

# Phytochemical Screening and Acute Toxicity Evaluation of Leaves Extract of Two Fabaceae's Species: *Sesbania pachycarpa* DC. and *Indigofera berhautiana* Gillett

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#### Abstract

Objective: The aim of this study is to screen chemical composition and to evaluate the acute general toxicity of leaves aqueous extracts from Sesbania pachycarpa DC. (Fabaceae), used in traditional medicine in Burkina Faso to treat malaria, helminthiasis, and aqueous extracts of the leaves from Indigofera berhautiana Gillet (Fabaceae) used to treat hepatitis and typhoid fever. Methods: Aqueous decoction of leaves from Sesbania pachycarpa DC. or Indigofera berhautiana Gillett which are the form of use recommended by traditional health practitioners, were used for tests. Aqueous extracts used to evaluate the acute toxicity, were studied on mice of strain NMRI. For the toxicity study, the doses of the aqueous extracts were respectively 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg of body weight. Phytochemical screening was done to identify the phytochemicals contained in extracts. Results: Aqueous extracts of two species: Sesbania pachycarpa DC., Indigofera berhautiana Gillet were not toxic at the maximum dose of 2000 mg/kg of body weight. Phytochemical screening, showed coumarins, flavonoids, tannins, polyphenols compounds, steroids and/or triterpenes and saponosides in the aqueous extracts of the two species. Alkaloids were identified in Sesbania pachycarpa aqueous extract.

## **Keywords**

*Sesbania pachycarpa* DC., *Indigofera bergautiana* Gillet, Aqueous Extracts, Acute General Toxicity, NMRI Strain, Phytochemicals Compounds

#### **1. Introduction**

Medicinal plants are widely used in Burkina Faso to treat the most diverse diseases possible. However, very few toxicological studies are conducted around these plants. The most vulnerable people who are sick, and among them children, pregnant women, are exposed to all kinds of plants that are recommended by traditional healers. It is necessary to carry out scientific studies on the plants used, in order to evaluate their toxicity.

During our ethnobotanical studies in Ouagadougou (Burkina Faso), leaves of *Indigofera berhautiana* Gillett are used internally to treat jaundice and typhoid fever.

According to Nacoulma [1], different parts of *Sesbania pachycarpa* DC., are used for therapeutic purposes. In internal use, the leaves are used to treat malaria, helminthiasis (nematodes), febrile body aches, gastritis. In external use, the leaves are used for the following cases: wounds, varicose ulcers, ear infections, dracunculiasis.

The antipicultural and toxic activity of *Sesbania pachycarpa* seeds has been demonstrated by Fafioye [2].

The biological activities of *Sesbania pachycarpa* have been described by several authors: antioxidant activity described by Cook *et al.* [3], Atawodi [4]. Ouattara *et al.* [5], antibacterial in the preventive treatment of syphilis, bacterial infection caused by *Treponema pallidum*, described by Berhaut [6], anti-malaria activity, antihypertensive, antibacterial for example against coughs, described by Nadembega *et al.* [7]. The increase in the use of medicinal plants as therapeutic agents is accompanied by an increased demand for evidence of the safety, efficacy and quality of products. The objectives of this study are to assess the acute toxicity of leaves aqueous extract of *Sesbania pachycarpa* and *Indigofera berhautiana* which are medicinal plants widely used in the traditional health system in Burkina Faso.

#### 2. Material and Methods

#### 2.1. Plant Material

The studies were conducted at Ouagadougou's University Joseph KI-ZERBO, (Burkina Faso), at UFR/SVT, in the Department of Biochemistry-Microbiology, in the Laboratory of Biochemistry and Applied Chemistry, specializing for the study medicinal plants.

The leaves of *Sesbania pachycarpa* DC. and *Indigofera berhautiana* Gillet were harvested in Ouagadougou. The samples were formally identified by botanists from the plant ecology laboratory of Ouagadougou's University Joseph Ki-Zerbo. After harvest, the samples are dried, protected from light, at room temperature.

#### 2.2. Decoction

The dried leaves are pulverized and sieved. We proceeded with the extraction by decoction of 50 g of powder of *Sesbania pachycarpa* DC. boiling under reflux in

500 ml of water for one hour, then the mixture was filtered on Wattman paper after cooling. The decoction is lyophilized and kept in a box, for studies.

#### 2.3. Assessment of Acute General Toxicity

The method is that described by Done [8], Lompo *et al.* [9]. Female NMRI strain mice, 8 to 10 weeks old, weighing between 25 and 35 g were used for the tests.

