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Research article

Biocidal activity of the extracts of *Vernonia amygdalina* against ticks responsible for livestock diseases

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Abstract. Biocidal activity of *Vernonia amygdalina* was assessed on *Rhipicephalus appendiculatus* ticks responsible for livestock diseases in North and South-Kivu provinces. In vitro trials were conducted in the laboratory of Lwiro Research Center for Natural Sciences to determine the lethal dose for different days after ticks contact with extracts. Five different dosages of 1.25, 2.5, 5, 10, 20 g/mL were evaluated. Water was considered as positive control and Batycol as negative control. The phytochemical screening of extracts of *Vernonia amygdalina* plant shows that this plant contains several substances responsible for acaricidal activity, mainly saponins, terpenoids, steroids and tannins. The experiment shows a mortality rate that varies with the concentration of the extracts; the most fatal is 20 g/mL and decreases with exposure time. The aqueous extracts showed a progressive decrease in mortality compared to ethanol extracts. This is due to the dissolution of the substances responsible for this insecticidal activity. The study recommends the application of aqueous extracts of *Vernonia amygdalina* in the fight against cattle ticks. Indeed, this is a natural insecticide available and easier to prepare, non-toxic to humans and is rapidly degraded in the environment.

Key words: biocidal, *Rhipicephalus appendiculatus*, *Vernonia amygdalina*, aqueous extract, ethanolic extract

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Introduction

Ticks are ectoparasites that directly affect animal production and are responsible for the transmission of several diseases such as Babesiosis, Theliosis, Anaplasmosis [1]. They are also vectors of serious parasitic or viral diseases such as the various rickettsioses of domestic animals or humans. These diseases represent a real obstacle to livestock development in endemic areas [1]. Ticks, because of the many infectious diseases that they transmit to animals and the trauma caused by their bites, constitute a real obstacle to the promotion and the profitability of the breeding. These diseases are responsible for more than 63% of animal mortality in several countries [1]. Indeed, if the morbidity rate is only 15 to 20% and the mortality rate of 5 to 10% in the traditional or native cattle, it is not the same for the cattle of exotic origin, introduced into enzootic zone. In these animals, the morbidity rate is higher in the order of 30 to 95% and the case fatality rate can reach 100% in unstable enzootic status [1, 2].

In Central and Eastern Africa, the tick distribution of *Rhipicephalus appendiculatus*, *Haemaphysalis leachi*, *Boophilis decoloratus* and *Ambliyomma* brings about the outbreak of various livestock diseases such as anaplasmosis, babesiosis and bovine theileriosis [3]. In the Democratic Republic of Congo (DRC), several cattle sectors are affected by anaplasmosis, babesiosis and theileriosis. In South Kivu cattle breeding has been around for a long time. It is one of the major concerns for the population in rural areas in general and particularly in the Bushi Mountains [4]. In this region, several parasitic diseases hinder the promotion of animal husbandry, including sometimes preventing the potential improvement of the local breed of cattle by crossing exotic breed of brood stock which, unfortunately, are very sensitive to these diseases. Faced with this menace, various wherewithal of control has been applied with mixed success, including those using acaricides. Indeed, the fight against ticks uses mainly synthetic chemicals but it is currently facing the problems of cost, efficiency, environmental pollution and use. It remains difficult to apply in our region of study given the poor income of breeders, the difficult accessibility to products and ignorance of application techniques. A better control strategy should therefore take these parameters into account. Thus, in order to find a solution to the problems of livestock in South Kivu and in Bugorhe, Irhambi/Katana sub-counties in particular, a study of acaricide effect of the plant *Vernonia amygdalina* on ectoparasite cattle ticks *Rhipicephalus appendiculatus* Neumann was conducted in vitro to determine the fatal and recommendable dose.

