rats following the limit test procedure at the doses 2000 and 5000mg/kg. Nine female rats aged between 8 and 12 weeks, weighing between 150 and 178 g were used. Prior to dosing, the rats were selected at random, marked to permit individual identification, and kept in their cages to acclimatize for a week. The rats were divided randomly into four groups of 3 per group each. The test groups were treated with the different aqueous extract while control group received distilled water as described by Etame-Loe et al. [20]. The rats were starved of food overnight prior to dosing. Following the fasting period, they were weighed, and the dose of the test substance was calculated. Using a feeding probe, the rats were treated with the aqueous extract at doses of 2000, 5000mg/kg of body weight, for a limit test in accordance with the OECD guidelines 423. The control group received distilled water. After administration of the aqueous extract of C. tora leaves, each rat was monitored continuously for the first hour followed by every 2 hours till 12 h and then weighed and observed every day for 14 days. Besides death, signs of toxicity were observed such as changes in mobility, coat, eyes, stool, posture, response to light, grooming, response to noise, salivation, sensitivity, appetite, sleep and breathing. After the 14th day, all animals were deprived of food for 12 hours and then sacrificed after mild ketamine anaesthesia (IM injection at 1ml/kg). Animals were subjected to partial post-mortem examination to evaluate the toxicity of the plant notably through the assessment of the change in total body mass, relative mass of organs (liver, kidney, heart, lungs and the spleen).

II.2.4. TEST OF ANTIOXIDANT CAPACITY
In a volume of 20 mL of methanol, increasing amounts of Cassia tora dry extract (80, 160, 240, 320, 360 and 440 mg) were added, after complete dissolution for 2 hours, the resulting solutions (40, 80, 120, 160, 180 and 220 mg/mL) were kept at 4 °C until used for the determination of the antioxidant activity.

II.2.4.1. FREE RADICAL SCAVENGING TEST USING DPPH
The free scavenging capacity of Cassia tora leaves was determined using the ability of a given antioxidant to scavenge a free radical or donate a hydrogen atom according to the method of Zhang and Yasumori [21], with some modifications. This DPPH (N, N-diphenyl-N'-picrylhydrazyl) method can be used in liquid and solid matrices and is not specific to any particular class of compounds (water and fat soluble). Radical DPPH has a high absorbance at 517 nm (purple) with a drop in absorbance during the reaction, the decolouration result is stoichiometric to the number of electrons captured by the antioxidant. In the analytical protocol, 2 mL of 0.1 mM DPPH (prepared in methanol) was introduced into a test tube containing 0.5 mL of Cassia tora. The mixture was then shaken well for 5 min and incubated in the dark for 60 min at room temperature (24°C). For the control tube, methanol was used instead of extract. Butyl-hydroxyanisol (BHA) was used as a positive control. The absorbance was read at 517 nm. The free radical scavenging of the Cassia tora extract was expressed as percentage inhibition according to the following equation:

\[ A.A(\%) = \frac{A_{control} - A_{Sample}}{A_{control}} \times 100 \]

II.2.4.2. TOTAL REDUCING POWER TEST
The antioxidant capacity of Cassia tora extract was assessed by determining their ability to reduce iron (III) to iron (II) by method of Duh and Yen [22]. Briefly, in test tube, 0-220 mg/mL of Cassia tora extract was mixed with 2.5 mL of buffer solution (0.2 M pH 6.6) and 2.5 mL of potassium hexacyanoferrate solution [K₃Fe(CN)₆] to 1 %. The whole was incubated for 30 min at 50°C in a water bath. Then 2.5 mL of 10% trichloroacetic acid was added and the mixture centrifuged at 3000 rpm for 10 min. Then 2.5 mL of supernatant was taken and mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous FeCl₃ solution. The absorbance was read at 700 nm. The percentage of decrease in blood glucose was calculated according to the following formula:

\[ A.A(\%) = \frac{A_{control} - A_{Sample}}{A_{control}} \times 100 \]

