International Journal of Agriculture and Forestry 2015, 5(2): 146-150
DOI: 10.5923/j.ijaf.20150502.08

In Vitro Comparative Anthelmintic Activity on *Hæmonchus contortus* of Two Natural Fodders (*Cassia obtusifolia* and *Piliostigma reticulatum*) Extracts Used in Burkina Faso

Wadré Saidou[1], Kaboré Adama[2,*], Bayala Balé[3], Traoré Amadou[2], Tamboura H. Hamidou[2], Belem A. M. Gaston[4]

[1]Ministère des Ressources Animales, Ouagadougou, Burkina Faso
[2]Laboratoire de Biologie et Santé Animales-DPA/INERA, Ouagadougou, Burkina Faso
[3]Université de Ouagadougou/UFR-SVT, Ouagadougou, Burkina Faso
[4]Université Polytechnique de Bobo-Dioulasso/IDR, Ouagadougou, Burkina Faso

Abstract The objective of this study was to evaluate the anthelmintic properties of aqueous extracts leaves of *Cassia obtusifolia* and *Piliostigma reticulatum* against *Haemonchus contortus*. For this purpose, phytochemical screening of two plants followed by eggs hatching and larval development tests subjected to five increasing concentrations (3.125, 6.25: 12.5: 25, 50 mg / ml) of aqueous extracts in comparison with a control group were conducted. We notified the presence of flavonoids, tannins and polyphenols, coumarins and alkaloids in the two extracts. All concentrations of two extracts caused a significant ($P<0.05$) percentage inhibition of eggs hatching and larval development of *H. contortus* compared to the control group. These *in vitro* results suggested that extracts of *C. obtusifolia* and *P. reticulatum* possess ovicidal and larvicidal properties and may be used in the control of gastrointestinal nematodes of small ruminant. However, it is suggested that *in vivo* tests must be conducted in order to secure rural farmers on the use of these extracts plants as natural deworming.

Keywords Cassia obtusifolia, Piliostigma reticulatum, Antiparasitic activity, Haemonchus contortus

1. Introduction

In Burkina Faso, national ruminant herd is numerically important and composed of 8 566 448 cattle, 8 490 513 sheep and 12 712 705 goats [1]. These animals and particularly small ruminants constitute an important factor in food and nutritional security of the population and also a fight against poverty by increasing the incomes of farmers in the order of 3% per year [2]. They represent a source of sure savings and a checking account easily converted into cash for farmers. However, their raising is still confronted with many development constraints which paralyze ruminant productivity [2]. Indeed, dominant raising system in Burkina Faso is extensive where malnutrition and gastrointestinal parasitism were permanent and cause low performances of animals [3, 4]. This situation led rural farmers with low-income to seek alternative solutions through the use of natural fodder plants with anthelmintic effect as *Cassia obtusifolia* [5] and *Piliostigma reticulatum* [6, 7] to control gastrointestinal parasitism.

Formerly called *Cassia tora* L., *C. obtusifolia* is of the Caesalpiniaceae family and native to tropical Africa and India. This is an annual woody herbaceous shrub that can grow to a height of 2.5 m and presents alternate, composed and paripinnate leaves. According to Farah et *al.* [5], the plant has several pharmacological activities in modern and traditional medicine.

P. reticulatum belongs to the family of Caesalpiniaceae. It is a shrub with rounded and bushy tops [8] that is encountered in Sahel and Sudan-sahel regions. These leaves are composed of two lobes and are usually simple, alternate and green matt gray [9]. In herbal medicine, *P. reticulatum* is involved in the treatment of many diseases [10, 11].

In this context, the objective of this study was to evaluate the potential *in vitro* anthelmintic effects of leaves extract of these two plants against eggs and infective larvae of parasitic nematode *Haemonchus contortus* of sheep.