Four concentrations of dry extracts diluted in water are prepared: 0.5 mg/ml, 5 mg/ml, 30 mg/ml and 200 mg/ml. Five batches of mice are made as homogeneous as possible including a batch of control mice. The test mice and the control groups of mice are fasted 12 hours before the test. The administration of the extracts is done by gavage according to the doses: 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg to be administered to each mouse.

The evaluation of the  $LD_{50}$  (lethal dose) is done between 24 hours and 72 hours in order to draw the dose-mortality regression line. The interpretation of the curve makes it possible to know if the extracts are an extremely toxic substance, a very toxic substance or a weakly toxic substance. The observation of the mice was still done for two weeks, according to current protocols.

#### 2.4. Phytochemical Studies

Methods of Ciulei [10] were used to determine phytochemicals compounds: alkaloids, coumarins, flavonoids, tannins, triterpenes, steroids, saponosides.

#### 2.5. Statistical Analyzes

All experiments are performed in triplicate and the results are expressed in means  $\pm$  standard deviation using Microsft excel 2013.

#### 3. Results and Discussions

#### 3.1. General Acute Toxicity for Sesbania pachycarpa DC.

The results of the toxicity tests for *Sesbania pachycarpa* DC. are shown in **Table 1**. The results indicate that there were no dead animals in any lot of mice up to a dose of 2000 mg/kg. The mortality rate is therefore 0%. For body weight, controls were increased from 35 g to 36 g, mice receiving 2000 mg/kg aqueous extracts of *Sesbania pachycarpa* leaves increased from 34 to 36 g in 3 days. This result indicates that the aqueous extracts of *Sesbania pachycarpa* were not toxic. In the literature, toxicity was observed for *Sesbania pachycarpa* seeds, studied on fish [2]. This work has even contributed to the search for toxicity in the aqueous extracts of *Sesbania pachycarpa* leaves used by our populations. At the end of our work, we found no toxicity on mice, with aqueous extracts of leaves.

#### 3.2. Acute General Toxicity for Indigofera berhautiana Gillett

The results of toxicity tests for *Indigofera berhautiana* are shown in **Table 2** for the two batches of mice: controls and 2000 mg/kg.

There were no dead animals, which means that the mortality is 0% at the

| Mice    | Weight (g)     | Administered<br>volume (ml) Number of dead animals |              |                      |            | Weight (g)    |              |             |  |
|---------|----------------|--|--------------|----------------------|------------|---------------|--------------|-------------|--|
|         |                | Day 0  | 24 H         | 48 H                 | 72 H       | 24 H          | 48 H         | 72 H        |  |
|         |                |  | Contr        | ols mice             |            |               |              |             |  |
| 1       | 34.91          | 0  | 0            | 0                    | 0          | 35.13         | 36.33        | 36.03       |  |
| 2       | 34.77          | 0  | 0            | 0                    | 0          | 34.55         | 35.28        | 35.77       |  |
| 3       | 34.72          | 0  | 0            | 0                    | 0          | 35.36         | 35.69        | 36.18       |  |
| Average | $34.80\pm0.10$ |  | 0            | 0                    | 0          | 35.01 ± 0.42  | 35.77 ± 0.50 | 35.99 ± 0.2 |  |
|         |                | Results for 5 mg/Kg                                | with extract | s from <i>S. pa</i>  | chycarpa,  | (0.5 mg/ml)   |              |             |  |
| 1       | 33.07          | 0.33   | 0            | 0                    | 0          | 34.12         | 34.62        | 34.44       |  |
| 2       | 32.02          | 0.32   | 0            | 0                    | 0          | 32.34         | 31.48        | 31.85       |  |
| 3       | 32.83          | 0.33   | 0            | 0                    | 0          | 31.29         | 32.55        | 33.28       |  |
| Average | 32.64 ± 0.55   |  | 0            | 0                    | 0          | 32.58 ± 1.43  | 32.88 ± 1.60 | 33.19 ±1.3  |  |
|         |                | Results for 50 mg/K                                | g with extra | cts from S. J        | pachycarpa | , (5 mg/ml)   |              |             |  |
| 1       | 34.62          | 0.35   | 0            | 0                    | 0          | 35.35         | 36.23        | 35.16       |  |
| 2       | 34.81          | 0.35   | 0            | 0                    | 0          | 35.94         | 36.04        | 36.99       |  |
| 3       | 33.85          | 0.34   | 0            | 0                    | 0          | 32.40         | 32.55        | 31.93       |  |
| Average | 34.43 ± 0.51   |  | 0            | 0                    | 0          | 34.56 ± 1.90  | 34.94 ± 2.07 | 34.69 ± 2.5 |  |
|         | 1              | Results for 300 mg/K                               | g with extra | cts from S. J        | pachycarpa | , (30 mg/ml)  |              |             |  |
| 1       | 32.46          | 0.33   | 0            | 0                    | 0          | 33.00         | 33.42        | 32.85       |  |
| 2       | 33.44          | 0.34   | 0            | 0                    | 0          | 34.16         | 34.05        | 34.59       |  |
| 3       | 34.41          | 0.35   | 0            | 0                    | 0          | 34.20         | 32.43        | 33.37       |  |
| Average | 33.44 ± 0.98   |  | 0            | 0                    | 0          | 33.79 ± 0.68  | 33.30 ± 0.82 | 33.60 ± 0.8 |  |
|         | R              | esults for 2000 mg/K                               | g with extra | cts from <i>S. j</i> | pachycarpa | , (200 mg/ml) |              |             |  |
| 1       | 33.90          | 0.34   | 0            | 0                    | 0          | 34.49         | 35.42        | 35.95       |  |
| 2       | 34.36          | 0.35   | 0            | 0                    | 0          | 33.63         | 34.03        | 34.67       |  |
| 3       | 34.82          | 0.35   | 0            | 0                    | 0          | 35.23         | 36.21        | 36.24       |  |
| Average | 34.36 ± 0.46   |  | 0            | 0                    | 0          | 34.45 ± 0.80  | 35.22 ± 1.10 | 35.62 ± 0.8 |  |

