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Moctar Chaibou
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Habibou Hama Hamadou
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Abdoul Nasser Moussa Bamba
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Zakari Chaibou Ousmane
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Moussa Idrissa
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Arzika Tanimoune
Botanical Laboratory, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Khalid Ikhiri
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

Corresponding Author:
Moctar Chaibou
Laboratory of Natural Substances and Organic Synthetics, Faculty of Science and Technology, University A. Moumouni (Niamey-Niger), BP 1062 Niamey-Niger

In vitro study of the anthelmintic effects of ethanolic extracts of *Bauhinia rufrescens* Lam. (Fabaceae) and *Chrozophora brocchiana* (Vis.) Schweinf (Euphorbiaceae) two plants used as antiparasitic in Azawagh area in Niger

Moctar Chaibou, Habibou Hama Hamadou, Abdoul Nasser Moussa Bamba, Zakari Chaibou Ousmane, Moussa Idrissa, Arzika Tanimoune and Khalid Ikhiri

Abstract
The search for alternatives to anthelminthics drugs becomes essential in order to control gastrointestinal worms because of resistance developed by helminths with regard to synthetic anthelmintics available and the high cost of these. The aim of this work is to evaluate the vermicidal properties *in vitro* of ethanolic extracts of *Bauhinia rufrescens* (Fabaceae) and *Chrozophora brocchiana* (Euphorbiaceae) two plants used as antiparasitic in Niger. Adult worms (*A. galli*) of approximately equal sizes are used. Different concentrations (2g / L, 5g / L and 10g / L) were prepared and tested. The phytochemical screening revealed the presence of tannins, flavonoids, steroids and terpenoids in both. There were not alkaloid and saponins in all extracts. All extracts showed vermicidal activity with 100% inhibition of motility before 24 hours. Compared with the negative control (NaCl 0.9%) which gives 100% inhibition of motility, after 72 hours, these extracts are significantly effective ($p<0.05$). These results confirm their traditional uses as dewormer. Studies on other types of worms will be conducted. Also *in vivo* tests are possible. Further studies will be done in order to elucidate active compounds structure.

Keywords: Anthelminthic, *Bauhinia rufrescens*, *Chrozophora brocchiana*, Azawagh, Niger

Introduction
Helminthiasis or worm infection is one of the most prevalent diseases in the world [1]. Recent estimates of the World Health Organization (2019) suggest that about 1.5 billion people suffer of helminthiases [2]. This infection is most prevalent in tropical and subtropical areas and pose a great threat to both human and animal health [3]. Parasitic worm infection may result in many health disorders such as anaemia, malnutrition, diarrhea, eosinophilia and pneumonia as well as organ damage and death in severe cases [3]. The control of these diseases is based on the use of synthetic anthelmintic drugs. Unfortunately, there are several shortcomings in the use of these drugs. These products are expensive and are still not available. Moreover, anthelminthic drugs resistance is now a global challenge for the control of these disesases [4]. This phenomena of resistance against anthelmintics have led to the proposal of screening medicinal plants for their anthelminthic property. The aim of this study is to evaluate *in vitro* the anthelmintic activity of the ethanolic extracts of *Bauhinia rufrescens* (Fabaceae) and *Chrozophora brocchiana* (Euphorbiaceae) on adults worms (*Ascaridia galli*), dominant poultry nematode in Niger [5, 6]. These plants are known for their many therapeutic properties. Thus, *C. brocchiana* has antimicrobial, antifungal and antioxidant activity due to phenols compounds [7]. *B. rufrescens* has antimicrobial, antioxidant properties due to tannins [8]. In Niger, they are used in the treatment of several types of diseases mainly bacterial and parasitic infections of the gastrointestinal sphere in humans and animals [9-11].

Material and methods
Preparation of extracts
The bark of *B.rufrescens* (Fabaceae) and whole plant *C. brocchiana* (Euphorbiaceae) were harvested during the month of January 2019 in the region of Niamey (Niger). The identification of the plant was made in the department of Biology (Faculty of Science and Technics of University Abdou Moumouni of Niamey). These different parts have been separately washed, dried at room temperature (37 °C) and then powdered.

