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Effect of Saponins Extracted from *Canavalia ensiformis* (*L*) DC (Leguminosae) on Ectoparasites of Cattle and Pigs in Congo-Kinshasa

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Abstract

A number of plant secondary metabolites such as Saponins are reported to display insecticidal effects and medicinal plant species have the advantage of low cost and local availability and offer a sustainable alternative to conventional chemical insecticides. The aim of this study was to evaluate *in vitro* the effect of saponins extracted from *Canavalia ensiformis* (*L*) DC (Leguminosae) on ectoparasites of Cattle and Pigs. Concerning microscopic observation, three genera of ticks were identified in cattle: *Amblyamma*, *Hyalamma* and *Ixodes*. While only one species of lice *Haemotipinus suis* has been observed in pigs. The treatment of these ectoparasite model systems by saponins extracted from the above mentioned plant revealed the insecticidal effect of this plant secondary metabolites. Indeed, the total saponins extracted from saber beans, applied at the doses of 6% and 10% respectively have an insecticidal effect against lice. As for ticks, these extracts resulted in the death of 88.3% of individuals for the dose of 6% of total saponins against 100% for the dose of 10%. For the best of our knowledge, this is the first report on the insecticidal activity of *Canavalia ensiformis* on ectoparasites of Cattle and Pigs occurring in Democratic Republic of the Congo. It is thus preferable that this biological experiment be carried out *in vivo* in order to study the appropriate dose and to evaluate its harmful effect on the domestic animals in treatment.

Keywords

Bionsecticide, Secondary Metabolites, *Canavalia ensiformis*, Ectoparasites, Domestic Animal

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1. Introduction

Recent findings indicate that pests and pathogens threaten animal production all over the world and are difficult to control by the mean of conventional methods like insecticides because of the development of insecticides resistance [1]. A number of plant secondary metabolites such as Saponins are reported to display insecticidal effects and medicinal plant species have the advantage of low cost and local availability. Their pharmacological action is related to several constituents working in synergy [2]. Domestic animals are subject to various pest attacks. This is the case of pathogenic microorganisms (bacteria, fungi, etc.) and ectoparasites (lice, ticks, flies, fleas, etc.) responsible for many diseases in animals [3, 4]. If it is true that the social and economic development of a country is measured by the importance and quality of these farms, it should be noted that the tropical regions are lagging behind in this point.

The efforts of the livestock farmers of the tropical regions face the insufficiency of the means of improvement of the animal health following the parasites which are there; the climatic conditions favorable to their proliferation.

Ectoparasites such as ticks and lice are vectors of several pathogenic germs responsible for many diseases in human and domestic animals [5-8]. This is the case of genera like *Rhicephalus* causing East Coast Fever in beef, pork and mutton; *Hyalomma* transmitting *Piroplasma bigeminum*, Piroplasma equi, hemogloburin biliser of beef and the genus *Anablyomma* that transmits heart water in beef and sheep ticks have a considerable importance in human and veterinary pathology. In fact, apart from the neurotropic or even paralyzing action of saliva developed by a female during oviposition, they act as a reservoir and vector for many viral (arboviroses), bacterial (bonnelioses, rickettioses) and parasitic diseases. (babesiosis, ehrlichiosis, filariasis) [3, 9, 10]. Although lice have a minor role in the transmission of both classical and African swine fever viruses [11], their important role as carriers of many serious diseases such as typhus and relapsing fever is recognized [4, 10].

This parasite condition poses a universal problem for farms. For that, the specialization and the improvement of the production in the breeding of the domestic animals must nevertheless count on the possibilities of control or control of insects and mites and profit that one can draw from them. Thus, to fight against pathogens and to ensure good health for livestock, which would lead to the assurance of food security for the population, control drugs were developed and marketed. The fight against ectoparasites consists in the use of insecticides: organochlorine and organophosphorus (malathion), carbamates, etofenprox, used in powder, lotion, shampoo, diffuser, etc. [3, 9; 10]. But, since introduction of *Rhipicephalus (Boophilus) microplus* in West Africa, breeders seem not to be satisfied of the traditional acaricides used [12].

Although the use of insecticides remains the basic means of controlling most ectoparasites, various methods are being developed to complement or synergize these products in order to increase their effectiveness and reduce their adverse effects by obtaining ecologically acceptable strategies [13].

