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Phytochemical Test and Larvicidal Activity of Three Organic Extracts Exits of the Stems of *Indigofera pilosa* on Larvae of Mosquitoes

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Abstract

In the search for alternative method beside the use of dangerous insecticides for health and the environment, the vegetable kingdom offers many possibilities. Work is carried out in this direction and showed an effectiveness of the extracts of plants. Our results indicate that the chloroformic contents present a better larvicidal activity than the other extracts. In addition, the analysis by Thin Layer Chromatography (TLC) of the three extracts of stems showed their wealth of flavonic compounds before compounds, alkaloids and tannins thus suggesting a correlation between the larvicidal activity and the contents in secondary metabolites of stems.

Subject Areas

Plant Science

Keywords

Indigofera pilosa, Stems, Chloroformic Extract, Extraction, CCM, Larvicidal Activity

1. Introduction

Paludism is the most widespread protozoose in the world. There exists in an endemo-epidemic state in the intertropical zone of the sphere [1]. According to WHO, more than 2 billion individuals are exposed throughout the world; 300 - 500 millions a year are infected including 270 - 480 millions in Africa [2]. In Senegal, paludism, parasitic disease of hydrous origin continues to pose public

health problems [3].

In the anti-mosquito fight, the active matters of insecticides of synthesis used present several disadvantages indeed. Besides their raised cost, they can be at the origin various environmental problems [4].

To ensure a better intervention, while preserving to the maximum the natural environment, of new preventive methods as new products are constantly required [5]. Then, the natural substances as bioactives molecules resulting from the plants currently arouse a very particular interest by their multiple biological activities (antibacterial, antioxidant and insecticides) [6].

The objective of this work is to identify the families of chemical compounds present in the stems of *Indigofera pilosa* in order to explain the larvicidal activity of the various extracts.

2. Material and Methods

2.1. Vegetable Material

Vegetable material is composed of stems of *Indigofera pilosa*. The plant was collected in the zone of Niayes in April 2015.

2.2. Animal Material

Animal material consists of larvae of mosquitoes.

2.3. Method

2.3.1. Preparation and Preservation of Vegetable Specimens

After having collected the plant, we separated the various parts of the plant. Thus we have the stems of the plant dried at the room temperature of the laboratory.

Following three weeks of drying, we crushed the samples of the plant using an electric crusher.

2.3.2. Collection and Preservation of Animal Specimens

The larvae of mosquito are collected on the level of channel IV in Fass (Dakar-Senegal). For the occasion, a surmounted pot of a long sleeve is introduced into water while inclining its edge of with 45˚, under the effect of the forces of tension, the surface layer of water is thus attracted as well as the specimens who survive it. The larvae are preserved in jars of 1 L filled at the three quarter with distilled water. At the laboratory, we used the larvae of mosquitoes of stages 3 and 4.

2.3.3. Extraction

The technique of practiced extraction is maceration. Indeed, the samples are impregnated in solvents (1 g/10mL) of increasing polarity during 72 hours. The solvents are in the order: cyclohexan, chloroform, butanol, methanol and distilled water.

The extracts obtained are concentrated using a rotary evaporator about 30 to 45min at temperatures around boiling points of solvents according to extract.

Thereafter, the concentrated extracts are dried safe from the light with the room temperature of the laboratory for one duration going from 2 to 6 days.

2.3.4. Identification of the Chemical Groups

1) TLC of alkaloids

For the identification of alkaloids we used silica gel like stationary phase; eluant is a mixture of chloroform and diethylamin (45V/5V); the witness used is Cinchonin. The revelation is performed through Draggendorf. Red few coloring would indicate the presence of alkaloid in the extracts. The reagent is carried out at room temperature and the atmospheric pressure.

2) TLC of tannins

The acetic mixture of ethyl/methanol/water in proportions (40V/8V/5V) is used like eluant. Plates of glass covered with silica gel are used like stationary phase. The revelation is made by a ferric chloride solution after drying. Coloring chestnut of the spots is synonymous of the presence of tannins in the extracts. The chromatography is carried out at room temperature and the atmospheric pressure.

