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Anthelmintic Activity Assessment of *Melanthera albinervia*, *Conyza sumatrensis* and *Cyperacium nathera* Used in Kalemie (DR Congo) Against the Goat Gastrointestinal Parasites

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Abstract: Study aim: This study compares the anthelmintic activity of three medicinal plants namely *Melanthera albinervia* (Asteraceae), *Conyza sumatrensis* (Asteraceae) and *Cyperacium nathera* (Cyperaceae) used in Kalemie and its surroundings against the gastrointestinal parasites beside the therapeutic effect of the ivermectin. Method: Each plant dried away from the sunlight, crushed and 30g of each drug is administered to goats, *in vivo*, to evaluate the therapeutic effect by comparing the reduction of the eggs number per gram of feces in the group of goat treated by ivermectin and the untreated group. Result: The coprological analysis before treatment revealed that the helminths prevalence in all studied goats with an average parasite load of 3850 ± 240.44 eggs per gram of feces. After treatment, the lab analysis showed that the therapeutic effect rate of the studied plants was ranging from 72.9% on the 12th day to 44.3% on the 20th day for *Conyza sumatrensis*, 84.8% on the 12th day to 85% on the 20th day for *Melanthera albinervia* and 89.5% on the 12th day to 79.5% on the 20th day for *Cyperacium nathera* against an average therapeutic effect rate of ivermectin ranging from 75.1% on the 12th day to 92.8% on the 20th day. Conclusion: The plant studied, by their ability to reduce the number of parasites eggs in the feces of goats studied and by their therapeutic efficacy, appear to be the alternative to the parasites control in the goat breeding.

Keywords: Anthelmintic-Goats-Kalemie-Gastrointestinal-Phytochemical Vermifuge

1. Introduction

The goats breeding is one of the cost-effective pastoral activities in the Kalemie and Moba territories and their surroundings, because the reproductive cycle of these animals is short and their diet is without any great requirements [1, 2]. This goats breeding helps to diversify and to increase the household incomes and to secure the family economy by allowing savings of farmers [1].

In the territory of Kalemie and its surroundings in DR Congo, the goat usually breeded for its meat, source of animal protein, building economic tissues and social values in rural areas [3]. Unfortunately their production and profitability remain low. This weakness is due, among others reasons, the poor livestock management and the health situation rather poor [4].

The goat gastrointestinal parasites goats are a problem that

comes back periodically in the farms; they are the permanent goat pathologies in the breeding environment because the vaccination and the deworming are rare, if not non-existent. The losses in animal heads per farmer per year are considerable; the use of antibiotics and synthetic vermifuges purchased on the local markets is almost non-existent [3, 5].

The gastrointestinal parasitic diseases are the most pronounced and the most formidable of all sixteen groups and 227 villages of the Territory of Kalemie [5]. These pathologies pushed ranchers and farmers to sell sick animals cheaply and for others to completely abandon the breeding career while she was among the main sources of income and savings; this is due to the lack of anthelmintic products, the ignorance of the existence of veterinary products, the ignorance of the veterinary herbal remedies or the ineffectiveness of some alleged chemicals for the symptomatic treatment of the goat gastrointestinal parasites and certain other animal diseases [3, 5].

In the villages of the territory of Kalemie, the synthetic vermifuges, although tolerated for occasional use which represents the short term solution in the farm, is not used because there is not the goat veterinarians who could monitor the framework map as everywhere in the DRC [5, 6]. Moreover, the synthetic anthelmintics are the usual means to fight against these parasites. However, the appearance of helminths populations resistant to these synthetic anthelmintic is increasingly frequent [7, 8]. The research of the new anthelmintic molecules, alternative or complementary, with the novel action mechanisms, was the subject of several studies [9, 10].

The populations of the city of Kalemie and its surroundings, in fact, use many recipes for the treatment of the gastrointestinal parasites. That is why the WHO says that nearly 75% of the African population has recourse to plants to treat the human or the animal diseases and has no access to "modern" drugs [11].

Many plants used in traditional medicine are as effective as the drugs imported by Africa which are unknown for the most of the African population [12-14]. Thus, it is for scientists to identify these traditional recipes, experiment *in vitro* and *in vivo* and to determine the most effective recipes. In this context, the ethnobotanical survey beside herders, traditional healers, veterinarians and agronomists of the territories of Kalemie and Moba was done between May 2013 and July 2015. Three plants were selected from those that were most cited or more considered effective by respondents. Then, in vivo assessment of the anthelmintic activity was done on the goats to evaluate their therapeutic effect on the gastrointestinal parasites in comparison with the therapeutic effect of the ivermectin, a synthetic anthelmintic for which the effectiveness was already demonstrated.

