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In Vitro phytochemical screening and anthelmintic activity of *Viscum congolensis* and *Galiniera coffeoides* against adult earthworm *Alma emini*

Jean-Louis BAHIZIRE KAYEYE[1], Bertin NDEGEYI KABALE[1], Pierre BATUMIKE CISHIBANJI[1], Jean-Jacques BAGALWA MASHIMANGO[1], Jean-Pierre BALUKU BAJOPE[1], Augustin BASHWIRA SANVURA[1-2], Augustin BASABOSE KANYUNYI[1], and Bahati BAGALWA[1]

[1]Département de Biologie, Centre de Recherche en Sciences Naturelles, RD Congo

[2]Département de Chimie, Institut Supérieur Pédagogique de Bukavu, RD Congo

ABSTRACT: This study was conduct at the Centre de Recherche en Sciences Naturelles de Lwiro to evaluate the anthelmitic activity of aqueous and ethanolic extracts of *Viscum congolensis* and *Galiniera coffeoides*, two plants collected in Kahuzi-Biega National Park. The objective was to assess their activity against earthworms *Alma emini*. The aqueous extracts are efficacy than ethanolic extracts. Their concentrations varied between 2 and 0.2 mg/mL. These results were compared to positives control (Mebendazole, Albendazole and Decaris) and negative one (water). The detailed are necessary for the isolation, characterization and evaluation of the substances responsible of anthelmitic activity and the action mode of the substance identified.

KEYWORDS: Anthelmitic, *Viscum congolensis, Galiniera coffeoides*, aqueous, ethanolic extracts, *Alma emini*.

1 INTRODUCTION

Helminth infections are extremely common intestinal parasites in children more than adults. It is one of the major prevalent diseases in the world, particularly in the tropical countries which affect more than a billion people around the world and many cases are lethal [1], [2], [3]. It is a real public health problem and even for animals productivity [4]. They are both cause and effect of malnutrition in some agroecological zones in Africa [5]. This situation is exacerbated in developing countries where the socio-economic situation is a serious constraint [6], [7]. This situation has not spared in Democratic Republic of Congo in general and the South Kivu province in particular where several cases are observed and where parasites identified hitting the majority of the population of all ages in rural areas [8].

In recent times, there has been an increasing interest in ethnomedical and ethnoveterinary practices across the world especially as it relates to the use of medicinal plants in treating various diseases. The control of helminth infections was started long time ago in the Occidental and Asian regions, and its result in the establishment of some anthelmintics drugs known in the modern international pharmacopoeia such as mebendazole, piperazine, albendazole, etc... Most people in developing countries use traditional medicine for their primary needs of primary health care due to the high cost of these synthetic products [9], [10], [11]. This is quite evident in communities that are geographically, economically and culturally isolated and in which drugs have difficulty not often at everyone. This data is favored by several factors including socio-economic factors, ignorance of modern anthelmintics, the lack of health infrastructure, lack of qualified and pharmacists [6].

However, traditional healers use herbal extracts to treat helminth infections without knowing the active ingredients that act effectively, the secondary effects of the solution administrated as well as doses to be used. The symptoms which they refer are general and not enough for a reliable diagnosis. It follows from this state of affairs many cases of groping without exposing patients to the risk of poisoning. Treatments are available but costs limit their application.

Corresponding Author: Jean-Louis BAHIZIRE KAYEYE

Anthelmintic plants have rarely been specific inventory work in the Democratic Republic of Congo in general and South Kivu in particular. Some authors have only flown over the issue during the ethnobotanical surveys that focused on traditional medicine as a whole [5], [12], [13]. The listed plants having anthelmintic activity are *Momordica foetida*, *Indigofera arecta*, *Celosia trigyna* [5, 13]. *Viscum congolensis* and *Galiniera coffeoides* has been suggested as a plant used by wild chimpanzees and gorillas for self-deparasitization [14], [15], [16], [17]. In the present work, we examine in vitro anthelmitic effect of the two plants *Viscum congolensis* and *Galiniera coffeoides* consumed by great apes for self-medication against the earthworms *Alma emini*.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND PHYTOCHEMICAL SCREENING

Viscum congolensis and *Galiniera coffeoides* plants which are the subject in this study were collected in Kahuzi-Biega National Park (02°14'0.28'' S, 028°47'6.26'' E and 2000 m of altitude). The plants were identified at the Laboratory of Systematic Botany, Department of Biology, Centre de Recherche en Sciences Naturelles of Lwiro, Democratic Republic of Congo were double samples are kept in the herbarium sheets.