* Corresponding author:
ade1_bf@yahoo.fr (Kaboré Adama)
Published online at http://journal.sapub.org/ijaf

2. Materials and Methods

2.1. Plant Material and Preparation of Extracts

Leaves of *C. obtusifolia* and *P. reticulatum* were harvested at Saria station of "Institut de l'Environnement et de Recherches Agricoles" in Burkina Faso. The specimens of two plants were then identified from the herbarium of the "Centre National de la Recherche Scientifique et Technologique" in Ouagadougou.

Then, harvested leaves were washed with water and dried for a week before being pulverized to achieve the maceration according to the procedure of traditional healers in the country. To this end, 25 g of powder of each plant were prepared in 500 ml of distilled water for 24 h. Macerated extracts were filtered over cotton wool and filter paper and concentrated by freeze dryer at 50°C for 4-5 days before being stored at refrigeration (at 4°C) for *in vitro* tests. One quarter of hour before the tests, these obtained extracts were dissolved in phosphate buffered saline (PBS pH: 7.2) to obtain six increasing concentrations (3.125 - 50 mg/ml) and used immediately. In addition, a part of each plant's powder was used for the characterization and phytochemical.

2.2. Animal Material

Stomachs of sheep naturally infected were collected in refrigerated slaughterhouse in Ouagadougou and used to collect adult of the *H. contortus* parasite. Then, *H. contortus* eggs were recovery using method previously described by Jabbar et *al.* [12] for *in vitro* tests.

2.3. Phytochemical Analysis

Powders of the two plants were subjected to phytochemical screening to identify key groups of secondary metabolites including alkaloids, flavonoids, tannins and polyphenols, saponins, coumarins and glycosides anthracenesusing standard procedures described by Ciulei [13].

2.4. In Vitro Tests Methodology

For the tests, inhibition egg hatch and larval development tests were applied and adapted from Jackson and Hoste [14].

2.4.1. Inhibition of Egg Hatch

The inhibition of egg hatch test was performed using approximately 100 eggs per 1 ml in Nunc cryotube (3.6 ml). After that, 1 ml of each extract of two plants (*C.obtusifolia* and *P. reticulatum*) at different concentrations (3.125, 6.25, 12.5, 25 and 50 mg / ml) prepared with PBS was added in each Nunc cryotube and PBS only as control group. Three replicates for each concentration of extract and control were performed. All Nunc cryotube were incubated under humidified condition at ambient temperature (27°C) for 48 h. Then, three drops of formalin solution (10%) was added to each Nunc cryotube to stop further hatching and the percentages of inhibition and larvae L1 were counted under an inverted microscope.

2.4.2. Inhibition of Larval Development

The test was conducted using approximately 100 eggs in 1 ml in Nunc cryotube (3.6 ml) and subjected to incubation at room temperature for 48 h in the obscurity to obtain egg development in larvae of first stage (L_1). Then, 200 µl of nutrient Agar (DIFCO: 2%) were added into each Nunc cryotube before being subjected to the same treatments of the previous test with the extracts of both plants and control. Three repetitions were performed. All tubes were further incubated for 7 days before three drops of formalin solution (10%) were used to stop all development in Nunc cryotube. Futher, all infective larvae obtained (L_3) was determined microscopically.

2.5. Statistical Analyses

Results of the phytochemical analysis of the two plants tested were noted - (negative) or + (positive). Data from egg hatching and larval development inhibition tests were transformed to log (x + 1) before being submitted to one-way analysis of variance. The comparison of means values obtained (expressed as mean ± standard deviation) was performed using Tukey-Kramer test. Kruskal-Wallis test was used to evaluate the effect of extracts. All analysis was performed with Costat software (version 6.20.4) at 0.05.

3. Results

3.1. Phytochemical Composition of Plant Extracts

Table 1 presents the result of phytochemical analysis of both two plants. Leaves of C. obtusifolia contain more chemicals than those of *P. reticulatum*. In all plants, applied analysis revealed the presence of flavonoids, tannins and polyphenolsand coumarins.