For each plant fifty grams of powder was macerated in 500 mL of ethanol for 48 hours. The various macerates were filtered and concentrated using a rotavapor. Then for each extract by dissolving in 0.9% NaCl solution (medium survival worms), 25mL of solutions (2g/L, 5g/L and 10 g/L) were obtained. The extraction yields have been determined by the following formula:

$$\text{Yield} = \frac{\text{mass of extract}}{\text{mass of powder}} \times 100$$

Phytochemical screening

The phytochemical screening was carried out according to the standard phytochemical screening protocol [12, 13]. The compounds sought are the alkaloids, tannins, saponins, flavonoids, quinones, steroids and terpenoids.

Anthelminthic activity evaluation

Adult *Ascaridia galli* worms from freshly slaughtered poultry intestines at the Harobanda (Niamey) poultry market were kept in 0.9% NaCl solution and divided into five batches including a negative control receiving only the survival medium (0.9% NaCl), a positive control receiving a solution of levamisole (1g / L) and the last three batches have been treated with plant extracts in concentrations of 2 g/L, 5g/L and 10 g/L. Each batch contains six worms. The number of dead worms has been notified every two hours. The death of a worm is characterized by a lack of mobility even if it is shaken followed by fading away of their body colors. Worms considered dead are put in distilled water to confirm their death [14-17]. The experiment was done in triplicate. Origin 6.0 software was used to calculate means and variances, and Microsoft Excel software was used to generate the illustration graphics. The dose-response effect was determined by considering the statistical level of significance $p < 0.05$.

Results and discussion

Extract yields

The yield of extraction are calculated as percentage (Table I). The yield is higher with extracts of *B. rufrescens* extracts (32.84 %) than *C. brocchiana* extracts (15.44 %).

Table 1: Differents extracts yields

Species (Family)	Yields (%)
B. rufrescens (Caesalpinaceae)	32,84
C. brocchiana (Euphorbiaceae)	15,44

Phytochemical screening

The results of phytochemical screening are reported in Table II. Alkaloids and saponins are absent in the two plants. Tannins, flavonoids, steroids and terpenoids are present in both. Quinones are only present in *B. rufrescens.*

Table 2: Phytochemical screening results.

Species (Family)	Parts	Phytochemical compounds						
		Alcaloids		Tannins	Flavonoids	Saponins	Quinones	Steroids-terpenoids
		M	D					
B. rufrescens (Fabaceae)	Bark	_	_	+	+	_	+	+
C. brocchiana (Euphorbiaceae)	Whole plant	_	_	+	+	_	_	+

M: Mayer; D: Dragendoorf; +: Present; - : Absent

Anthelmintic activity

The anthelmintic activity evaluation test reveals the nematicidal potential of the plant parts involved. Table III shows the nematicidal effect of the ethanolic extract of the bark of *B. rufrescens* and whole plant of *C. brocchiana* on *Ascaridia galli* adult worms. Inhibition of the motility of the worms is observed before 24 hours of contact with the extracts (Fig.1, Fig.2, and Fig.3). The effects are observed from 2 hours of contact for high concentrations and from 6 hours for low concentrations.

At a concentration of 2g / L, there is an inhibition of 16% after 6 hours of exposure, 50% after 10 hours and 100% after 22 hours with *B. rufrescens* extracts. *C. brocchiana* extracts exhibited an inhibition of 16% is after 8 hours of exposure, 50% after 12 hours and 100% after 22 hours of exposure.

At a concentration of 5g / L, an inhibition of 16% is observed after 4 hours of exposure, 50% after 8 hours and 100% after 18 hours with *B. rufrescens* extracts. *C. brocchiana* extracts had shown an inhibition of 16% after 6h, 50% after 8h and 100% after 20h of exposure.

At a concentration of 10g / L, we observed an inhibition of 16% after 2 hours of exposure, 50% after 6 hours and 100% after 10 hours with *B. rufrescens* extracts. However, with *C. brocchiana* extracts, an inhibition of 16% is observed after 4h, 50% after 8h and 100% after 16h of exposure. There is a non-significant difference between the effects of the different species ($p>0.05$). Similarly, there is also a non-significant difference between the extracts (10g / L) and levamisole ($p>0.05$).

Phytochemical screening has shown the phytochemical composition of the two parts studied. These results are similar from those of Ikhiri *et al*., who showed the presence of same compounds in *C. brocchiana* whole plant [18]. In the same way Usman and al. also signaled the presence of similar compounds in *B. rufrescens* bark [19].