Study areas. Irhambi/Katana and Bugorhe Sub-Counties are located between 2°15' and 2°30' S and between 28°48'and 28°85'E, on the Eastern flanks of the Kahuzi-Biega massif in South-Kivu, in the Democratic Republic of Congo where the samples were collected. Several species of ticks are found in these Sub-Counties: *Ambliyoma variegatum*, *Haemaphysalis leachi*, *Rhippicephalus appendiculatus* and *Boophilis decoloratus* [2, 5]. Among these different species of ticks, *Ripicephalus appendiculatus* is the vector of the *Theileria parva*, causal agent of theileriosis, *Boophilis decoloratus* is the basis of *Babesia bovis*, *Babesia bigemina*, causative agent of Babesiosis and Anaplasma marginal causative agent of East Coast fever.

Materials and methods

Plants sampling and preparation. Samples of *Vernonia amygdalina* were collected in Lwiro, South Kivu Province, Democratic Republic of Congo in July 2016. The plant was identified at the Botany Laboratory of Natural Science Research Center of Lwiro in comparison with the specimens of the herbarium. The leaves were sun dried in the open air and then pounded in a mortar to obtain a fine powder. Organic and aqueous extracts were prepared according to conventional methods by maceration, techniques most frequently used by breeders. A sample of 30 g of vegetable powder is macerated in deionized water and another sample of 30 g in a hydro-alcoholic mixture (ethanol/water 70/30: V / V) for 24 hours. The aqueous and hydro-alcoholic (organic) extracts are separately evaporated under vacuum at a temperature of 40 °C. The residues obtained in the two extracts are dissolved in deionized water to prepare the different concentrations. A phytochemical screening was carried out on the aqueous and organic extracts following the procedures proposed by Sofowora [6]. The alkaloids were detected by the Drangedorff and Wagner reagents, the saponins were detected by the persistence of the foam after stirring, the terpenoids were detected by Lieberman Buchard and Hirschson reagents; steroids — by Lieberman-Buchard reagents and flavonoids — by the reagents of Dechene, Murthi and Briggs [6, 7]. The results were noted as follows: (+) reaction in trace or weakly positive; (++) positive reaction; (+++) strongly positive reaction.

Harvesting ticks. The tick harvest was carried out as described by Pamo and his collaborators [8]. Ticks were identified in the laboratory following the keys of Walker and his collaborators [9] and only *Rhipicephalus appendiculatus* was selected for this study.

Preparation of different doses for bioassays. After several preliminary tests, the doses were chosen such that each concentration was twice the previous or the 1/10th and five dilutions (1.25; 2.5; 5; 10; 20 g/mL) were prepared. After homogenization, using a syringe, each of the solutions thus prepared was uniformly spread over a Whatman N°1 filter paper washer placed in a petri dish where the ticks were deposited in these suspensions for 2 minutes. The ticks were removed and put in observation tubes for 4 days. Every 24 hours, a tick count was carried out until the fourth day. One test consisted of deionized water alone and another one of Bayticol Miticide as a control dose.

Toxicity study by contact of extracts. The evaluation tests of the toxicity by contact of the extracts were carried out by depositing in the previously prepared petri dishes, 15 ticks were selected for the bioassays. Three repetitions were performed for each dose. The number of dead ticks was counted every 24 hours during the 4 days of each trial and the mortality rate was calculated using the Abbott formula [10].

$$\mathrm{Mc}=\frac{\mathrm{Mo}-\mathrm{Me}}{100-\mathrm{Me}}\times 100,$$

where Mo = mortality recorded in treated lots (%); Me = mortality recorded in controls (%); Mc = corrected mortality (%).

Data analysis. The data obtained were subjected to analysis of variance after correction of the observed mortalities compared to those of the controls and the differences between the extracts. Regression of the dose logarithm of mortality was used to determine the DL_{50}.

Results and discussion

The biocidal activity of *Vernonia amygdalina* on the tick population *Rhipicephalus appendiculatus* was evaluated in the laboratory by controlling tick mortality according to the extracts but also the controls used (deionized water and Batycol Miticide). The results of in vitro tests of the aqueous and organic extracts of the plant *Vernonia amygdalina* against the tick species *Rhipicephalus appendiculatus* are presented in Table 1.