Table 1. Acute toxicity tests performed with aqueous extracts of leaves from Sesbania pachycarpa DC.

maximum therapeutic doses of 2000 mg/kg.

Since the mortality rate is 0% at 2000 mg/kg, it can be concluded that the leaf extracts of *Indigofera berhautiana* were not toxic at the therapeutic doses used. Further studies will determine whether the observed weight loss is related to the condition of certain organs during treatment. We did not find references in the bibliography on the toxicological study of *Indigofera berhautiana* Gillett to compare our results with the work of other researchers.

Medicinal plants can be toxic, [11] hence the interest of carrying out toxicity studies.

| Mice    | Weight (g)   | Administered<br>volume (ml) Number of dead animals |                |                    |             | Weight (g)                |              |            |
|---------|--------------|--|----------------|--------------------|-------------|---------------------------|--------------|------------|
|         |              | JO   | 24 H           | 48 H               | 72 H        | 24 H                      | 48 H         | 72 H       |
|         |              |  | Contr          | ols mice           |             |                           |              |            |
| 1       | 34.91        | 0  | 0              | 0                  | 0           | 35.13                     | 36.33        | 36.03      |
| 2       | 34.77        | 0  | 0              | 0                  | 0           | 34.55                     | 35.28        | 35.77      |
| 3       | 34.72        | 0  | 0              | 0                  | 0           | 35.36                     | 35.69        | 36.18      |
| Average | 34.80 ± 0.10 |  | 0              | 0                  | 0           | 35.01 ± 0.42              | 35.77 ± 0.50 | 35.99 ± 0. |
|         | Resu         | ults for 5 mg/Kg with                              | h extracts fro | om <i>Indigofe</i> | era berhau  | <i>tiana</i> , (0.5 mg/ml | )            |            |
| 1       | 33.43        | 0.33   | 0              | 0                  | 0           | 35.8                      | 35.59        | 36.99      |
| 2       | 33.74        | 0.34   | 0              | 0                  | 0           | 38.3                      | 36.64        | 38.26      |
| 3       | 35.25        | 0.35   | 0              | 0                  | 0           | 37.14                     | 37.68        | 38.54      |
| Average | 34.14 ± 0.97 |  | 0              | 0                  | 0           | 37.08 ± 1.25              | 36.64 ± 1.25 | 37.93 ± 0. |
|         | Res          | ults for 50 mg/Kg wi                               | ith extracts f | rom <i>Indigo</i>  | fera berha  | <i>utiana</i> , (5 mg/ml) | )            |            |
| 1       | 31.49        | 0.32   | 0              | 0                  | 0           | 32.65                     | 33.7         | 35         |
| 2       | 34.6         | 0.35   | 0              | 0                  | 0           | 35.08                     | 35.86        | 37.06      |
| 3       | 35.57        | 0.36   | 0              | 0                  | 0           | 34.93                     | 36.93        | 37.73      |
| Average | 33.89 ± 2.13 |  | 0              | 0                  | 0           | 34.22 ± 1.36              | 35.50 ± 1.65 | 36.60 ± 1. |
|         | Resu         | lts for 300 mg/Kg wi                               | th extracts f  | rom <i>Indigo</i>  | fera berha  | <i>utiana</i> , (30 mg/m  | 1)           |            |
| 1       | 31.93        | 0.32   | 0              | 0                  | 0           | 32.47                     | 33.82        | 34.81      |
| 2       | 34.62        | 0.35   | 0              | 0                  | 0           | 34.83                     | 36.25        | 36.83      |
| 3       | 32.35        | 0.32   | 0              | 0                  | 0           | 33.87                     | 34.34        | 35.75      |
| Average | 32.97 ± 1.45 |  | 0              | 0                  | 0           | 33.72 ± 1.19              | 34.80 ± 1.28 | 35.80 ± 1. |
|         | Result       | s for 2000 mg/Kg w                                 | ith extracts f | rom <i>Indigo</i>  | ofera berha | <i>utiana</i> (200 mg/r   | nl)          |            |
| 1       | 34.89        | 0.35   | 0              | 0                  | 0           | 31.55                     | 35.1         | 37.26      |
| 2       | 33.4         | 0.33   | 0              | 0                  | 0           | 29.38                     | 33.21        | 37.34      |
| 3       | 31.77        | 0.32   | 0              | 0                  | 0           | 30.83                     | 33.43        | 34.8       |
| Average | 33.35 ± 1.56 |  | 0              | 0                  | 0           | 30.59 ± 1.11              | 33.91 ± 1.03 | 36.47 ± 1. |