Then, the aim of this work is to determine which one among these three plants (*Melanthera albinervia* O. Hoffm. (Asteraceae), *Conyza sumatrensis* (Retz.) E. Walker. (Asteraceae) and *Cyperacium nathera* (Cyperaceae)) will be more efficient in the ability of the reduction of number of eggs per gram of feces and could be recommended to farmers after approved phytochemical and biological studies.

2. Study Area

The experimental work was conducted on goats in confinement in the city of Kalemie in the neighborhood Kichanga on Kahinda Avenue at the lab of anti-tuberculosis and leprosy center (CATAL) in the Democratic Republic of Congo, from 20/06 to 10/07/2015.

Geographically, the neighborhood Kichanga is situated at the north-east of the city of Kalemie, on the Lake Tanganyika coastal plain and borders the valley almost at the estuary of the Lubuye river (5°53'22,8" South latitude and 29°13'37,9" East longitude). The coprology samples were collected at the farm before being analyzed in the lab of anti-tuberculosis and leprosy center (CATAL) from Kalemie General Hospital (5°55'10'' South latitude and 29°11'22,55"de East longitude).

3. Material and Methods

3.1. Plant and Animal Material

In this study, for the experiment, we used:

- Three plant species including *Melanthera albinervia* (Asteraceae), *Conyza sumatrensis* (Asteraceae) and *Cyperacium nathera* (Cyperaceae) as phytochemical anthelmintic.
- Twenty Goats (age, sex and different physiological states).
- Ivermectin as synthetic anthelmintic [15, 16].

The block system (4 blocks corresponding to 4 groups each comprising 4 goats) was adopted to evaluate the therapeutic effect of three medicinal plants considered more effective by the respondents against the gastrointestinal parasites compared to that of ivermectin.

The 30g of powder in 250mL of water was administered to each animal once to compare with the dose of 0.2mg/kg of ivermectin. The aim was to put all the animals in the same conditions of treatment but during the survey, the administration of the drug recipe was done three days successively [17]. For this study, we choose the single dose like Dorchies (2000) [18].

3.2. Evaluation of the Anthelmintic Activity

The evaluation of the anthelmintic activity of the solutions based on *Conyza sumatrensis*, *Melanthera albinervia* and *Cyperacium nathera* was made by OPG reduction test [2,10,19-20]. In fact, the following treatment groups were formed:

Group 1 (T0) received no treatment (Negative control);

Group 2 (T1) was treated with *Conyza sumatrensis* (P1);

Group 3 (T2) was treated with *Melanthera albinervia* (P2);

Group 4 (T3) was treated with *Cyperacium nathera* (P3);

Group 5 (T4) was treated with ivermectin at a dose of 0.2 mg / kg (Positive control).

During the experiment, the goats were fed a ration based on palm kernel cake and rice bran and would not graze until

1:00 up to 5:00 pm. The goats were weighed using a balance at the beginning and at the end of the experiment to assess weight variation.

The techniques of coprological analysis before and after administration of drug (Phytochemical and synthetic vermifuge) have been used to determine the number of eggs per gram of feces (OPG) and the prevalence of different species of gastrointestinal helminths. The coprology began by the collect of dung directly in the rectum, in the morning in all 20 goats of the experimentation. Each animal was previously marked by shearing and each sample was placed in an isothermal box labeled with the goat number and then analyzed in the medical lab. These samples were kept in the refrigerator until preparation for the coprological analysis. The fecal analysis was carried out by flotation techniques in a saturated solution of NaCl 40%. In this test, two methods were used: qualitative enrichment method and the quantitative enrichment method. Indeed, the qualitative method of enrichment was used to assess the taxonomic egg quality of the gastrointestinal parasites visible to the photonic microscopy. For this, we used the flotation technique in the test tube (Willis Technique). For quantification of eggs per gram of feces (OPG), we used the technique of Mc Master [21]. For this purpose, by the lack of the Mc Master cells, an automatic micropipette was used to collect 0.15 mL of the filtrate which corresponds to the volume of both chambers of the Mc Master cell. As feces are diluted in the ratio of 1÷15 and we withdrew 0.15 ml in 60 ml of total volume, then counted the number of eggs that is contained in one hundredth of a gram of feces. For the number of eggs per gram, the result obtained when counting is multiplied by 100.