3) TLC of the flavonoids

Eluant used is a mixture of ethyl acetate and water at 15%. The silica gel is used as stationary phase. The revelation is made with aluminum chloride and the observation under UV with 254 nm. Yellow coloring would indicate the presence of flavonoids.

4) Identification of the saponosids

In test tubes, one poured 10 ml aqueous total extract. Each tube is agitated vigorously during 15 seconds, and then left at rest during 15 minutes. A height of persistent foam, higher than 1 centimeter would indicate the presence of saponosids.

2.4. Biological Activities

The larvicidal activity was undertaken according to the method of the tests of sensitivity standardized by the World Health Organization, adopted to test the sensitivity of the larvae, with respect to insecticides used in fight campaigns [7] [8].

2.4.1. The Experimental Protocol Is the Following One

Starting from each dry extract, we prepared five solutions with amounts of increasing concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL). 10 larvaes of stage 3 and 4 were taken using a flexible grip and were put in goblets of 5 cm diameter, each one containing 40 ml of water. The same number of larvae was placed in a pilot goblet containing 50 ml of water. Four repetitions were carried out for each dilution like for the witness. The formula of Abbott is then used to correct mortalities observed.

2.4.2. Statistical Analysis

The studied factors are time, the number of died of insects, the amount and the

nature of the extracts like their interaction. The method General Linear Model in Minitab 17 was used for the statistical analysis of the collected data.

3. Results

3.1. Phytochemical Tests

The results of phytochemical tests are consigned in Table 1.

3.2. Extraction

The results of extractions are gathered in Table 2.

3.3. Identification of the Chemical Groups

The results of the tests of identification revealed by thin layer chromatography (TLC), of the various chemical groups announced by the phytochemical tests on the extracts, are photographed and presented hereafter: (Photograph 1(a)-(c)).

3.4. Biological Activities

Table 1. Results of phytochemical tests on the stems *Indigofera pilosa.*

Part of the plant	Extract	Flavonoids	Alkaloids	Tannin	Saponosids
Stem	Cyclohexan	-	+	-	-
	Chloroform	-	+	-	-
	Butanol	+	+	-	-
	Methanol	+	+	+	-
	Water	-	-	-	-

+ Presence means; - Absence means.

Table 2. Results of the extractions of the stems of *Indigofera pilosa.*

Part of the plant	Extracted	Initial mass (g)	Mass of the extract (g)	Output (%)	Aspect of the extract	Color of the extract
Stem	Cyclohexane	35.00	0.47	1.34	Pasty	Gray greenish
	Chloroform	34.30	1.85	5.40	Powder	Green
	Butanol	32.46	0.08	0.24	Pasty	Yellowclear
	Methanol	29.94	1.29	4.30	Pasty	Orange

Eluant: acetic acid 15% in water (a) | Ethyl acetate/methanol/water (40v/8v/5v) (b) | Ethyl acetate/water (15%) (c)

Photograph 1. (a) TLC of alkaloids photograph, (b) TLC of tannins photograph, (c) TLC of the flavonoids.

Table 3. Results of the treatments with extracts of the stems of *Indigofera pilosa.*

Source of variation	Mortality		
	DL	F	P
Doses	3	0.14	0.936
Time	3	191.90	0.000
Extracts	2	109.42	0.000
Doses-time	9	2.59	0.009
Doses-extracts	6	0.16	0.987
Extracts-times	6	47.17	0.000
Doses-time-extract	18	0.93	0.546
Error	143		
Total	190		

DL: Degree of freedom; F: Frequency; P: Probability.

4. Discussion

The outputs of the extraction obtained are more important for chloroform (5.4%) and methanol (4.3%). Methanol more polar than the others made up organics being used like solvent, one can think that the stems of *Indigofera pilosa* contain manypolar substances but also of the dissolved non-polar substances in chloroform[9].