Table 1. Main phytochemical groups in two analyzed plants

Chemical Groups	Extracts	
	C. obtusifolia	*P. reticulatum*
Flavonoids	(+)	(+)
Tanins et polyphenols	(+)	(+)
Saponins	(-)	(-)
Alkaloids	(+)	(-)
Coumarins	(+)	(+)
Heterosids anthracenic	(-)	(-)

(+): presence (-): absence

3.2. Inhibition of Hatching Eggs

The percentage of inhibition of *H. contortus* hatching eggs observed by the concentrations of both extract plants and control is presented in Figure 1 and Table 2. The percentage of inhibition achieved with control was 9.3% and those of plant extracts ranged from 28% to 48.5% and 17.3% to 46% for *C. obtusifolia* and *P. reticulatum,* respectively. The percentage of inhibition of control was significantly ($P < 0.05$) lower compared to those presented by all

concentrations of two plant extracts. Between the extracts, only the concentration of 6.25 mg/ml did not show a significant difference (P <0.05).

The inhibition effect on egg hatching decreased significantly (P = 0.0214) with increasing concentrations of *P. reticulatum* extract unlike that of *C. obtusifolia* (P = 0.1580).

Table 2. Inhibition mean percentages on *H. contortus* egg hatching at different concentrations of two plant extracts

Concentrations (mg/ml)	Tested extracts	
	C. obtusifolia	*P. reticulatum*
50	38.6 ± 7.0 aA	18 ± 2 bB
25	38,0 ± 10 aA	17,3 ± 1,1 bB
12.50	48,5 ± 5.2 aA	25,3 ± 6,4 abB
6.25	41,3 ± 7,0 aA	46,0 ± 13,1 aA
3.125	28,0 ± 7,2 aA	40,6 ± 6,1 aB
Control (PBS)	9,3 ± 5,7 b	9,3 ± 5,7 c

Small letters (abc) compare means in the columns and capital letters (AB) in the lines. Different letters indicate significantly values (P <0.05).

Table 3. Inhibition mean percentages on *H. contortus* larval development at different concentrations of two plant extracts

Concentrations (mg/ml)	Tested extracts	
	C. obtusifolia	*P. reticulatum*
50	84 ± 5 aA	79,3 ± 2,3 aA
25	80,6 ± 5 aA	72,6 ± 4 aA
12.5	67,3 ± 13,6 aA	79,3 ± 4,1 aA
6.25	71,3 ±4,1 aA	80 ± 9,4 aA
3.125	76,6 ± 6 aA	84,6 ± 4,1 aA
Control (PBS)	40,6 ± 6,1 b	40,6 ± 6 ,1 b

Small letters (ab) compare means in the columns and capital letter (A) in the lines. Different letters indicate significantly values (P <0.05).

3.3. Inhibition of Larval Development

Table 3 and Figure 2 present the percentage of inhibition of *H. contortus* larval development obtained with the concentrations of extract plants and control. The percentage of inhibition of control (PBS) was 40.6%, while those of the extracts ranged from 67.3% to 84% for *C. obtusifolia* and 72.6% to 84.6% for *P. reticulatum*. The percentage of inhibition of control was significantly (P <0.05) lower compared to those of the extract concentrations plant of two plants between which no significant difference (P <0.05) were observed. At the end of the test, all developped eggs to the stage LI and L2 died in each plant extract.

Kruskal-Wallis test found no significant difference between the concentrations in *C. obtusifolia* (P = 0, 121) and *P. reticulatum* (P = 0.7074).

4. Discussion

The main aims of this study were to determine the ovicidal and larvicidal effects of *C. obtusifolia* and *P. reticulatum* on H. contortus which is commonly used as animal model to evaluate the anthelmintic activity of medicinal plants [12, 15]. In addition, it is one of the most dominant digestive parasites in Burkina Faso [16, 17] where the anthelmintic activity of *C. obtusifolia* and *P. reticulatum* was not studied. Also, these plants are present throughout the whole country and are natural fodders that are little used as ruminant food notably in the extensive raising which is dominant in the country [1].