Nevertheless, in rural areas of developing countries, the use of these products remains limited for various reasons such as; the high cost of imported pharmaceutical products, the lack of technical knowledge of application, the lack of adequate infrastructure for their use, etc. the degrading effects due to the misuse of this product in these environments lead to the proliferation of numerous resistant strains [14].

In a context where chemical pesticides are increasingly criticized, new crops protection strategies that do not affect agriculture efficiency and productivity, must be found [15]. To do this, the international organizations as well as the United Nations system agencies (ADRA, first Emergency, FAO, WHO...) advocate the search for substitutes for these products by resorting to natural products of the plant kingdom.

According to the study carried out in Burkina-Faso (West Africa), plant species constitute promising source of pesticidal compounds (biopesticides) and offer a sustainable alternative to conventional chemical insecticides [16]. Some biologically active compounds like alkaloids, saponins and glucoside-containing compounds were identified from plant species such as *Canavalia ensiformis*, *Tephrosia vogelii* and *Crimumbulbis permum* [17, 18]

Canavalia ensiformis is an annual tropical plant belonging to Leguminosae family [19], vigorous with climbing stalk, able to fixe atmospheric nitrogen, in its roots and produce a nematicidal substances. So, it limits the nematode populations which parasite banana roots (*Meloïdogyne incognita* and *Rotylenchulus reniformis*) [20]. The whole plant is used in folk medicine for the treatment of vomiting abdominal dropsy, kidney related lumbago, influmentry diseases and swelling [21].

The aim of the present study is to evaluate the effect of Saponins Extracted from *Canavalia ensiformis (L)* DC (Leguminosae) on Ectoparasites of Cattle and Pigs.

2. Material and Methods

2.1. Study Area

The experiment was carried out in the institute Faculty of Agricultural Sciences of yangambi (IFA-YANGAMBI) center of Kisangani. Kisangani, capital of the eastern province, is located in the central basin of the Democratic Republic of Congo between 25° 11 east longitude and 0° 31 north latitude and at an altitude between 396 and 410 m. Its area is about 1910 km^2 with a population on average 1186479 inhabitants which gives a density of 621.19 inhabitants per km^2.

The climate of the experimental site is that of Kisangani which belongs to the type Af according to the classification of KOPPEN. It is a hot and humid equatorial climate. The average temperature is close to 25° C and the temperatures are higher than 1800mm year. The annual sunstroke is 1925 hours. The relative humidity is high; it varies between 80 and 90%.

The soil of the experimental site is a well-drained ferralitic soil, belonging to the order of oxisols of the "soil taxanomy" and corresponding to ferralsols of the FAO-UNESCO system [22]. The physical and chemical characteristics of the soil at the experimental site are summarized as follows: 19.7% clay, 10.6% silt and 69.6% sand. The contents of total nitrogen, organic carbon and assimilable phosphorus are respectively 0.5%; 072% and 0.289%. This soil has an acid pH of about 4.9. The primitive vegetation of Kisangani is a savannah aspect following the action of the man but at the beginning, it is anombrophilous vegetation.

2.2. Material

Bean seeds swept domestic pig lice and cow ticks and constituted the essential material in this study.

Saber bean seeds were harvested from the experimental field of the Faculty of Agricultural Sciences Institute of Yangambi in Kisangani. The pods were dried in the sun for four weeks during the day to reach the constant weight. The treated seeds are pounded in a wooden mortar and sieved through a sieve of 0.25 mm mesh to obtain fine flour which is put in a plastic package pending the extraction of total saponins.

Lice, insects with dorso-ventral flattened bodies [9, 10], were harvested from live pigs at the O.N.G PRODAM piggery, located in the second avenue, Boyoma plateau district in the municipality of Makiso. The recollect was in hand raised after restraint of the animal. They were kept directly in petri dishes for the text in the laboratory. The ticks were harvested from live cattle at Scolasticat Sr. Déhon farm in Motumbe district, Makiso commune and Mandombe junior seminary farm at Kabondo district, 13 km away, towards the road ofBangboka International Airport in Makiso Commune. These ticks were kept in petri dishes in the laboratory.