II.2.4.3. FERROUS ION CHELATING CAPACITY TEST
Ferrous ion chelating capacity is a method of assessing antioxidant capacity based on the chelation of pro-oxidants (Fe²⁺) by the product analysed. It was evaluated using the method of Suter and Richter [23] with slight modifications. The reagent solution (100 µL ferrous chloride (2 mM) + 400 µL potassium ferrocyanide (5 mM)) was mixed with 200 µL of the extract (at different concentrations) and distilled water (1 mL). The mixture was then incubated at 20°C during 10 min. The formation of the potassium hexacyanoferrate complex was measured at 700 nm using a spectrophotometer (Genesys spectronic 2PC, USA). The analysis was conducted at 20°C to prevent any possible oxidation Fe²⁺. In this protocol, low absorbance indicated high chelating power. The negative control was performed without extract, EDTA was used as a positive control. The chelation capacity of ferrous ion by Cassia tora extract was calculated by comparing the absorbance of the samples with that of the negative control according to the following formula:

\[ \text{Chelation Capacity (\%)} = \frac{A_{control} - A_{Sample}}{A_{control}} \times 100 \]

II.2.5. TESTING THE ANTIDIABETIC EFFECT OF CASSIA TORORA LEAF
II.2.5.1. EVALUATION OF THE HYPOGLYCAEMIC EFFECT OF CASSIA TORORA LEAF EXTRACT
Twenty normal glycaemic animals were divided into 5 batches of 4 rats each, and fasted for 12 hours. At time T₀, the initial blood glucose level was determined. Distilled water, glibenclamide (10 mg/kg body weight) and three doses of Cassia tora extract (100, 200, 400 mg/kg body weight (BW)) were administered to the animals (fig. 1). Blood was collected every 30 min for 3 hours. A small incision at the distal end of the rats’ tails allowed a drop of blood to be obtained which was placed on the reactive range of an ONETOUCH ULTRA 2 strip and the blood glucose reading was automatically taken using an ONETOUCH SELECT Plus glucometer. The percentage of decrease in blood glucose was calculated according to the following formula:

\[ \text{Percentage of decrease in blood glucose} = \frac{G_0 - G_t}{G_0} \times 100 \]

G₀: Initial Blood glucose and Gₜ: blood glucose at time t= 30; 60; 90; 120, 150 and 180 min.
**Figure 1.** Distribution of animals by group for the exploration of hypoglycaemic and anti-hyperglycaemic potential of *Cassia tora* leaf extract

### II.2.5.2. EVALUATION OF THE ANTI-HYPERGLYCAEMIC EFFECT OF CASSIA TOR A LEAF EXTRACT

As before, 20 rats divided into 5 batches of 4 rats each were fasted in 12 hours and baseline blood glucose measured (figure 1). At the time T0, 30 min after administration of the different treatments, the animals were force-fed with a glucose solution (3 g/kg). The blood glucose levels of the rats were measured just before the administration of the glucose solution and after the treatment at 30 min intervals for 2 h 30 min using a glucometer as before. The animals were kept fasting during the experiment. The percentages of inhibition of induced hyperglycaemia were calculated as follows:

\[
\text{Percentage of inhibition} = \frac{G_0 - G_t}{G_0} \times 100
\]

where:
- \( G_0 \): Initial blood glucose
- \( G_t \): Blood glucose at time \( t = 30; 60; 90; 120 \) and 150 min.

**TABLE 1**: Clinical signs observed in rats after a single dose of *Cassia tora* leaf extract (2 and 5 g/kg)

<table>
<thead>
<tr>
<th>Signs of toxicity</th>
<th>Do</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
<th>D9</th>
<th>D10</th>
<th>D11</th>
<th>D12</th>
<th>D13</th>
<th>D14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Coat</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Eyes</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Stool</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Posture</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Response to light</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Grooming</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Response to noise</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Sensibility</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Appetite</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Sleep</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Salivation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

### II.2.6. STATISTICAL ANALYSIS

Data were entered into Excel (Microsoft Office 2016, USA) and analysed with Statgraphics Plus Version 5.0. Sigma Plot version 12.0 were used to draw curves. Quantitative data were presented as mean ± standard deviation (n=4). One- and two-factor ordered analysis of variance (ANOVA) was used to compare group means. Tukey and Bonferroni post hoc tests were used for multiple comparisons. The significance level was set at \( p < 0.05 \).