In the study, the obtained results during the two tests show that all the concentrations of two plant extracts inhibit egg hatching and larval development of *H. contortus* compared to control. In addition, eggs and inhibited larvae died, denoting the ovicidal and larvicidal activity of the two extracts. Similar studies have reported the effectiveness of plant extracts such as *Spigelia anthlemia* on *H. contortus* by Assis et *al.* [18] and *Chenopodium ambrosioides* on the parasitic nematode *Heligmosomoides bakeri* by Wabo Pone et *al*. [19].

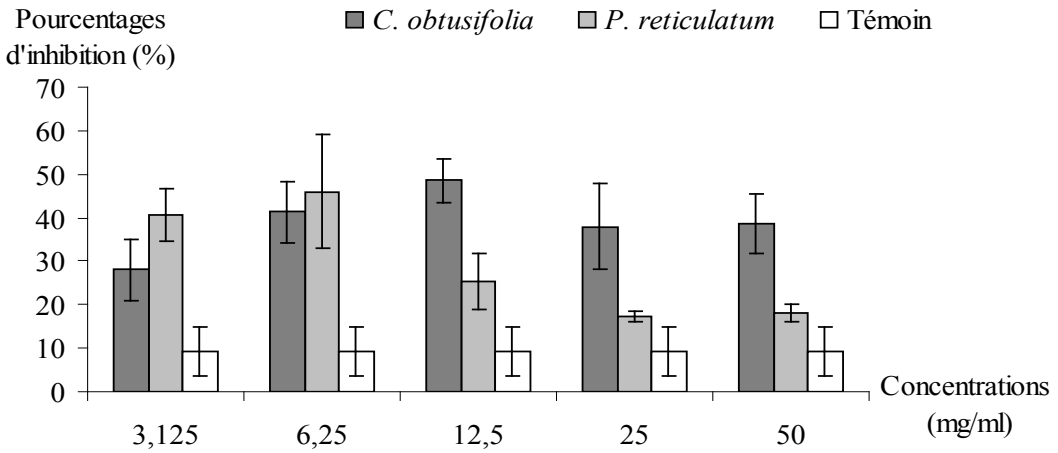

Figure 1. Response profile of egg hatching of *H. contortus* submitted at the concentrations of *C. obtusifolia* and *P. reticulatum* compared to the control

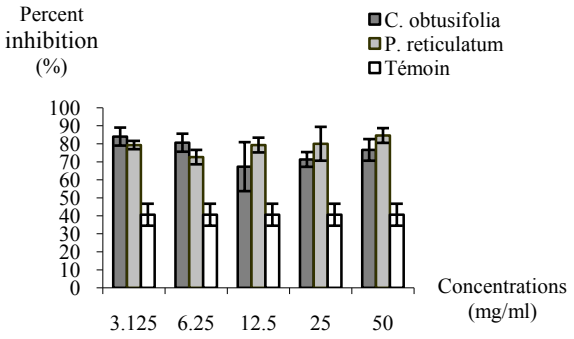

Figure 2. Response profile of larval development of *H. contortus* submitted at the concentrations of *C. obtusifolia* and *P. reticulatum* compared to the control

The anthelmintic activity observed during the study may be due to the presence of secondary metabolites contained in the two plant extracts that would act synergistically or individually on the eggs and larvae of *H. contortus*. Indeed, our studied extracts contain secondary metabolites as flavonoids, tannins and polyphenols, coumarins and alkaloids which contain various pharmacological properties that are reported by Cowan [20] and several authors. According to Wabo Pone et *al.* [21], basicity condition created by alkaloids is detrimental to the survival of larvae that prefer acidic conditions. The works of Lee et *al.* [22] on *C. elegans* and Reddy and Seetharam [23] on *Pheretima posthuma* report that flavonoids have anthelmintic activity. For tannins, including condensed tannins, their anthelmintic activities have been mentioned by Hoste et *al.* [24]. In all cases, active secondary metabolites observed in the two extracts probably penetrated the egg shell and the cuticle of larvae by osmosis through the circulatory system to stop the development process of segmentation of blastomeres and larvae and then cause death by starvation [25] or paralysis [26]. In the study, the inhibition of larval development is due especially to the L_1 larval mortality in the egg shell. In addition, the comparative analysis of the relative percent inhibition of tested concentrations shows that low doses (3.125mg/ml) of *P. reticulatum* extract is more ovicidal and larvicidal than *C. obtusifolia* extract although it would contain less secondary metabolites. Similar deductions on the mechanisms of anthelmintic activity of medicinal plants were made with *Chenopodium ambrosioides* and *Tithonia diversifolia* extracts on the parasitic nematode *Heligmosomoides bakeri* by Wabo Poné et *al.* [19] and *Leuceana leucocephala* and *Gliricidia sepium* extracts by Kaboré et *al.* [27].

