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HPLC ANALYSIS OF FRACTIONS FROM CHLOROFORMIC EXTRACT OF *Indigofera pilosa* (PEAR) LEAVES TESTED ON MOSQUITO LARVAES

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INTRODUCTION

Indigofera alone represents more than 700 species in tropical and subtropical regions of Africa, Asia, Australia, South and North America.[1] *Indigofera*, apart from their predominant roles in animal fodder, and in the textile industry, are now an important sourceof secondary metabolites (flavonoids, rotenoids and phenol acids).[2] Based on the biological properties of each of the compounds identified or characterized (rotenoids, terpenoids, sterols, saponosides, isoflavonoids) in the genus *Indigofera*,it would be important to study one of the species of thisgenus: *Indigofera pilosa* (Poir) and compare it, in terms of its content, to other species of the same family. In addition, secondary metabolites characterized in the genus are currently tracks of plant-based compounds used as repellents or toxic products against insects and pests.[3] Indeed, researchers are currently concerned about their harmful role in diseases such as malaria and crop destruction. Also, the active ingredients of synthetic insecticides used have several drawbacks. To ensure better intervention, while preserving the natural environment as much as possible, new preventive methods and new products are constantly sought.[4] It is in these contexts that this study on *Indigofera pilosa*,which has been the subject of little literature, takes place. This study will focus on bioactivityon mosquito larvae and HPLC-UV analysis of fractions from the chloroformic extract of the leaves of this plant.

MATERIAL AND METHODS

1. Material

1.2. Technical Material

1.2.1 Plant material

The plant material and composed of leaves of *Indigofera pilosa*. The plant was harvested in the Niayes area of Dakar in April 2015.

1.2.2. Biological material

The animal material consists of mosquito larvae harvested from Channel IV in front of the Amadou Hampaté Ba University, which adjoins the Blaise Diagne High School in Dakar.

2. Methods

2.1. Preparation and conservation of plant material

After harvesting the plant, the different parts of the plants were separated. Thus, the leaves were dried in the shade at the ambient temperature of the laboratory. After three weeks of drying, they were crushed using an electric shredder. The grinder is made of blackish powder of *Indigofera pilosa* leaves.

2.2. Extraction and splitting of active secondary metabolites in *indigofera pilosa* chloroformic extract by column chromatography

30 grams of *Indigofera pilosa* leaf powder are macerated in 300 mL of chloroform for 72 hours.After filtration and evaporation of the solvent, the recovered extract is subjected to a silica column chromatography with elvation gradient.

2.3. HPLC-UV analysis methods

Fractions that were found to be more effectivewere analyzed by high-performance liquid chromatography (HPLC-UV). The following method was used:

A thermo-grade liquid chromatograph was used. It is equipped with an automatic pump and coupled with a UV-absorbing detection system with diode bar. The set is controlled by a computer equipped with the data acquisition and operating software (Chromnav).

A column in reverse phase (silica is grafted by linary carbon chains and the chosen one used is polar) of the Type Hypersil-keyston C18 (particle size: 5 degrees;

250mm length; 4.6mm internal diameter) allows the separation of molecules and elition in isocratic mode was made by a binary mixture composed of acetonitrile and water at 60 and 40% respectively, with a flow of 1mL/min. Under these conditions, the duration of the analysis is 20min. The detectionwavelength and 230nm.

The standards (Quinine, Rutine, Gallic Acid, Emodine, Cinchonine) are solubilized in ethyl acetate. The analysis by HPLC was carried out on the basis of comparing the retention times of the extracts with those obtained with the same corresponding standards.

3. RESULTS

3.1. Split results

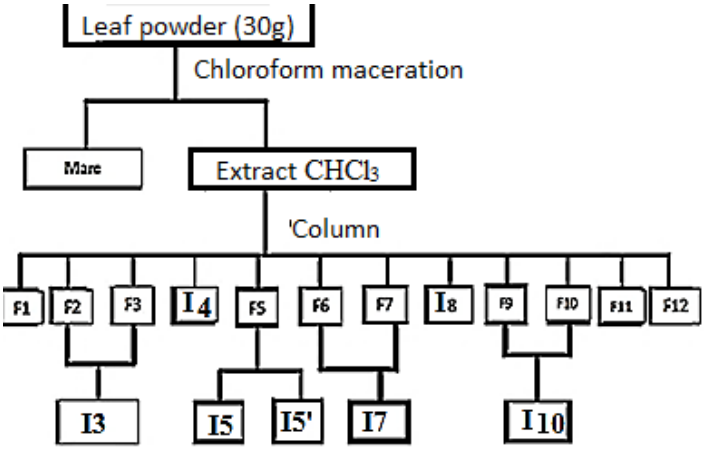

Figure 1: Chloroformic leaf splitting diagram of *Indigofera pilosa.*

The resulting fractions are analyzed by CCM with as elected ethyl/Hexane acetate. The CCM is made on Silica plate 60 F254 Merck, 0.1 mm on aluminum support. The reading is done under UV lamp at 254 nm and 360 nm. The results showed that it was possible to put together certain fractions as shown in Figure 1. Subsequently, the new fractions obtained are tested on mosquito larvae.