### III. RESULTS AND DISCUSSION

#### III.1. ORAL ACUTE TOXICITY

Table 1 shows the clinical signs observed in animals for the 14 days after oral administration of limit doses of the extract of *Cassia tora* leaves (2 and 5 g/kg). From the first hours of treatment to day 14, mobility, coat, eyes, stool, posture, response to light, grooming, response to noise, salivation, sensitivity, appetite, sleep and breathing were normal regardless of the limit dose used. Throughout the treatment and observation period, the animals continued to grow normally, no rat died. Furthermore, the autopsy performed did not show any abnormalities. Following the weighing of the organs, a balance between organ mass and body mass was noted. The median lethal dose was greater than 5 g/kg BW (LD50 > 5 mg/kg). According to Globally Harmonized System of Classification and Labelling of Chemicals (GHS), *Cassia tora* leaf extract is not classified as acutely toxic [19]. In addition, Koubé et al. [24] consider any substance or mixture of substances with a median lethal dose (LD50) above 5000 mg/kg as lacking acute oral toxicity. Therefore, the aqueous extract of *Cassia tora* leaves is devoid of acute oral toxicity.
Breathing

<p>| | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Number of deaths

0 0 0 0 0 0 0 0 0 0 0

N= Normal, D= day.

III.2. BIOCHEMICAL ACTIVITIES OF CASSIA TORA LEAF EXTRACT

III.2.1. ANTIOXIDANT ACTIVITY OF CASSIA TORA LEAF EXTRACT

III.2.1.1. FREE RADICAL SCAVENGING POWER OF CASSIA TORA LEAF EXTRACT

The ability of Cassia tora leaf extract to scavenge free radicals was analysed in function of concentration, with Butylhydroxyanisol (BHA) as the reference antioxidant (fig. 2). For concentrations of 40 to 120 mg/mL, the free scavenging power of Cassia tora extract was significantly lower than the reference (P < 0.05). Above this level, the free scavenging effect of Cassia tora leaf extract was similar to that of BHA. However, from 180 mg/mL onwards, the free scavenging power of the extract became constant, with a DPPH neutralisation capacity of over 89%. This suggest that Cassia tora leaf extract acts as type I antioxidant. The secondary metabolites (alkaloids, tannins, flavonoids, anthraquinones, phenols, sterols, coumarins and saponins) of Cassia tora leaf extract convert DPPH into a stable molecule. Cassia tora extract can limit the onset of oxidative stress and related diseases [25].

![Graph showing free scavenging effect of Cassia tora extract and BHA](image)

Figure 2. Evolution of the free scavenging effect of Cassia tora leaf extract

Values represent the mean of three trials, and the error’s bar of standard deviation.

BHA: Butylhydroxyanisol

III.2.1.2. REDUCING POWER OF CASSIA TORA LEAF EXTRACT

Figure 3 shows the reducing power of Cassia tora leaves as function of concentration. The reducing power of this extract increased with concentration and was statistically higher than that of BHA up to 180 mg/mL (P < 0.05). The strong reducing power of the extract would be due to the high number of hydroxyl groups they contain in the bioactive substances [26]. The concentration in phenolic compounds and mucilages would justify the reducing effect of the Cassia tora leaf extract. Overall, extract had a greater ability to prevent free radical formation by reducing ferric ion to ferrous ion, limiting the pro-oxidant effect of the latter. This action of extract is specific to type 2 antioxidant.
Figure 3. Evolution of the reducing power of *Cassia tora* leaf extract

*Values represent the mean of three trials, and the error’s bar of standard deviation*

*BHA*: Butylhydroxyanisol

### III.2.1.3. CHELATING POWER OF *CASSIA TORA* LEAF EXTRACT

The variation of the chelating of ferrous ion by the extract of *Cassia tora* leaves is presented in fig. 4. The chelating power increased as a function of the concentration, independently of the treatment. No statistical difference was noted between Ethylene diamine tetra-acetic acid (EDTA - reference) and *Cassia tora* leaf extract (*P* > 0.05). From 200 mg/mL, the latter had the capacity to complex more than 80% of metals to limit pro-oxidation. This extract would possess the biopolymers that form complex with transition metals [27]. This chelating capacity is all the more important as metal concentrations can reach higher than normal proportions in the body and trigger oxidation process in the cells. For example, as result of the Fenton reaction, these reduced metals can form highly reactive hydroxyl radicals and cause oxidative stress [28]. The oxy-radicals formed damage cellular lipids, nucleic acids, amino acids, carbohydrates and lead to cell weakening, or sometimes death.