Tests of the evaluation of anthelminthic activity of the various extracts showed the efficacy of *C. brocchiana* whole plant and *B. rufrescens* bark on *A. galli*. These plants are as efficient as *Polygonum minus* that shows approximately same time (25±3.39 hours) of mortality at 2g/L [20]. However, they are more effective than extracts of *Acacia oxyphylla* on *A. galli* having higher times: 78.86 hours ± 0.79, 76.47 hours ±0.82 at the concentrations of 5mg / mL and 10mg / mL respectively [21].

Several studies have shown the efficacy of plants against *A. galli* [22-24]. These plants owe their biological properties to their chemical compositions. These compounds may be responsible for the anthelmintic properties of studied species.

Various studies have shown the effect of these compounds on parasitic worms. Thus, polyphenols may be responsible for anthelmintic activity [25]. The flavonoids could be responsible for anthelmintic properties of plants [26] by inhibiting oxidative phosphorylation of helminths [27]. In addition, they bind to a glycoprotein, collagen, which plays the protective role of the parasite's cuticle. This fixation induces damage to the cuticle, followed by death of the helminths [28]. Tannins also possess anthelminthic activity. Tannins will bound to free proteins in digestive tract of worms or glycoproteins in cuticle which then causing physiologic impairments in motility, nutrition absorption, and reproduction [29, 30].

Table 3: Anthelmintic activity test result

Species (Family)	Conc (g/L)	Percentage of dead worms (%)											
		0h	2h	4h	6h	8h	10h	12h	16h	18h	20h	22h	24h
B. rufrescens (Fabaceae)	2	0	0	0	16	33	50	50	60	80	80	100	100
	5	0	0	16	33	50	60	80	80	100	100	100	100
	10	0	16	33	50	60	100	100	100	100	100	100	100
C. brocchiana (Euphorbiaceae)	2	0	0	0	0	16	33	50	60	60	80	100	100
	5	0	0	0	16	33	50	60	80	80	100	100	100
	10	0	0	16	30	50	60	80	100	100	100	100	100
Levamisole	1	0	100	100	100	100	100	100	100	100	100	100	100

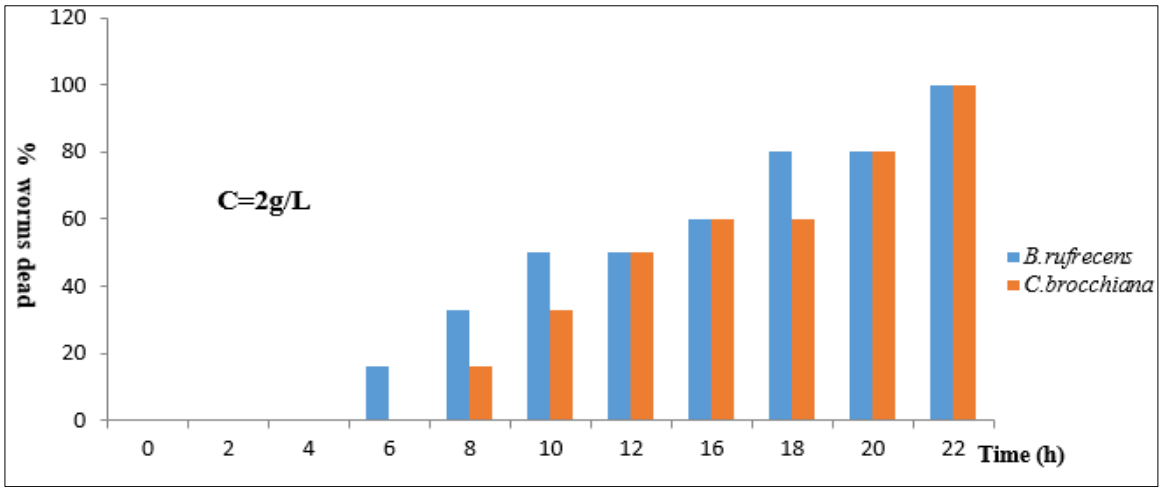

Fig. 1. Average *in vitro* efficacy of different plants against *Ascaridia galli* at 2g/L