3.3. OPG=Number of Eggs Counted x 100

To assess the effectiveness of treatments, analysis of OPG for each animal before treatment and the 12th and 20th days after treatment was done. And effectiveness of treatment was calculated according to the Presidente method (1985) [22] which considers the means OPG before and after treatment using the formula:

$$E(\%) = \left[1 - \left(\frac{T_1}{T_2} \times \frac{C_2}{C_1}\right) \times 100\right] \quad (1)$$

With E%: efficiency rates; T1: OPG nth day after treatment; T2: Initial OPG of the control group; C1=: OPG nth day after treatment in the control group; C2: Initial OPG of the control group.

3.4. Statistical Analysis

The results of the experiment were processed and analyzed using the Statview software (1997) by the analysis of variance test (ANOVA) at the 5% threshold completed by the Fisher LSD test when ANOVA showed a significant difference.

4. Results

The results below present the prevalence of gastrointestinal parasites before treatment, the number of eggs per gram of feces (OPG) before treatment and on the 12th and 20th days after treatment with the vermifuges. The therapeutic efficacy of each drug on the 12th and 20th days is noted respectively E1% and E2%. They also relate to the weight variation during the experiment.

4.1. The Prevalence of the Different Species of Gastrointestinal Helminths

The table 1 shows the prevalence of gastrointestinal parasites in all goats studied. The most frequent helminths species are *Haemonchus contortus* and *Avitellina centripuncta* which have the prevalence of 100%. They are followed by *Strongyloïdes papillosus* (75%), *Monieza expansa* (50%) and *Fasciola hepatica* (25%) in the goats of 3-6 months. Furthermore, in the goats whose the age varies between 7 and 25 months, in order of their prevalence, we found *Haemonchus contortus* (100%), *Avitellina centripuncta* (60%), *Strongyloïdes papillosus* (50%), *Monieza expansa* (10%), *Fasciola hepatica* (10%) and *Schistosoma bovis* (10%).

Table 1. *The prevalence of different gastrointestinal parasites species.*

Age (Month)	*Strongyloïdes papillosus*		*Haemonchus contortus*		*Avitellina centripuncta*		*Schistosoma bovis*		*Monieza expansa*		*Fasciola hepatica*	
	Pre%	OPG	Pre%	OPG	Pre%	OPG	Pre%	OPG	Pre%	OPG	Pre%	OPG
3-6	75	370	100	700	100	800	00	00	50	350	25	100
7-25	50	390	100	950	60	610	10	100	10	100	10	10

Legend: Pre: prevalence

4.2. Evolution of OPG and Therapeutic Efficacy of the Plants Used

Table 2. *Evolution of average OPG during the study.*

OPG nth day	Treatment					Probability
	T0	T1	T2	T3	T4	
D0	3850^{a}	3825^{a}	4175^{a}	3500^{a}	3900^{a}	0,9938
D12	4075^{a}	1250^{b}	625^{b}	325^{b}	900^{b}	0,0001
D20	3925^{a}	1975^{b}	625^{c}	500^{c}	250^{c}	0,0001

Legend: $^{a, b, c}$: the average values by different letters in the same row are significantly different at $p < 0.05$; D: day

The table 2 above, concerning the OPG, shows that before to start the experiment (D0), no significant differences between different groups of goats at p <0.05 was found. Furthermore, the 12th day after treatment, the OPG of all the treated groups have a very highly significant difference compared with those of untreated group (p <0.0001) but there is no significant difference between the OPG of all treated group. Finally, on the 20th day after treatment, the OPG of the untreated group are highly superior to those of the groups T2, T3, T4 (p <0.0001) and superior to those of the group T1 (p=0.006). We observed a significant difference in the OPG of the group T1 compared to the groups T2, T3 and T4 but there was no significant difference between OPG of the groups T2, T3 and T4 (p <0.05).