To conclude, the results from this study showed that the extracts from *C. obtusifolia* and *P. reticulatum* have shown active *in vitro* anthelmintic effect against eggs and larvae of *H. contortus*. These anthelmintic efficacies could justify their use against gastrointestinal parasites of small rural ruminants by smallholders and pastoralists in farming areas. However, further *in vivo* toxicological and parasitological studies may be done because results obtained from *in vitro* conditions could be different from *in vivo* conditions [28].

ACKNOWLEDGEMENTS

Authors acknowledge PAES / UEMOA (VPMAP: P Z1 - IAD - 002) and IAEA-BKF 5014 projects for the support provided to the study implementation.

REFERENCES

[1] Ministère des Ressources Animales (MRA), 2012. Statistique du secteur de l'élevage. Annuaire 2011. Ouagadougou. 151 pages.

[2] Ministère des Ressources Animales (MRA), 2010. Plan d'Actions et Programme d'Investissements du Sous-secteur de l'Elevage (PAPISE 2010-2015). Ouagadougou. 73 pages.

[3] Krecek R.C., Waller P.J., 2006. Towards of implementation of the "basket of option" approach to helminth parasite control of livestock: Emphasis on the tropics/ subtropics. *Vet. Parasitol.,* 139: 270-282.

[4] Agence Internationale de l'Energie Atomique (AIEA), 2006. Improving Animal Productivity by Supplementary Feeding of Multinutrient Blocks, Controlling Internal Parasites and Enhancing Utilization of Alternate Feed Resources. AIEA-TECDOC-1495, FAO/AIEA Division, Vienne. 280 pages.

[5] Farah N., Srivastav S., Singh P., Mishra G., 2011. Phytopharmacological Review of *Cassia tora* Linn. (Fabaceae). *Asian Journal of Plant Science and Research*, 1(1): 67-76.

[6] Kaboré A., Tamboura H. H., Belem A. M. G., Traoré A., 2007. Traitements ethno-vétérinaires des parasitoses digestives des petits ruminants dans le plateau central du Burkina Faso. *International Journal of Biological and Chemical Sciences*. Vol. 1, n°3, 297-304.

[7] Yelemou B., Bationo B. A., Yameogo G., Millogo-Rasolodimby J., 2007. Gestion traditionnelle et usages de *Piliostigmareticulatum* sur le Plateau central du Burkina Faso. *Bois et forêts des Tropiques* , n° 291 (1).

[8] Arbonnier M., 2000. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. CIRADIMNHNIUICN. 541 p.

[9] Maydell H.J.Von., 1983. Arbres et arbustes du Sahel. Leurs caractéristiques et leurs utilisations. GTZ. 532 p.

[10] Dosso K., N'guessan B.B., Bidié A.P., Gnangoran B.N., Méité S., N'guessan D., Yapo A.P., Ehilé E.E., 2012.Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigma reticulatum* (Caesalpiniaceae) in rats.*Afr. J. Tradit. Complement. Altern. Med*., 9(2):242-249.

[11] Tira-Picos V., Nogueiro J. M., Gbolade A. A., 2010. Comparative analysis of leaf essential oil constituents of *Piliostigma thoningii* and *Piliostigma reticulatum*. *Int. J. Green Pharm*. 4:67-70.

[12] Jabbar A., Iqbal Z., Nisar Khan M., 2006. *In vitro* anthelmintic activity of *Trachyspermum ammi* seeds. *Pharmacognosy Magazine*, Vol 2, 6: 126-129.