Table 1

Effect of extracts of *Vernonia amygdalina* on *Rhipicephalus appendiculatus* as a function of time

Exposure time in days	Plant material	Control used water	Control used Bayticol®	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	20 g/mL
1	Aqueous extract	5.6 ± 0.1	100 ± 0.0	12.5 ± 9.7	17.0 ± 3.1	19.6 ± 7.1	22.7 ± 0.0	100 ± 0.0
	Organic extract	5.6 ± 0.1	100 ± 0.0	43.5 ± 1.5	37.5 ± 0.0	27.2 ± 0.0	15 ± 0.0	4.7 ± 0.0
2	Aqueous extract	13.9 ± 2.4	100 ± 0.0	7.8 ± 1.4	18.2 ± 2.1	19.1 ± 9.4	28.9 ± 12.0	0.0 ± 0.0
	Organic extract	13.9 ± 2.4	100 ± 0.0	0.0 ± 0.0	3.0 ± 10.0	42.8 ± 0.0	19.5 ± 8.8	22.5 ± 2.5
3	Aqueous extract	23.8 ± 15.8	100.0 ± 0.0	7.6 ± 10.2	13.0 ± 11.3	35.9 ± 16.0	37.0 ± 18.5	0.0 ± 0.0
	Organic extract	23.8 ± 15.8	100.0 ± 0.0	4.5 ± 4.5	5.3 ± 1.6	28.0±15.5	65.5 ± 15.8	73.0 ± 16.4
4	Aqueous extract	29.8 ± 119	100 ± 0.0	26.6 ± 18.8	12.8 ± 10.4	16.6 ± 12.2	11.4 ± 2.5	0.0 ± 0.0
	Organic extract	29.8 ± 11.9	100.0 ± 0.0	9.5 ± 0.4	36.6 ± 6.6	2.0 ± 1.8	0.0 ± 0.0	0.0 ± 0.0

Generally, the mortality increases with the increasing concentration in the aqueous extracts and in the organic extracts according to the days. High concentrations cause high tick mortality. At the concentration of 20 mg/l, the aqueous extracts caused a mortality rate of 100% while the ticks showed resistance in the first two days to organic extracts. For the positive control, the “Batycol”, 100% of the tick mortality rate was observed at the concentration used by farmers for the treatment of grazing animals. While the negative control showed a low rate of about 5% on the first day of counting.

Increasing the exposure period decreases the overall mortality rate in both extracts (aqueous and organic). Table 1 shows that after exposure of ticks to extracts, the mortality rate fluctuated with the concentrations.

Mortality rate averages and their standard deviation show differences depending on the concentration of the products used. It is observed that the cumulative mortality percentages of ticks change according to the extracts (Figure 1 and 2).

For aqueous extracts, 100% mortality rate was observed at 20 mg/mL concentration for the 3 days of tick observation. The mortality rate rises 100% on the fourth day for the concentration of 10 mg/mL.