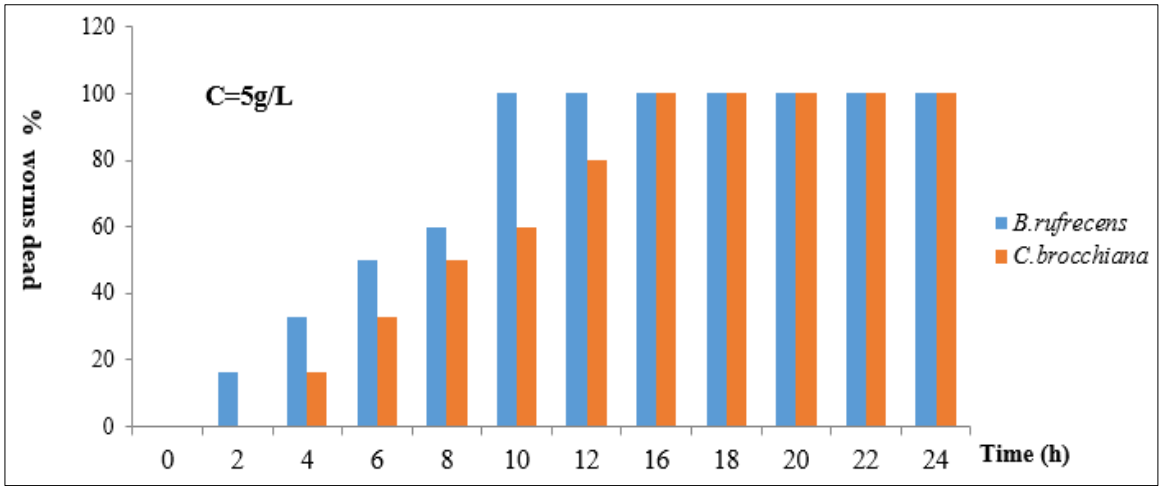

Fig. 2. Average *in vitro* efficacy of different plants against *Ascaridia galli* at 5g/L

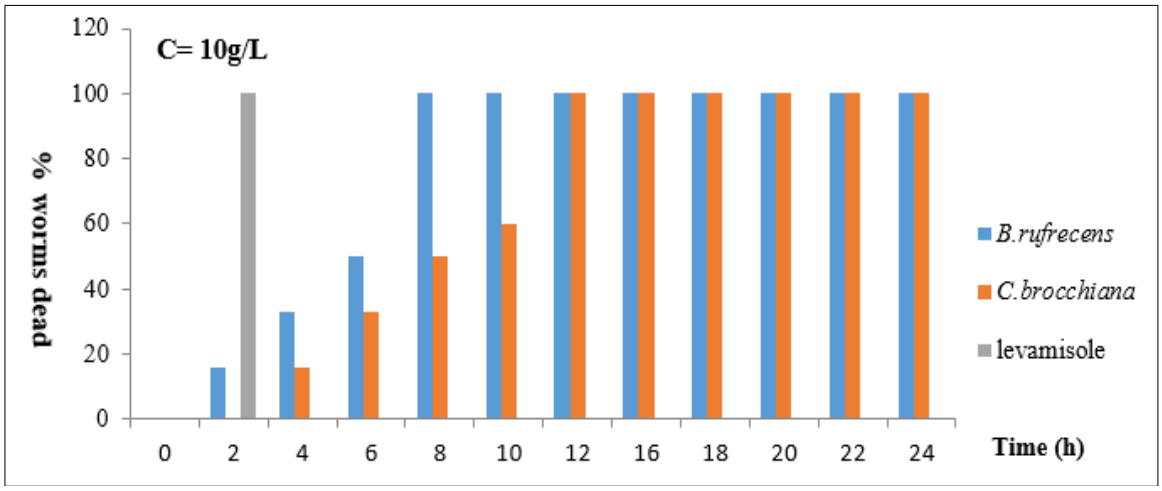

Fig. 3. Average *in vitro* efficacy of different plants and levamisole against *Ascaridia galli* at 10g/L

Conclusion

Ethanolic extracts from *C. brocchiana* whole plant and *B. rufrescens* bark have shown nematicidal activity on *A. galli*. The effects observed with these extracts confirm the uses in traditional medicine of these plants as dewormer. This plant is a potential candidate in the development of improved phytomedicines. Further studies will be carried out to isolate the compound (s) responsible for this activity. This study contributes to the valorization of Niger medicinal plants.

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