[13] Ciulei I., 1982. Methodology for analysis of vegetable drugs. Bucharest: Ministry of Chemical Industry, Bucharest, 67p.

[14] Jackson F., Hoste H., 2010. *In vitro* methods for the primary screening of plant products for direct activity against ruminant gastrointestinal nematodes. In "*In vitro* screening of plant resources for extra nutritional attributes in ruminants: nuclear and related methodologies" P. E. Vercoe, H. P. S. Makkar and A. C. Schlink Editors FAO/IAEA Springer Edition. P: 24-45.

[15] Eguale T., Tilahun G., Gidey M., Mekonnen Y , 2006. *In vitro* anthemintic activities of four ethiopian medicinal plants against *Haemonchus contortus*. *Phamacologyonline*, 3:153-165.

[16] Belem A. M. G., Kaboré A., Bessin R., 2005a. Gastrointestinal helminths of sheep in the central, eastern and northern parts of Burkina Faso. *Bull Anim. Hlth. Prod. Afr.*, 53: 13-23.

[17] Belem A. M. G., Kaboré A., Bessin R., 2005b. Variations saisonnières des helminthes gastro-intestinaux chez la chèvre du plateau central du Burkina Faso. Rev. Elev. Méd. Vét. Pays Trop. 58: 37-43.

[18] Assis L. M., Bevilaqua C. M. L., Morais S. M., Vieira L. S., Costa C. T. C., Souza J. A. L., 2003. Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmia* Linn.extracts on *Haemonchus contortus*. *Vet. Parasitol.,*117 : 43-49.

[19] Wabo Poné J., Yondo J., Fossi T. O., Komtangi M. C., Bilong Bilong C. F. and, Mpoame M., 2011a. The *in vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri. J. of Pharmacognosy and Phytoth.,* 3 (4):56-62.

[20] Cowan M. M., 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, Vol. 12, n°4, 564-582.

[21] Wabo Poné J, Fossi T. O., Yondo J., Komtangi M. C., Mpoame M., Bilong Bilong C. F., 2011b. The *in vitro* effects of aqueous and ethanolic extracts of the leaves of *Ageratum conyzoides* (Asteraceae) on three life cycle stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). *Vet. Med. Inter*. doi: 104061/2011-140293.

[22] Lee Y., Kawasaki I, Lim Y, Oh W. S., Paik YK and Shim YH (2008). Inhibition of developmental processes by flavones in *Caenorhabditis elegans* and its application to the pinewood nematode, *Bursaphelenchus xylophilus*. *Mol.Cells,* 26: 171-174.

[23] Reddy U. B., Seetharam Y. N., 2009. Anthelmintic activity of Trikatu churna and its ingredients. *Ethanobotanical leaflets*, 2(2): 181.

[24] Hoste H, Jackson F, Athanasiadou S, Thamsborg SM, Hoskin SO. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*., 22: 253-261.

[25] Athanasiadou S., Kyriazakis I., Jackson F., Coop R. L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodos of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology*, 99: 205-219.

[26] Dobson R. J., Donal A. D., Waller P. J., Snowdon K. L., 1986. An egg hatch assay for resistance to levamisole in trichostrongyloid nematode parasites. *Veterinary Parasitology*, 19: 77-84.

[27] Kaboré A., Traoré A., Nignan M., Gnanda B. I., Bamogo V., Tamboura H. H., Bélem A. M. G., 2012. *In vitro* anthelminthic activity of *Leuceana leucocephala (Lam.)* De Wit. (Mimisaceae) and *Gliricidia sepuim (Jacq.)* Kunth ex Steud (Fabaceae) leave extracts on *Hæmonchus contortus* ova and larvae. *J. Chem. Pharm. Res*., *4 (1): 303-309.*

[28] Marie-Magdeleine C, Udino L, Philibert L, Bocay B, Archimede H., 2010. *In vitro* effect of cassava (*Manihot esculenta*) leaf extracts on four development stages of *Haemonchus contortus. Vet. Parasitol.*, 173: 85-92.