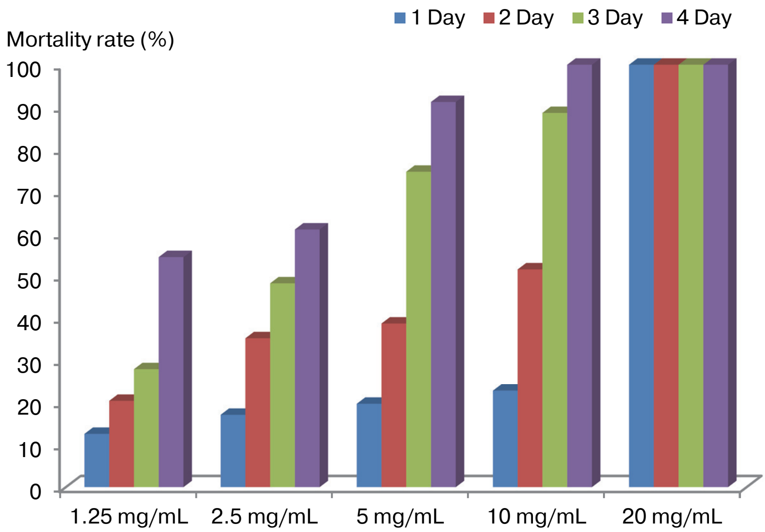

Fig. 1. Cumulative mortality rates of aqueous extracts based on days of exposure

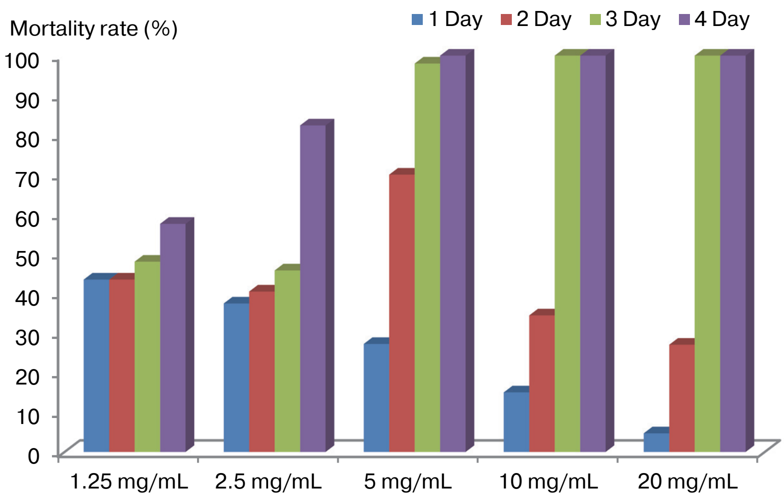

Fig. 2. Cumulative mortalities of ethanolic extracts by days of exposure

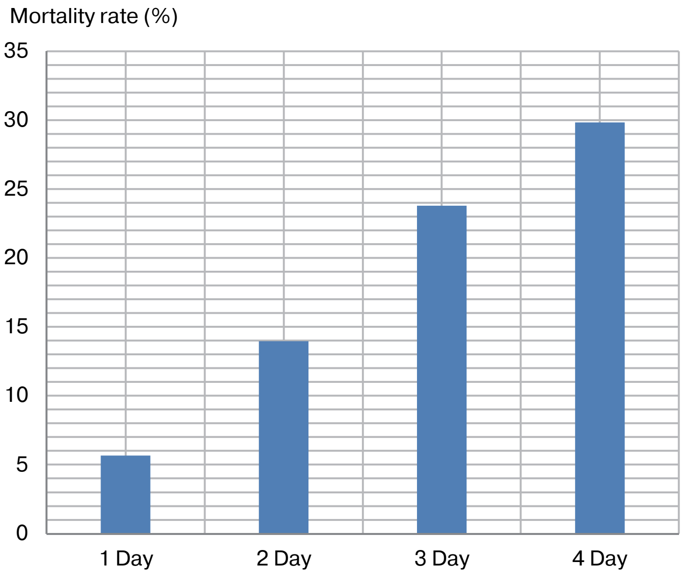

Fig. 3. Mortality rate in the control (water) as a function of days of exposure

There are significant differences between the cumulative daily mortality rates as a function of the concentrations ($p < 0.05$) with an increase in the mortality rate at the end of the fourth day of observation in aqueous extract. But, for ethanol extracts, 100% mortality rate was recorded on the third day at the concentration of 10 mg/mL and on the fourth day at the concentration of 5 mg/mL.

The mortality rate of ticks *Rhipicephalus appendiculatus* (Figure 3) brought into contact with water as a control is low and varies with exposure time.

The mortality rate of ticks in the control was less at the fourth day of exposition. It was less than 30% as reported by other authors [11, 12]. This low rate would probably be due to the manner in which the ticks were harvested (uprooting) from the animals, they would have been traumatized and some would have been injured.