Table 3. *Therapeutic efficacy after treatment on the 12th and 20th days.*

Therapeutic efficacy E (%) at nth day	Treatment				Probability
	T1	T2	T3	T4	
D12 (E1%)	72,9[a]	84,8[ab]	89,5[b]	75,1[a]	0,0384
D20 (E2%)	44,3[a]	85[b]	79,5[b]	92,8[b]	0,0019

Legend: [a, b]: the average values by different letters in the same row are significantly different at p <0.05; D: day

The table 3 above, relative to the therapeutic efficacy of different plants on the 12th and 20th days after treatment, shows that the therapeutic efficacy on the 12th day after treatment of *Cyperacium nathera* (T3) is slightly greater than that of all other treatments. The variance analysis at p <0.05 illustrates that there is no significant difference between the efficiency of goats of the groups T3, T2 and T4, but a significant difference with the therapeutic efficacy of the group T1 (p=0.0384).

On the 20th day after treatment, the therapeutic efficacy of ivermectin (group T4) appears higher than all other treatments. The analysis of variance at the threshold of 5% indicates no significant difference in the therapeutic efficacy between *Melanthera albinervia* (P2) *Cyperacium nathera* (P3) and ivermectin (Positive control); by cons there is a very highly significant difference between the therapeutic efficacy of the group T2 and the group T4 compared to that of the group T1 (p <0.001) and a highly significant difference in the therapeutic efficacy of groups T2 and T3 facing the group T1 (p=0.0042).

4.3. The Average Daily Weight Variation During the Experiment (ADWV)

During the experiment, the goats studied showed an ADWV of 122.08 ± 44,8g overall weight/day. The groups T2, T3 and T4 presented an ADWV very highly superior to that of untreated group (T0) after analysis of variance (p <0.05).

The analysis of variance at the same threshold of significance (5%) indicated no significant difference between the ADWV of group T1 and that of the untreated group (T0) and between the ADWV of the groups at T2, T3 and T4. By cons, a highly significant difference of the ADWV of the group T2 compared to the group T1 (p=0.0104) and a significant difference in ADWV for the group T3 and T4 compared with the group T1 (p=0.03) were observed.

Table 4. *Average daily weight variation (ADWV) of goats after treatments with vermifuges.*

Parameter	Treatment					Probability
	T0	T1	T2	T3	T4	
ADWV (g/d)	16,2 ±45,3[a]	81,2 ±55,4[a]	183,7 ±69,9[b]	162,5 ±25[b]	166,6 ±28,8[b]	0,001

Legend: [a, b]: the average values by different letters in the same row are significantly different at p <0.05

5. Discussion

5.1. Prevalence of the Different Species of Gastrointestinal Helminths

The qualitative microscopic examination of feces showed a prevalence of gastrointestinal parasites in 100% goats studied. Some species of helminths such as *Haemonchus contortus* and *Avitellina centripuncta* were observed in all the goats. The prevalence of *Strongyloides papillosus*, *Monieza expansa* and *Fasciola hepatica* are respectively 75%, 50% and 25% among the goats of 3-6 months while for the goats whose the age ranged between 7-25 months, the prevalence of gastrointestinal parasites species was 100% for *H. contortus*, 60% for *Avitellina centripuncta*, 50% for *Strongyloïdes papillosus* and 10% for *Monieza expansa*, *F. hepatica* and *Schistosoma Bovis*.

These results are similar to those reported in the research conducted by Okombe et al (2013) [20] in the farm of the Veterinary Faculty of the University of Lubumbashi (DR Congo). They found in goats an average prevalence of 81 to 86% of the gastrointestinal strongyles species of goats and specifying that this prevalence varies by season and age.

This prevalence of gastrointestinal helminths is linked not only to the fact that the identified parasites are common for ruminants, but also to poor breeding conditions and lack of veterinary care [23].

The presence of *Schistosoma bovis*, a bovine specific trematode in goats, could be explained by the fact that the goats of this experiment jointly share the pasture with cows;

there would be a mixed infection of ruminants as demonstrated by Doumenc (2003) [24] in his researches on the goat helminth faun in Saone-et-Loire, which is influenced by the mixed pasture with cows in France.

5.2. Evolution of OPG and Therapeutic Efficacy of the Plants Used

The goats of different groups showed a heavy infestation at the beginning, with an overall average of 3850±240.44 OPG. These OPG are lower than those reported by Monolaraki (2011) [25], but considered as sufficient to undertake the evaluation of anthelmintic activity [26].

