In vitro study of the anthelmintic effects of ethanolic extracts of Bauhinia rufrescens Lam. (Fabaceae) and Chrozophora brocchiana (Vis.) Schwein. (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 Ikhirı

Abstract
The search for alternatives to anthelmintics 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 in vitro anthelmintic 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: Anthelmintic, 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]. Anthelmintic drugs resistance is now a global challenge for the control of these diseases [4]. This phenomenon of resistance against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic 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 adult 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.

**Anthelmintic 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 noticed 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: Different extracts yields

<table>
<thead>
<tr>
<th>Species (Family)</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. rufrescens</em> (Caesalpiniaeae)</td>
<td>32.84</td>
</tr>
<tr>
<td><em>C. brocchiana</em> (Euphorbiaceae)</td>
<td>15.44</td>
</tr>
</tbody>
</table>

**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>
<thead>
<tr>
<th>Species (Family)</th>
<th>Parts</th>
<th>Alcaloids</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Quinones</th>
<th>Steroids-terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. rufrescens</em> (Fabaceae)</td>
<td>Bark</td>
<td>+ M</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>C. brocchiana</em> (Euphorbiaceae)</td>
<td>Whole plant</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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 Ikhir 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 anthelmintic 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 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 anthelmintic 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

<table>
<thead>
<tr>
<th>Species (Family)</th>
<th>Conc (g/L)</th>
<th>Percentage of dead worms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>2h</td>
</tr>
<tr>
<td>B. rufescens (Fabaceae)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>C. brocchiana (Euphorbiaceae)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Levamisole</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

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. brochiana 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.

References