The plant material was dried in the open air and reduces to powder. Extracts were prepared using exact weighed sample powder in the measured volume of solvents like, deionized water, ethanol, hexane and benzene. Qualitative assay, for the presence of plant phytoconstituents such as alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, quinine, phenols and saponins were carried out on following standard procedure Brain and Turner [18] and Trease and Evans [19].

2.2 ANTHELMINTIC ACTIVITY

2.2.1 COLLECTION OF EARTHWORMS

Earthworms that we used in the bioassay of anthelmintic activity are the species *Alma emini*, an oligochaete glossoscolecid worm. These worms are used for the test because of their anatomical and physiological resemblance with the intestinal round worm parasite of human being [7], [20], [21]. These worms were collected in the wetland surrounding the Centre de Recherche en Sciences Naturelles of Lwiro. They were caught in the juvenile stage (3-5 cm in length and 0.1 to 0.2 cm in diameter) and transported to the laboratory in jars in the mud.

2.2.2 PREPARATION OF DILUTIONS

The dried residue extracts containing the active ingredients previously weighed, were taken up in 5 ml of deionized water. Various concentrations were made ($1/10^{em}$) with deionized water. Three concentrations were made for each plant extracts and other intermediate solutions were performed following dilution technique.

The positive control solutions were separately formed by crushing 100 mg of mebendazole tablet (100 mg) and albendazole (400 mg) using a mortar to obtain fine powders which dissolve each of powder in 100 ml of distilled water to obtain a solution of mebendazole respectively (1mg/ml), a solution of decaris (5mg/ml) and a solution of albendazole (4mg/ml) [11], [22], [23], [24].

2.2.3 ANTHELMINTIC ACTIVITY

The test was performed on adult earthworms of the species *Alma emini* following method Sollman which is a safe and reproducible method requiring any anthelmintic substance is toxic to earthworms and a substance that is toxic to earthworms can be considered as an anthelmintic [11], [20], [21]. Thus, five earthworms *Alma emini* are placed in a petri dish containing 5 ml of each of the aqueous extracts (3 dilutions), ethanolic extracts (3 dilutions) doses, deionized water (negative control) and reference standard albendazole, decaris and mebendazole (positive control). The mortality rate was noted after 24 hours. The counting was to remove separately to different petri dishes and then immerse them in containers with deionized water. After 30 minutes, the earthworms *Alma emini* killed were separated with others. Each test was repeated at least three times. Mortality rates was computerized to give the lethal concentration (LC_{00}, LC_{50} and LC_{100}) determined by probit statistical curves analysis [25].

3 RESULTS

The results of this study are presented in the following tables: these are the results of the phytochemical screening, testing anthelmintic activity and the determination of the lethal dose.

3.1 PHYTOCHEMICAL SCREENING

The phytochemical screening of two plants showed the presence of substances such as tannins, flavonoids and terpenes from the other substances (Table 1). These substances are strongly present in these two plants. Other substances such as saponin, alkaloids, phenols, steroids are also found in both plants.

Table 1: Results of phytochemical screening

Organic Natural constituants	Plants	
	Viscum congolensis	*Galiniera coffeoides*
Alkaloides	+ + +	+ + +
Tannins	+ +	+ + +
Terpenoids	+ + +	+ + +
Steroids	+ + +	+ + +
Saponins	+ + +	+ + +
Flavonoids	+ + +	+ + +
Quinons	+ + +	+ +
Glycosides	+ + +	+ + +
Lipoides	+ + +	+
Phenols	+ + +	+ + +

Legend: *- + + +: strong positive reaction*
- + +: Positive reaction
- +: Reaction slightly positive

The presence of these natural organic substances in the two plants would be responsible for anthelmintic activity.

3.2 ANTHELMINTIC ACTIVITY

The anthelmintic activity of aqueous and ethanolic extracts of *Viscum congolensis* and *Galiniera coffeoides* are presented in Table 2.