According to **Table 1**, the phytochemical studies showed that most extracts of stems of the plants contain secondary metabolites. One indeed notes the presence of flavonoids in the butanolic and methanolic extracts, of alkaloids in all the extracts. Only the methanolic extracts contain tannins. Also the aqueous extracts of the stems of *Indigofera* do not contain saponosides. These results were indeed corroborated by the tests of identifications revealed by thin layer chromatography (TLC). The presence of these metabolites would explain their use with fine therapeutic and ichtyotoxic larvicide activities. Indeed some flavonoids contain repulsive compounds such as aldehyds of flavonoids used as repulsive against the insect's harmful (flies, cockroaches, plant lousealeurodes, mosquitoes, ticks, chips…) [10]. According to Sylvie Morel [11] the role of the flavonoids in the interactions plant-insects is very largely accepted. In practice the rotinoids, and in particular the rotenone were largely studied for their insecticidal activity.The degueline and the tephrosin (rotinoïdes) seem good parricides against *Aedes aegypti*, [12]. Lastly, some pterocarpans have insecticidal properties against *Anopheles gambiae* (neodulin, 4-méthoxynéoduline) [13]. The stems of *Indigofera pilosa* contain alkaloids, which consist of a large number of chemical compounds which have almost both toxicological and pharmacological activities [14] [15] [16]. Besides their antifungal and antiviral activity [17], tannins present a toxic direct effect for certain species of insects [18]). And our study revealed well the presence of this compound in the stems.

For the analysis of the variance corresponding to the effect of the extracts of the stems on the larvae of mosquitoes, **Table 3** shows that mortality has a very

significant variation according to time and extracts ($P < 0.001$).Also the interactions time-extracts and amount-times are very significant ($P < 0.05$). On the other hand the factors amounts and amount-extracts are not significant ($P > 0.05$). Thus the larvicidal effect depends on time, the extract and the amount. With regard to times, twenty-four hours prove to be sufficient so that the extract acts effectively on the larvae of mosquitoes. And the most effective extract is the chloroformic extract. (Figure 1 and Figure 2; Tables 1-3).

5. Conclusion

At the end of our research task, it is revealed that the stems of *Indigofera pilosa* contain secondary metabolites which would confer its biocide activities to the plant. What makes it possible as a result to justify their use as insecticides or ichthyotoxic. However, it should be recognized that the activity of an extract is

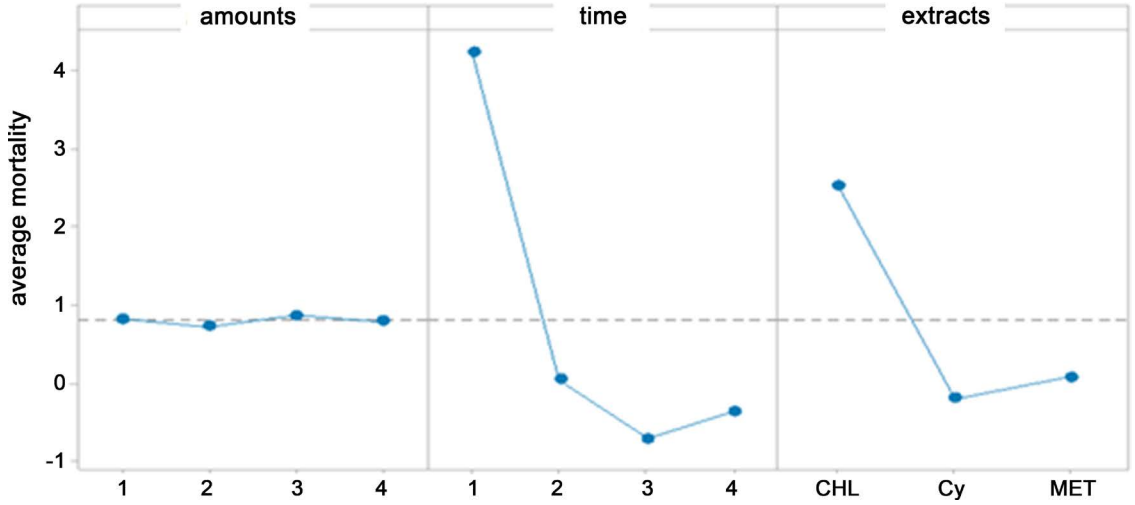

Figure 1. Curve of mortality according to the amounts, times and extracts of stem of *Indigofera pilosa* on the larvae of mosquitoes. CHL: chloroformic extract; Cy: cyclohéxanique extract; MET: méthanoliques extract.