Figure 4. Evolution of the chelating power of *Cassia tora* leaf extract

*Values represent the mean of three trials, and the error’s bar of standard deviation*

*EDTA*: Ethylene diamine tetra-acetic acid
III.2. HYPOGLYCAEMIC EFFECT OF THE AQUEOUS EXTRACT OF CASSIA TORA LEAVES

Table 2 shows the rate of decrease blood glucose after administration of treatments, including distilled water (negative control), Glibenclamide 10 mg/kg (positive control), different concentrations of Cassia tora leaf extract (100, 200 and 400 mg/kg). During the first hour after administration, there was no statistical difference between the treatments (P < 0.05). However, there was a statistically significant increase in the positive control compared to the negative control. In contrary, by the 90th minute, the difference had become highly significant between the negative control and the Cassia tora leaf extract (P< 0.05), with an inverse dose dependence effect. Two hours later, the dose dependence effect of extract was normal until the third hour. In the latter time frame, there was no longer any statistical difference between the positive control and the highest dose (400 mg/kg) of extract. The onset of action of the positive control on blood glucose was comparable to that of the extract. The efficacy of the extract became effective two hours after administration. Specifically, the percentage of decrease in blood sugar in normal animals was 72.17% for the positive control and 62.73% for the extract. The hypoglycaemic effect of Cassia tora leaf extract can be explained by its inhibitory action on the secretion of catabolism hormones (adrenaline and glucagon) or on the enzymes responsible for glycogenolysis (glycogen phosphorylase, glycogen transferase and α (1-6) glycosidase). Kumar et al. [17] reported that lowering of blood glucose is attributed to the alkaloids of Cassia tora seeds.

Table 2. Percentage decrease in blood glucose levels after treatment of the normal animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T30</th>
<th>T60</th>
<th>T90</th>
<th>T120</th>
<th>T150</th>
<th>T180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.09±0.05</td>
<td>5.26±0.45</td>
<td>8.02±4.91</td>
<td>5.84±0.51</td>
<td>8.54±3.13</td>
<td>4.67±0.91</td>
</tr>
<tr>
<td>Glibenclamide 10 mg/kg</td>
<td>9.63±3.27</td>
<td>22.18±2.90</td>
<td>36.13±6.15</td>
<td>50.77±3.37</td>
<td>59.85±10.10</td>
<td>72.17±5.32</td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td>4.26±1.49</td>
<td>14.36±5.20</td>
<td>29.38±8.38</td>
<td>37.92±3.14</td>
<td>46.59±2.72</td>
<td>49.49±6.38</td>
</tr>
<tr>
<td>Extract 200 mg/kg</td>
<td>6.80±3.41</td>
<td>13.38±5.64</td>
<td>27.27±8.64</td>
<td>38.14±4.92</td>
<td>52.47±5.16</td>
<td>55.92±10.46</td>
</tr>
<tr>
<td>Extract 400 mg/kg</td>
<td>3.29±1.15</td>
<td>13.61±2.33</td>
<td>24.51±7.40</td>
<td>39.56±5.05</td>
<td>53.89±4.92</td>
<td>62.73±10.89</td>
</tr>
</tbody>
</table>

The values of the percentage decreases are presented as means ± standard deviation. Means in the same column with the same superscript letter are not statistically different at the 5% level.