For the positive control, we used Bayticol which showed 100% mortality for the concentration diluted to 1% used in the treatment of livestock in veterinary medicine. As this product is synthetic, it kills ticks directly after contact. This observation was also made for ticks treated with a concentration of 20 mg/mL aqueous extracts. Although Bayticol is used in livestock treatment in South Kivu, ticks continue to cause significant livestock damage due to the lack of access to this product by most herders. Experimentation with extracts of *Vernonia amygdalina* in the laboratory shows a cumulative mortality rate of ticks which varies with the concentration of the extracts and decreases with the time of experimentation. The aqueous extracts show a gradual decrease in mortality as the ethanolic extracts. This would be due to the dissolution of the substances responsible for this insecticidal activity. Indeed, despite their low efficacy on ticks *Rhipicephalus appendiculatus*, these extracts were able to generate a mortality rate that exceeds that of the negative controls (water) after 4 days.

In the aqueous extracts, the variation in the percentages of mortality decreases as a function of the concentration of the products. Organic extracts varied also with concentration of the extracts tested as a function of time. This is due to the active acaricide substances responsible for the activity found in the plant *Vernonia amygdalina* [6]. These crude extracts of the aqueous phase of this plant *Vernonia amygdalina* which have shown an acaricidal activity, should be separated to isolate and concentrate the active substances which, certainly, could have much lower lethal concentrations and should be able to be promoted as an alternative acaridical substances in a region devastated by repetitive wars and cut off imports of acaride products. This plant would be a palliative solution insofar as it is accessible to the population. Indeed, active substances responsible for this activity is more soluble in water than in ethanol; which shows that the aqueous extracts are more active than the organic extracts based on ethanol that was used in the acaricide activity test of this plant. Determination of DL_{50} concentration versus the logarithmic dose of the aqueous extracts for the first day of exposure yielded the following regression equation:

$$Y = 18.07\,X - 19.85\ (R^2 = 0.77).$$

DL_{50} calculated from this equation is 15.44 mg/mL for the aqueous extracts of *Vernonia amygdalina*. For ethanolic extracts, the mortality rate varies for the first day of contact; it normalizes on the third day.

This would probably due to the amount of active substances soluble in these ethanolic extracts. The phytochemical screening (Table 2) of this plant shows that it contains more phenol, tannins, flavonoids, saponins and glycosides.

But also in the extracts, alkaloids and quinone which are known acaricides are present as found Chifundera and his collaborators [7]. This plant is used for different medicinal treatments because it contains several chemical substances that act differently [13]. Indeed, since plant extracts are composed of several compounds with a multiple action mechanism, they simultaneously solicit several physiological defense mechanisms as opposed to specific pesticides that may have only one molecular target.

Results of variance analysis of concentration-corrected daily mortality data from plant extracts *Vernonia amygdalina* showed significant differences between concentrations ($p < 0.05$) and extracts (aqueous and organic). This has also been observed in other tick studies [7, 14, 15] *Vernonia amygdalina* is a medicinal plant that contains other substances such as carvanol, flavonol, tymol that may be responsible for the insecticidal activity observed in some trials [13, 16]. Some substances endowed with biological activity have been isolated from the plant *Vernonia amygdalina* and would probably be the cause of the mite effect. Of these substances, Vernodalin and Vernosioside are known to have insecticidal, bacterial and anthelmintic activity [13]. It has been shown in previous studies that the activity of some plants may be due to the action of the 3, 4, and 7-trihydroxy-3-methoxy-7-glucoside flavone enzyme as in the plant *Deverra*

Table 2

Phytochemical Screening Results

Substances	*Vernonia amygdalina*
Saponines	+ + +
Alkaloids	+ +
Glucosides	+ + +
Flavonoids	+ + +
Tannins	+ + +
Lipids and lipoids	+
Steroids	+
Terpenoids	+
Phenols	+ + +
Quinone	+ +

Legend: + Test slightly positive
++ Positive test
+++ Strongly positive test

scoparia. This enzyme has been found to inhibit the carboxylesterase responsible for resistance observed in animals [17]. Thus, natural plant extracts can be the tools of choice in pest resistance management programs for pesticides such as Thiodan, Dithane, Dichloro diphenyl Trichloro Ethane.