The evolution of OPG and therapeutic efficacy showed that single treatment with *Conyza sumatrensis* (T1) resulted in a mean reduction of OPG with a therapeutically effective rate to 72.9% on the 12th day and 44.3% on the 20th day. This low activity manifested by the small reduction in OPG and low effectiveness rate may be explained by the fact that their bioactive substances such as tannins, flavonoids, saponins, steroids, terpenoids, phenolic acids and glycosides have a short-term action and would be eliminated quickly in the organism; there would be a possibility of infestation during the experimentation [27]. These results are different from those found by Hoste (2002) and Fayer (2010) on goats with most of the phytochemical vermifuges [19, 23]. This small reduction suggests that we should use *Conyza sumatrensis* (T1) in the short interval, for example three times as observed by Hounzangbe-Adote et al (2008) with the seeds of *Carica papaya* on gastrointestinal strongyles of small ruminants in Southern Benin [28].

We also observed a decrease in therapeutic efficacy with the treatment based on *Cyperacium nathera* (T3). This case led to a considerable reduction of OPG and therapeutic efficacy rate of 89.5% on the 12th day to 79.5% on the 20th day.

The single treatment with *Melanthera albinervia* (T2) resulted in a considerable reduction of OPG with therapeutic efficacy rate of 84.8% on the 12th day and 85.05% on the 20th day. This strong therapeutic efficacy is linked to the saponins and the tannins, especially to thiarubrine A and essential oil contained in this plant [29-31]. In addition, Kuiate et al. (1999) have shown that the essential oils obtained by the hydrodistillation of *Aspilia Africana* leaves (currently *Melanthera albinervia*) contain monoterpenes and sesquiterpenes [32]. Indeed, the saponins, the condensed tannins and the terpenes have potent antimicrobial and antiparasitic properties [33-37]. The chemical composition of this plant would justify its use in self-medication in chimpanzees against gastrointestinal parasites [38-40].

Finally, the single treatment with ivermectin (0.2mg/kg) resulted in a significant reduction in therapeutic efficacy rate of 75.1% on the 12th day to 92.8% on the 20th day. These results are similar to those found by Okombe et al. (2013) compared with the *Curcubita muscheta* seed powder. These researchers obtained an average efficacy of 93% with ivermectin on the 20th day in treatment of goats [20].

Compared with the positive control, from the 12th day, the therapeutic efficacy rates of *Melanthera albinervia* (84.8%) and *Cyperacium nathera* (89.5%) were higher than that of ivermectin (75.1%). By cons, on the 20th day, the rate of therapeutic efficacy of ivermectin is increased from 75.1% to 92.82% but for *Melanthera albinervia*, the therapeutic efficacy rates increased slightly from 84.8% to 85.05% and for *Cyperacium nathera*, it was observed a decrease of the therapeutic efficacy rates from 89.5% to 79.5% (A variance of 10%). These results are similar to those found by Fayer (2010) with the *Vernonia amygdalina* leaves and the *Carica papaya* seeds and those of Okombe et al. (2013) with the *Curcubita mouscheta* seeds [19, 20].

The similarity between the results observed for animals treated with ivermectin and the goats which received the macerate of *Melanthera albinervia* (T2), of *Cyperacium nathera* (T3) and *Conyza sumatrensis* (T1) compared to negative controls suggests that all three plants had an effect on the gastrointestinal helminths of the goats studied. The depth phytochemical studies will elucidate later the active principles responsible for anthelminthic activity of these plants.

5.3. The Average Daily Weight Variation During the Experimentation (ADWV)

During the experimentation, the goats studied have experienced an average daily weight variation of 122.08 ± 44.8 g (Table 4). All groups (T2, T3 and T4) showed an ADWV greater than that of the untreated group of goat studied (T0). This is explained by the fact that helminths have a large impact on food conversion by reducing enteric absorbent surface [23] and they are plunderers [2, 41]. These observations are consistent with those identified in the study of Jarrige (1999) on the digestive helminths of the ruminants in growing [42].

6. Conclusion

During the evaluation of the anthelmintic activity of *Conyza sumatrensis*, *Melanthera albinervia* and *Cyperacium nathera*, the decrease of the gastrointestinal helminths eggs in the goat fecal excretion after administration of solutions of these plants allows to assume the existence of bioactive chemical compounds in these plants studied.

The decrease of the gastrointestinal helminth eggs highlights the interest that can provide the use of these three plants in the control of gastrointestinal parasites in the goat breeding.

Indeed, the plants studied appear to have an ability to be retained among the alternatives to the parasites control in the goat breeding; however, it is necessary to complete this biological study by the phytochemical and toxicological studies and to better define the optimal conditions for their use in the breeding of goats.

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