III.2.3. ANTIHYPERGLYCAEMIC EFFECT OF THE AQUEOUS EXTRACT OF CASSIA TORA LEAVES

The percentages of hyperglycaemia inhibition after administration of the treatments, including distilled water, Glibenclamide and different concentrations of aqueous extract of Cassia tora leaves, are presented in table 3. From the thirty minute onwards, there was a significant difference between the high doses of Cassia tora aqueous extract (200 and 400 mg/kg) and negative control. The action of the extract was dose-dependent, and better than that of the positive control (11.12%), for the concentrations of 200 mg/kg (12.35%) and 400 mg/kg (14.46%). From minute 60 to minute 80 the trend was the same, with only one change at minute 90, where the concentration 400 mg/kg had a higher percentage of inhibition than glibenclamide. Two hours after treatment, the trend was reversed, the action of the positive control became better (54.19%), but not statistically different from that of 400 mg/kg dose extract (50.52%). The same pattern was observed 150 minutes after treatment, with an inhibition percentage of 73.59% and 64.47% for glibenclamide and extract of Cassia tora (400 mg/kg) respectively. The bioactive substances contained in the Cassia tora leaf extract would act by activating either insulin or anabolic enzymes, including glycogen synthase. The secondary metabolites of the extract can act directly on the bioavailability of blood glucose, by forming complexes with it.

Table 3. Percentage of inhibition of hyperglycaemia following treatment of hyperglycaemic animals with Cassia tora leaf extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T30</th>
<th>T60</th>
<th>T90</th>
<th>T120</th>
<th>T150</th>
<th>T180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>4.98±2.49</td>
<td>10.45±3.42</td>
<td>13.31±3.57</td>
<td>16.94±3.28</td>
<td>21.89±1.79</td>
<td>21.89±1.79</td>
</tr>
<tr>
<td>Glibenclamide 10 mg/kg</td>
<td>11.12±4.23</td>
<td>19.90±2.57</td>
<td>36.41±8.21</td>
<td>54.19±7.95</td>
<td>73.59±7.54</td>
<td>73.59±7.54</td>
</tr>
<tr>
<td>Extract 100 mg/kg</td>
<td>7.61±3.21</td>
<td>16.86±2.76</td>
<td>20.65±3.64</td>
<td>28.91±3.04</td>
<td>39.97±5.42</td>
<td>39.97±5.42</td>
</tr>
<tr>
<td>Extract 200 mg/kg</td>
<td>12.35±4.98</td>
<td>22.67±6.84</td>
<td>31.44±11.61</td>
<td>41.01±7.81</td>
<td>51.56±9.80</td>
<td>51.56±9.80</td>
</tr>
<tr>
<td>Extract 400 mg/kg</td>
<td>14.46±1.15</td>
<td>26.92±4.34</td>
<td>39.59±0.90</td>
<td>50.52±6.86</td>
<td>64.47±4.23</td>
<td>64.47±4.23</td>
</tr>
</tbody>
</table>

The values of the percentage decreases are presented as means ± standard deviation. Means in the same column with the same superscript letter are not statistically different at the 5% level.

T30: 30 min after administration; T60: 60 min after administration; T90: 90 min after administration; T120: 120 min after administration; T150: 150 min after administration.

IV. CONCLUSION
This study was performed to access the safety, antioxidant activity, as well as hypoglycaemic and anti-hyperglycaemic properties of Cassia tora leaves. We found that the Cassia tora leaf extract is safe in single oral dose, has secondary metabolites capable of acting as antioxidant of types 1 and 2, and inhibiting any increase of blood sugar. Cassia tora leaves, in addition to providing a tasty and nutritious sauce to the consumers, also known as Tasba, appear to be effective in treatment of diabetes, as well as other components of metabolic syndrome associated sugar metabolism and oxidative stress. The results of our research revealed the immense nutraceutical potential of Cassia tora leaves. This suggests that Cassia tora leaves are strongly recommended in the diet of diabetics or people prone to metabolic diseases.

**FUNDING:** This study was not funded by any research funding agency or public or private sector funder.

**ACKNOWLEDGEMENTS:** The authors would like to thank Professor Dongmo of the Faculty of Science, University of Douala for his technical and logistical assistance.

**ETHICAL CONSIDERATIONS**

**CONFLICT OF INTEREST:** The authors declare that they have no conflict of interest

**REFERENCES**