Indeed, the use of extracts of acaricide plants in the laboratory using the direct contact method is very effective at high concentrations (10 mg/mL). The results of this study show that the application of aqueous extracts is one of the methods to be used in the fight against cattle ticks. Indeed, natural insecticides are easily available and cost nothing for their preparation. In addition, they are not toxic to curative doses in humans and degrade rapidly in the environment [18].

The success of plant extracts in the fight against *Rhipicephalus appendiculatus* is certainly due to the fact that they combine certain advantages such as great protection for the environment while offering the possibility of being used in the manner of a chemical insecticide.

According to studies by Chiasson and Beloin [19], no case of resistance to plant extracts has been found. Thus, the use of *Vernonia amygdalina* in the fight against ticks is very interesting means control as this plant has already been used by breeders without knowing the lethal concentrations. Some other plants are used by breeders in other countries such as in Benin and has been proved their potential effect on ticks [20—22]. Plant extracts are made up of several chemical substances with different multiple action mechanisms; these directly result in several defense mechanisms of the target species, unlike synthetic pesticides with only one active molecule. This advantage of plant extracts allows us to consider that after thorough studies, the use of these natural extracts in the fight against ticks would be a path of integrated pest management solution.

Conclusion

This work confirms the potential of *Vernonia amygdalina* against ticks *Rhipicephalus appendiculatus* in the laboratory. The results of this study show that the aqueous and organic extracts of the leaves of this plant are toxic for ticks *Rhipicephalus (Boophilis) appendiculatus*. The percentage of mortality increases with the increase in the concentration of extracts and decreases with the exposure time. These results encourage us to carry out a study of the activity of these extracts for the treatment of ticks in livestock farming, especially in villages where access to synthetic products is a problem. The effective substances present in the extracts should first be identified for isolation and subsequent testing. It will thus be possible to develop preparations that can have acaricidal effects. This would allow for an integrated pest control program to effectively control ticks that target livestock. *Vernonia amygdalina* is a common plant in the region; its use by breeders would be less expensive, effective, having low environmental impact and would contribute to the fight against cattle diseases transmitted by ticks.

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Научная статья

Биоцидная активность экстрактов *Vernonia amygdalina* против клещей, вызывающих болезни скота

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Аннотация. Биоцидную активность *Vernonia amygdalina* оценивали на клещах *Rhipicephalus appendiculatus*, ответственных за заболевания скота в северных и южных провинциях Киву. Испытания в пробирке проводились в лаборатории Научно-исследовательского центра Лвиро, чтобы определить смертельную дозу для разных сроков после контакта клещей с экстрактами. Были оценены пять различных доз 1,25, 2,5, 5, 10, 20 г/мл. Вода рассматривалась как положительный, а Батикол — отрицательный контроль. Фитохимическая обработка экстрактов растения *Vernonia amygdalina* показывает, что это растение содержит несколько веществ, ответственных за акарицидную активность, в основном сапонины, терпеноиды, стероиды и дубильные вещества. Эксперимент показывает уровень смертности, который зависит от концентрации экстрактов (наиболее фатальна 20 г/мл) и уменьшается со временем воздействия. Водные экстракты показали прогрессивное снижение смертности по сравнению с этанольными экстрактами. Это связано с растворением веществ, ответственных за инсектицидную активность. В исследовании рекомендуется применение в борьбе с клещами крупного рогатого скота водных экстрактов *Vernonia amygdalina*, природного инсектицида, доступного и легко приготавливаемого, не токсичного для человека и быстро разлагающегося в окружающей среде.

Ключевые слова: биоцидный, *Rhipicephalus appendiculatus, Vernonia amygdalina*, водный экстракт, этанольный экстракт

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