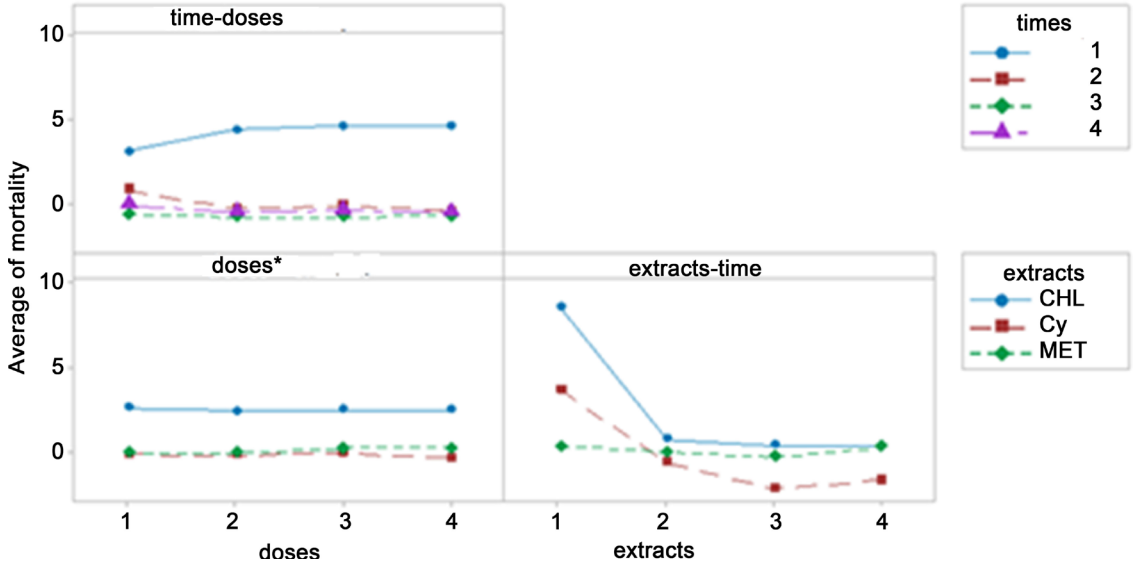

Figure 2. Interaction between the amounts, time and extracts of stem of *Indigofera pilosa* for mortality.

not always ascribable with a single principle but with a diversity of active ingredients from where need for continuing the investigations on this plant with the methods more elaborate such as the fractionation of the extracts even the insulation of the molecules to allot to one or the other of the components the actions biocides announced.

References

[1] Balam, M. (2010) Use of *Bacillus thuringiensis* (Bti) within the Framework of the Control of the Vectors of Paludism in Rural Environment of Banambani and Right Gabakoro in MALI. 18 p.

[2] OMS (2003) Guide of the Trainee. Entomology of the Paludism and Control of the Vectors. Guide of the Trainee WHO/CDS/CPE/SMT/2002.18 Revu.1, Part I.

[3] PNLP (2014) Strategic of Fight against Paludisme in Senegal. Report of the Direction of the Fight against Paludism. PNLP Senegal. pnlp.sn/rapport.html

[4] Barbouche, N., *et al.* (2001) Contribution under Investigation of the Biological Activity of Extracts of Sheets of *Cestrum parqui Hérit.* (Solanaceae) on the Locust Pilgrim *Schistoceraca gregaria* (Forsk.). *Biotechnol. Agron. Ploughshare Approximately*, **5**, 85-90.