#### Table 2. Acute toxicity tests performed with aqueous extracts of leaves from Indigofera berhautiana Gillett.

#### 3.3. Phytochemical Studies

The phytochemicals identified by the simple characterization tests are shown in **Table 3**. Apart from the alkaloids that we did not find in the aqueous extracts of *Indigofera berhautiana* leaves, both species contain small amounts of flavonoids, but rather tannins and polyphenols, steroids and triterpenes, saponosides.

The phytochemicals observed may be responsible for the biological activity of the extracts. Several studies have shown the antimicrobial activity of these phytochemicals [5] [12] [13] [14].

The identified compounds may be supplemented by subsequent HPLC analyzes, as well as by the determination of the active ingredients responsible for the

| Phytochemical<br>compounds              | Alkaloids | Coumarins | s Flavonoids | Tannins and<br>phenolic<br>compounds | Saponosides | Triterpenes<br>and/or<br>stéroïds |
|---|-----------|-----------|--------------|--------------------------------------|-------------|-----------------------------------|
| <i>Sesbania</i><br>pachycarpa DC.       | +++       | +++       | ++           | +++                                  | ++          | +++                               |
| <i>Indigofera<br/>berhautiana</i> Gill. | -         | +++       | +            | ++                                   | +           | +++                               |

**Table 3.** Phytochemicals identified in aqueous extracts of *Sesbania pacycarpa* DC. and*Indigofera berhautiana* Gillett leaves.

Legend: negative reaction (-), weakly positive reaction (+), moderately positive reaction (++), strongly positive reaction (+++).

recognized biological activities.

#### 4. Conclusions

We evaluated the acute general toxicity of two plants from Fabaceae's family: *Sesbania parchycarpa* DC. and *Indigofera berhautiana* Gillett, commonly used in traditional medicine in Burkina Faso.

According to our studies, leaves aqueous extracts from *Sesbania parchycarpa* DC. or *Indigofera berhautiana* Gillett, which are recommended for use by patients, were not toxic in NMRI strain mice, at the dose of 2000 mg/kg of body weight.

Our studies have shown that the phytochemicals present in *Sesbania parchy-carpa* DC. are: alkaloids, coumarins, flavonoids, tannins and polyphenols, steroids, triterpens and saponosides. Those present in the aqueous extracts of *Indigo-fera berhautiana* Gillett leaves are averagely flavonoids, tannins, polyphenols compounds, steroids, triterpens, saponosides.

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### **Authors Contributions**

Initiation and elaboration of protocole: OUATTARA Monique Brigitte, M. Kiendrebeogo, O. G. Nacoulma, J. H. Bationo/Tests toxicologiques: OUATTARA Monique Brigitte, J. H. Bationo/Antibacterial, antioxidant and phytochemicals tests: OUATTARA Monique Brigitte.

### **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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