2.3. Methodology

Experimentation and *in situ* and *in vitro* observation were highlighted for the collection of data from this work. The samples collected in the above-mentioned media were processed and analyzed in the scientific laboratory of Faculty of Agricultural Sciences Institute of Yangambi, Kisangani city.

2.3.1. Treatment of Saber Beans

(i). Presumptive Test and Extraction of Saponins

The foam test is used to confirm the presence of total saponins in sword bean kernels. This test consisted in placing in the oven-calibrated crucible at 105° C until its constant weight. Then take and introduce 5 g of this powder into each of 4 250 ml flasks containing a solution of 50 ml of methanol and 50 ml of distilled water which is allowed to macerate for 60 hours.

(ii). Gravimetric Determination of Saponins

The MUNDUNDU experiment was used with total saponin extract under the following procedure: the contents, refluxed for 30 minutes, are filtered while hot on the west. The filtrate collected in a 250 ml flask, previously tarred is concentrated to a small volume. The saponins are precipitated cold in the methanol solution. A double volume of diethyl ether is added thereto to further precipitate the saponins. The solvent is removed by decantation and distillation under vacuum using a Rotavapor. Finally, the precipitate is dried in an oven for 12 hours to obtain the brown saponins brown coloring. The weight of the total saponins is obtained by deducting weight from the balloon with the tare which is the weight of the empty balloon.

(iii). Preparation of *Test Solutions*

The preparation of test solutions was made by the solution of saponins in a given volume of distilled water. Two types of solutions were prepared: 6 and 10% total saponins and stored in vials for their distinctive application on ectoparasites in vitro.

2.3.2. Identification of Ticks and Lice

The Wild HEERBRUGG brand no. 105155 binocular loupe was used to identify sample. For ticks the determination key recommended by some authors was used for their taxonomy [10, 23, 24]. The tick genera were determined by taxonomic characters such as the presence or absence of the anal groove; his position; the presence or absence of the eyes; rostrum size; the size of coxa IV in the male; the form of coxa I; the shape of the base of the capitulum and the shape of the palpi. Ticks are divided into two groups: soft ticks in under-order of *Argasina* and solid ticks in under-order of Iodine [3, 4, 7, 25].

Stinging lice or Anoploura feeding on mammals only; are easily distinguished from Mallophaga by their head which is narrower than the thorax. The key to diagnosis of the main species of lice adopted in this study was that recommended by some authors [3, 10, 11]: eyes atrophied or absent; head retracted into the thorax; long horns; mammalian parasites; three pairs of substantially equal legs equal presence of temporal horns; presence of prominent pleural plaques on the abdomen: *Haematopinus suis* (large, 5 to 6 mm, parasites of pigs) *H. eurysternus* (2, 5 to 4, 5 mm, parasites of cattle) *H. asini* (measuring 2; 5 to 3, 5 mm (parasite of equines).

2.3.3. Modality of Application of Test Solutions

0.5 ml of saponin solution was taken using standard syringes 2 ml and applied dropwise on the ventral side, dorsal and dorso-ventral lice.

The treated lice and ticks were returned to the petri dish lined with filter paper.

The treatments applied were the following:

1. T0: (control) sample that has not received any application of the product (this is to ensure the viability of ectoparasites outside their hosts)
2. T1: application to 6 percent solution of total saponin saber bean;
3. T2: application to 10 percent of total saponins of saber beans

3. Results and Discussion

3.1. Types of Ectoparasites

The figure 1 gives the results of observation related to the identification of different types of ectoparasites collected during this study.

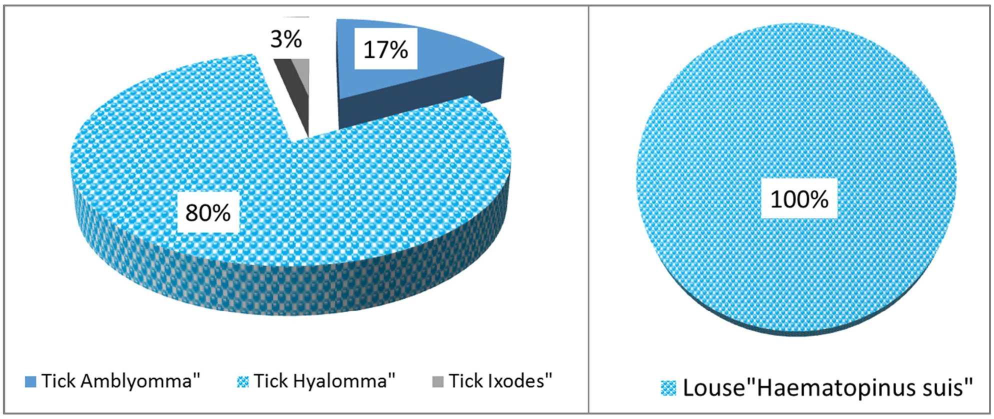

Figure 1. Ectoparasites frequency identified on cattles and pigs.