3.2. Results of effective fraction contact tests on mosquito larvae

The following graph highlights the most effective fractions on mosquito larvae over time in Indigofera pilosa.

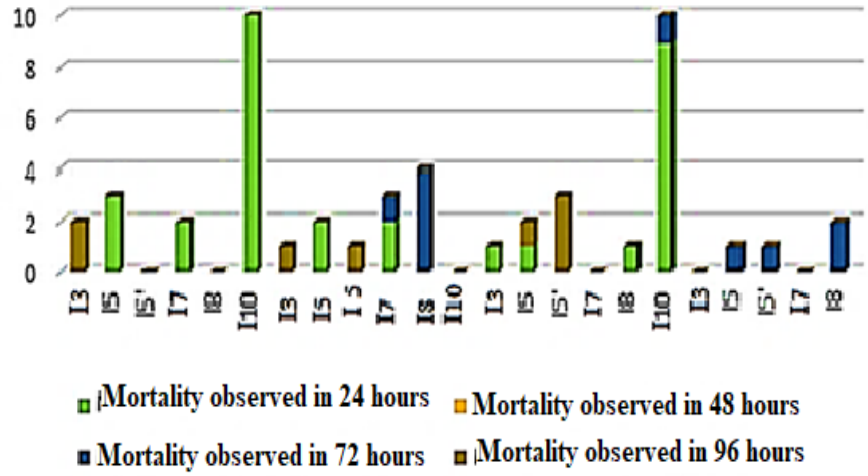

Figure 2: Larvae mortality based on fractions and time.

Analysis of Figure 2 shows that Fraction I10 is more effective with doses 1 and 3 after 24 hours, followed by I8after 72 hours with dose 2.

3.3. HPLC Fraction Analysis Results

3.3.1. Chromatographic profile of witnesses

The controls used, for the identification of the active ingredients responsible for the larvicide activity of fractions, are five: quinine (alkaloid), rutine (bioflavonoid), gallic acid (tannin), emodine (anthraquinone), and cinchonin (alkaloid). These controls can be divided into three families: alkaloid, flavonoid and tannin. The identification is bioguided because several bio pesticides are part of these latter families.

3.3.2. Results of the analysis of fractions from *Indigofera pilosa*

HPLC-UV analysis of chloroformic fraction is

Figure3 represents the chromatogram of the chloroformic fraction I3. It shows the existence of a single majority compound. Its surface percentage is 86.34%, its retention time is 2,982 minutes. This time corresponds exactly to the retention time of the ido.

The chloroformic fraction I3 would contain emodine.

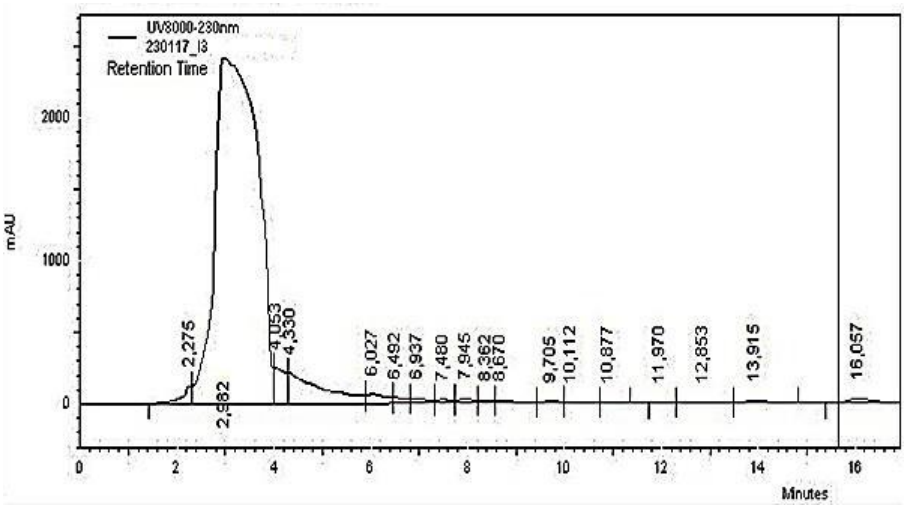

Figure 3: Fraction is Chromatography.