Table 2. Mortality (%) "in vitro" of aqueous and ethanol extracts of Viscum congolensis and Galiniera coffeoides against Alma emini (average results of three tests)

Plants	Aqueous extract dilutions		Ethanolic extract dilutions	
	Concentration (mg/mL)	Mortality rate (%)	Concentration (mg/mL)	Mortality rate (%)
Viscum congolensis	20	100	20	100
	2	10	2	80
	0.2	0	0.2	0
Galiniera coffeoides	15	100	15	100
	1.5	100	1.5	30
	0.15	0	0.15	0
Mebendazole	1	100		
Albendazole	4	100		
Decaris	5	100		
Deionized water	-	0		

From this table it appears that the total extracts of these two plants *Viscum congolensis* and *Galiniera coffeoides* are active against *Alma emini* after 24 hours of exposure. The mortality rate reached 100 % at the respective concentrations of 1.9 mg/mL and 1.5mg/mL in the aqueous extracts. However mortality was reduced in the organic extracts to a respective concentration of 19 mg/mL and 15 mg/mL. At lower concentrations these two plants do not have anthelmintic activity. Mortality rate of positive control consisting of Mebendazole, Albendazole and Decaris show a rate of 100% for 24 h of exposure. As for the water that made our negative control, the rate is 0% after 24 h of exposure.

3.3 LETHAL CONCENTRATION

The results of determining the lethal dose experimentally obtained are presented in Table 3.

Table 3: Dose lethal or lethal concentration (LC_{00}, LC_{50} and LC_{100}) in mg/mL

	Extracts		
Plants	LC	Aqueous	Ethanolic
Viscum congolensis	LC_{100}	2	3
	LC_{50}	0.2	1.65
	LC_0	0.2	0.2
Galiniera coffeoides	LC_{100}	1.5	5
	LC_{50}	0.02	0.2
	LC_{00}	0.15	0.5

Lethal concentrations of aqueous extract of these two plants are more active than the ethanolic concentrations. The LC_{50} of *Galiniera coffeoides* is 10 times lower LC_{50} *Viscum congolensis*. This shows that the plant *Galiniera coffeoides* contained anthelmintic virtues than *Viscum congolensis*.

4 DISCUSSION

The result of the phytochemical screening revealed that both plants contain substances such as tannins, alkaloids, flavonoids, glycosides and steroids that have anthelmintic activity [26], [27], [28], [29], [30], [31], [32].

[33] and [26] reported that the tannin extracts from *Acarcia nitotica* have a effect on *Hoemocus contortus* larvae of goats. Polyphenolic tannins extracted from bryophytes showed also anthelmintic activity on nematodes [28]. The anthelmintic activity of tannins is attributed to its ability of the related proteins in the gastrointestinal juice in the nutrients available for reducing noise and in parasite death follows [26], [34]. Earlier studies showed that tannins and terpenoids are responsible for anthelmintic activity [29], [35]. Tannins compounds show to interfere with energy generation in helminths parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite and cause death [34]. It is possible that tannins and terpenoids present in the extract of *Viscum congolensis* and *Galiniera coffeoides* produced similar effects as observed in the plant *Prunus domestica* [7].

For flavonoids, Lahlou, [29] show that flavonoids are substances that have an anthelmintic effect. Steroids have also been identified as active ingredients in the seeds of *Butea monosferma* and as anthelmintic activity [32]. Other substances such as quinones, terpenoids and saponins were under the control of and are widely used as an anthelmintic [7], [35]. Comparing our results with those of other researchers on other plants we find that our two plants have anthelmintic activity more pronounced than the ethanolic extracts *Latana camara* [36]. The concentrations used for testing in their work are 10 times higher than the lethal concentrations in our experiments. In this work the tannins are substances responsible for the death of earthworms.

5 CONCLUSION

The result of this study shows that both *Viscum congolensis* and *Galiniera coffeoides* have phytoconstituants which as effective anthelmintic activity comparable to some conventional products used in the modern pharmacopoeia. The details are still necessary to achieve isolate characterize and assess the substances responsible for the anthelmintic activity and mode of action of these phytoconstituants. The aqueous extracts are more effective than ethanol extracts. This justifies the

mode of administration of these products by the traditional healers and even their use by apes in the forest of self-medication in Kahuzi.

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