[5] Ndiaye, A. (2016) Phytochimical Tests and Larvicide Activity of Three Organic Extracts from the Seeds of Indigofera pilosa on Larvae of Mosquitoes. *European Newspaper of Pharmaceutical and Medical Research.*

[6] Bouderhem, A. (2015) Effect of Essential Oils of the *Laurus nobilis* Plant on the Toxicological and Morphometric Aspect of the Larvae of Mosquitos (*Culexpipiens et Culiseta longiarealata*). Memory of End of Study for Obtaining the Academic diploma of Master Degree Sector: Biological Sciences Speciality: Biochemistry Applied, p. 23.

[7] OMS (1963) Method to Follow to Determine the Sensitivity or the Resistance of the Larvae of Mosquitoes to Insecticides. Resistance to Insecticides and Fight against the Vectors. 13*th Report of Committee WHO of Experts of Insecticides*, WHO, Geneva, Technical Report 265, 55-60.

[8] Aouinty, B., Oufara, S., Mellouki, F. and Mahari, S. (2006) Preliminary Evaluation of the Larvicide Activity of the Aqueous Extracts of the Sheets of the Castor Bean (*Ricinus communis* L.) and of the Wood of Thuja (*Tetraclinis articulata* (Vahl) Mast.) on the Larvae of Four Culicides Mosquitoes: *Culex pipiens* (Linné), *Aedes caspius* (Pallas), *Culisetalongiareolata (*Aitken) and *Anopheles maculipennis* (*Meigen*). *Bases* [*Online*], **10**, 67-71.
http://popups.ulg.ac.be/1780-4507/index.php?id=1115

[9] Bassene, E. (2012) Initiation in Research on Natural Substances. Presses University of Dakar, Dakar, 23 p.

[10] Proguard, Inc. (1998) Repulsive Compositions Containing of Aromatic Aldehydes. Number of Publication WO1996039827 A1.

[11] Morel, S. (2011) Phytochimic Study and Biological Evaluation of *Derris ferruginea* Benth. (Fabaceae). Doctorate, 57.

[12] Yenesew, A., Twinomuhwezi, H., Kabaru, J.M., Akala, H.M., Kiremire, B.T., Heydenreich, M., Peter, M.G., Eyase, F.I., Waters, N.C. and Walsh, D.S. (2009) Antiplasmodial and Larvicidal Flavonoids from *Derris trifoliata*. *Bulletin of the Chemical Society of Ethiopia*, **23**, 409-414. https://doi.org/10.4314/bcse.v23i3.47665

[13] Jiménez-González, L., Álvarez-Corral, M., Muñoz-Dorado, M. and Rodríguez-

García, I. (2008) Pterocarpans: Interesting Natural Products with Antifungal Activity and Other Biological Properties. *Phytochemistry Reviews*, **7**, 125-154. https://doi.org/10.1007/s11101-007-9059-z

[14] Angenot, *et al.* (1978) New Alkaloids of the Sheets of *Strychnosus ambarensis*. *Day. Pharm. Belg.*, **33**, 11-23.

[15] Schakirov and Yunusov (1990) Steroidal Alkaloïds. *Natural Product Reports*, **7**, 549-564.

[16] Varadinova, T.L., Shishkov, S.A., Ivanovska, N.D., Velcheva, M.P., Danghaaghin, S., Samadanghiin, Z. and Yansanghiin, Z. (1996) Antiviral and Immunological Activity of a New Pavine Alkaloid (-)-Thalimonine Isolated from *Thalictrum simplex*. *Phytotherapy Research*, **10**, 414-417. https://doi.org/10.1002/(SICI)1099-1573(199608)10:5<414::AID-PTR876>3.0.CO;2-5

[17] Cadman, C.H. (1960) Inhibition of Plant Virus Infection by Tannis, Phenolics in Plants, In HEALTH and Disease. Plant Phenolics *Group Symposium*, 101-105.

[18] Raymond, V., Barbehenn, C. and Constabel, P. (2011) Tannins in Plant-Herbivore Interactions. *Phytochemistry*, **72**, 1551-1565. https://doi.org/10.1016/j.phytochem.2011.01.040