Analysis of chloroformic extract I8

Figure 4 below represents the chromatogram of fraction I8. This chromatogram identifies a majority peak with a retention time of 2,943 minutes for a surface percentage of 58.94%. This peak is attributed to gallic acid which appeared at the same retention time for a surface percentage of 60.70%.

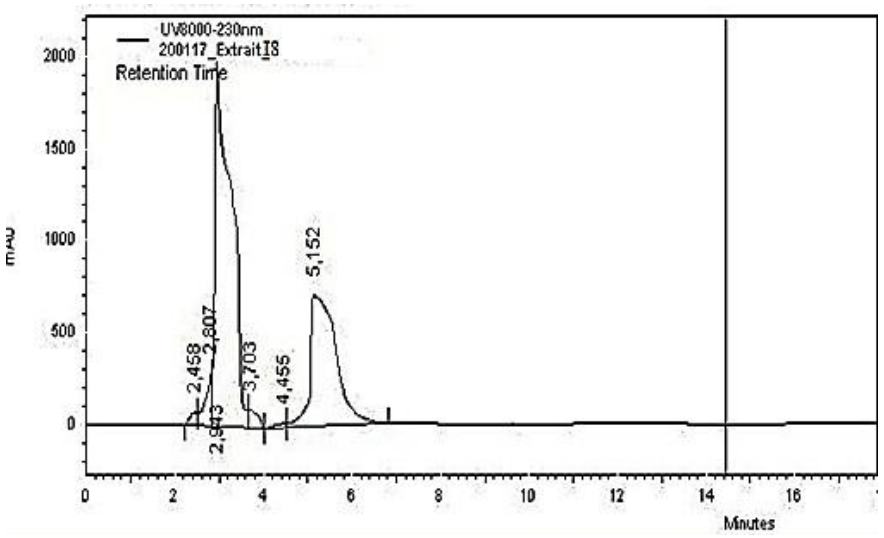

Figure 4: Chromatogram of I8.

Fraction analysis I10

Figure 5 represents the chromatogram of fraction I 10. It highlights a majority peak. Its retention time is 3,265 minutes with 42.86% confidence percentage. This peak could correspond to the cinchonin that appeared at the retention time 3,260 minutes, for a surface percentage of 40.56%.

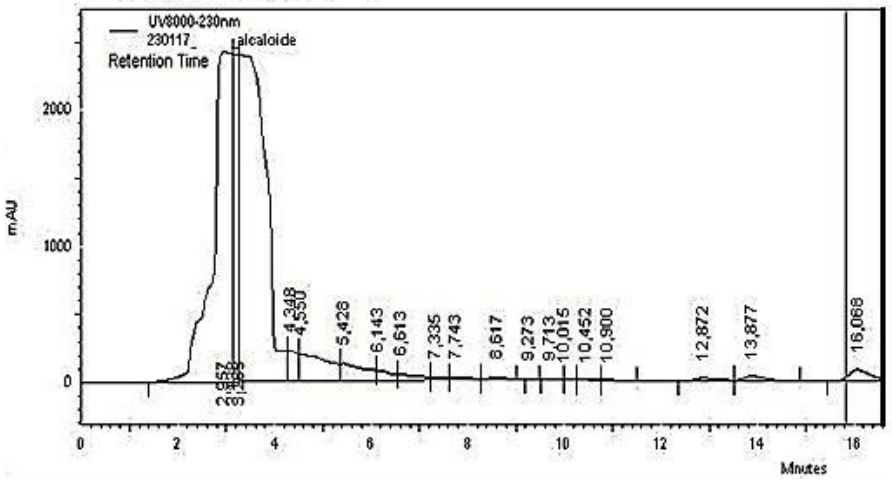

Figure 5: Fraction I10 Chromatogram.

4. DISCUSSION

4.1. Insecticide activity of *Indigofera pilosa* fractions

Fractions I10 and I8 (electing: hexane/ethyl acetate) that offer more efficacy on mosquito larvae as well as I3 (electing: hexane/ethyl acetate), with efficacy times ranging from 24 to72 hours.

4.2. Chromatography Liquid High Performance (CLHP)

Compared to controls, the chromatograms showed the existence, in fractions: gallic acid, rutin, emodine, cinchonin and quinine. There are also other more or less unidentified polar compounds.

5. CONCLUSION

Like most species of the *genus Indigofera*, *indigofera pilosa* exhibits larvicide activities on mosquito larvae. It would therefore contain bioactive substances. The results of the HPLC analyses confirmed the presence of tannin flavonoid, anthracenic drive and alkaloid.

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