The figure 1 revealed that for ticks, the *Hyalomma* genus is the most abundant of the sample collection medium at 80 percent, followed by *Amblyomma* at 17 percent, and *Ixodes* at 3 percent. The results below are applicable for these kinds of matters.

For lice; the only species found during this experiment was *Haematopinus suis* domestic pork parasites.

3.2. Total Saponins Extraction

Table 1. Average weight of total saponins extracted from sword bean seeds.

	Average weight (g)		
	Specimen 1	Specimen 2	Average
Total Saponins	0.57	0.6	0.585 ± 0.021

It can be seen from this table that the average weight of total saponins extracted from sword bean seeds is 0. 59 g.

Insecticidal effect of total saponins

The percentage of individuals killed following the application of total saponins on ectoparasites is presented in the table below.

Table 2. Insecticidal effect of total saponins on lice and ticks.

Saponins Application	Number tested	LICE				TICKS			
		Rate (%)				Rate (%)			
		A	B	C	Average	A	B	C	Average
T0	20	0	0	0	0	0	0	0	0
T1	20	100	100	100	100	80	85	100	88.3
T2	20	100	100	100	100	100	100	100	100

(Legend: A: backside; B: below side and C: back-below side)

The table 2 revealed that the total saponins extracted from saber beans, applied at the concentrations of 6% and 10% respectively have an insecticidal effect against lice. As for ticks, these extracts resulted in, on average, the death of 88.3% of individuals for the dose of 6% of total saponins against 100% for the dose of 10%. The difference in the results obtained could be due to the fact that some ticks are provided with chitin which confer them resistance to the effect of total saponins where they cover their body. The back-below side application has a deadly killing effect because of the body's exposure of the individual to the solution of total saponins to tick bodies. The solution at the dose of 10% of total saponins did not meet any resistance in ticks and resulted in the death of all ticks regardless of the application surface. The respective doses of 6% and 10% total saponins revealed insecticidal effect against ectoparasites of cattle and pigs.

Saponins are a group of steroidal or triterpenoidal secondary metabolites of plant origin. This type of molecules has an interesting pesticidal potential as previously reported [26]. Indeed, these natural products are known to display toxicity effect against harmful insects (anti-feeding, disturbance of the moult, growth regulation, mortality, etc.). The insecticidal activity of saponins is due to their interaction with cholesterol, causing a disturbance of the synthesis of ecdysteroids. These compounds are also protease inhibitors or cytotoxic to certain insects like *Ostrinia nubilalis, Spodoptera littoralis, Culex fatigans, Acrolepiosis assectella, Collosobruchus chinensis, Spodoptera littoralis and Shistocerca gregaria* [26]. Saponins were also reported to display several pharmacological properties like cytotoxic action to mammalian cells and their hemolytic properties [26, 27]. For the best of our knowledge, this is the first report on the insecticidal activity of *Canavalia ensiformis* on ectoparasites of Cattle and Pigs occurring in Democratic Republic of the Congo.

4. Conclusion

The aim of this study was to evaluate the effect of saponins extracted from *Canavalia ensiformis (L*) DC (Leguminosae) on ectoparasites of Cattle and Pigs. Concerning microscopic observation, three genera of ticks were identified in cattle: *Amblyamma*, *Hyalamma* and *Ixodes*. While only one species of lice *Haemotipinus suis* has been observed in pigs. The treatment of these ectoparasite model systems by saponins extracted from the above mentioned plant revealed the insecticidal effect of this plant secondary metabolite. It is preferable that this biological experiment be carried out *in vivo* in order to study the appropriate dose and to evaluate its harmful effect on the domestic animals in treatment.

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