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Anthelmintic Activity of Nine Varieties of *Cajanus cajan* (L.) Millsp on *Haemonchus contortus* from Sheep

Faustin Parfait Koutouan[1,2], Yapo Magloire Yapi[1,3],
Souleymane Kande[4], Eboua Narcisse Wandan[1,2]

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ABSTRACT

Background: Internal parasitosis is the most important parasitism in small ruminants in tropical region. Synthetic anthelmintics are usually used for their control. Due to the emergence of resistance in worm population, the use of alternative methods such as plants bioactive molecules are developed. This study aimed at assessing anthelmintic activity of nine varieties of *Cajanus cajan,* a taniferous plant cultivated in West Africa.

Methods: Leaves of the nine varieties of *Cajanus cajan* were dried, ground and hydroacetonic extracts were obtained by cold maceration at a concentration of 60 mg/ml. Live adult of *Haemonchus contortus* were obtained from sheep's stomach and exposed, in triplicate, to the following solutions: hydroacetonic extracts (60 mg/ml), hydroacetonic extracts (60 mg/ml) associated with polyvinylpyrrolidone (PVP) (50 mg/ml), Levamisole (20 mg/ml) and phosphate buffered saline (PBS) solution. Worm death time was recorded for each treatment.

Result: Worms exposed to Levamisole recorded the shortest death time about 9.73±1.77 min. The potent of the extracts induced worm death time of 64.83±4.73 min while the least efficient induced a death time of 156.50±23.20 min. Worms in the PBS solution were still alive after 24 hours of exposure. These results indicated that the four varieties of *C. cajan* used in this study have promising wormicidal. Besides that, the effect of tannins were not the only compound responsible for the anthelmintic activity.

Key words: Condensed tannin, Helminthosis, Hydroacetonic extracts, Polyphenols, Small ruminants, Taniferous plant.

INTRODUCTION

In tropical regions, internal parasitosis are the most important parasitism in small ruminants which constrain animals' survival and productivity (Mahieu *et al.,* 2009, Krecek and Waller, 2006). Their high frequency causes significant economic losses in small ruminant breeding (Hounzangbe-Adote *et al.,* 2005). Synthetic anthelmintics are used usually to control these parasitosis (Hoste *et al.,* 2006). However, requests for a reduction of their use are more and more recurrent (Jackson and Miller, 2006), due to the emergence of resistance in worm population, their ecotoxicity and residues presence in animal products (Hoste *et al.,* 2006; Krecek and Waller, 2006, Papadopoulos, 2008). For this purpose, alternative and or complementary methods are developed to control this gastrointestinal parasitism. Plant bioactive molecules use is one of these methods (Jackson and Miller, 2006; Githiori *et al.*, 2006; Meenakshisundaram *et al.*, 2017).

Cajanus cajan L., is an important legume forage (Kaboré *et al.*, 2016; Sarkar *et al.*, 2020) with several species (Sawargaonkar *et al.,* 2016; Talari and Shakappa, 2018) which are medicinal plants containing tannins. Traditional varieties of this species have a long development cycle (7-11 months) (Velay *et al.*, 2001) allowing sheep whose diet is composed of 20 to 50% ligneous and sub-ligneous species in the savannah (Bodji *et al.*, 1996; César and Zoumana, 1999) to have feed during the dry season. Odeny (2007) reported that *C. cajan* forage increased roughage consumption, which resulted in a high live weight of the animals. *In vitro* action of *Cajanus cajan* on nematode is currently documented. This anthelmintic action has been attributed to phenolic compounds (Singh *et al.*, 2010) such as tannins and flavonoids (Harris *et al.*, 2014). Tannins play an important role in gastrointestinal parasitism control (Hoste *et al.*, 2006; Williams *et al.*, 2014), but this anthelmintic activity of the tannins depend on plant species (Hoste *et al.*, 2006; Quijada *et al.*, 2015). In addition, phenolic compounds vary according to *Cajanus cajan* varieties (Koutouan *et al.,*

[1]Institut National Polytechnique-Félix Houphouët Boigny de Yamoussoukro, BP 1093 Yamoussoukro, Côte d'Ivoire.
[2]Laboratoire Sciences Société and Environnement, UMRI Sciences Agronomiques et Génie Rurale, BP 1093 Yamoussoukro, Côte d'Ivoire.
[3]Département Agriculture et Ressources Animales, Laboratoire de Zootechnie et Productions Animales, BP 1313, Yamoussoukro, Côte d'Ivoire.
[4]Laboratoire National d'Appui au Développement Agricole/ Laboratoire Régional de Bouaké, BP 639 Bouaké 01, Côte d'Ivoire.

Corresponding Author: Yapo Magloire Yapi, Institut National Polytechnique-Félix Houphouët Boigny de Yamoussoukro, BP 1093 Yamoussoukro, Côte d'Ivoire. Email: yapimagloire@yahoo.fr

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2019). This in turn, could lead to a varietal difference in anthelmintic activity (Nguyen *et al.,* 2005), as already noted by some authors in Sainfoin (Manolaraki, 2011; Azuhnwi, 2012). In previous anthelmintic tests performed with *C. cajan*, no indication was given on the varieties (Singh *et al.,* 2010; Kaboré *et al*., 2016).

So, this study aimed to investigate the anthelmintic properties of nine varieties of *Cajanus cajan* against adult *Haemonchus contortus* from sheep and to elucidate the role of tannins in this activity.

MATERIALS AND METHODS

The study was carried out at the Institut National Polytechnique Félix Houphouët-Boigny (INP-HB) of Yamoussoukro (Côte d'Ivoire), from May 2018 to June 2019. The climate of the region is characterized by annual rainfall of 900 to 1100 mm, temperatures ranging from 18°C to 35°C and a relative humidity between 75% and 85%. Five improved varieties (ICPL 87 119-ASHA, ICP 8863-MARUTI, ICP 7035-KAMICA, ICPL 87 119 GUIMU 3 (Asha), ICPL 332 ABAAYA), one Burkina Faso traditional variety (FKB-Red) and ivorian three traditional cultivars (white, red, black) of *C. cajan*, adapted of Côte d'Ivoire local agro-climatic conditions, were used in this study.

The characteristics of these varieties are described in Table 1. The plants were grown on the same site, under the same conditions. The leaves of these varieties were harvested at the early stage of ripening throughout the plant. Total polyphenols and tannins contents of the leaves were previously evaluated (Koutouan *et al.,* 2019). The collected leaves were shade dried and ground into a fine powder using an electric blender. Then, the powders were stored in a dry and dark room at ambient temperature. Hydroacetonic extract was prepared by a cold maceration method using a 70% aqueous-acetone solution as previously described by Makkar (2000). The resulting mixture was suspended in an ultrasonic bath and sonicated for 30 minutes (two times 15 minutes) at room temperature. The obtained macerate was filtered and the extract was dried in an oven at 5°C. The dry extracts were stored at 4°C until use.

Leaf extracts of the nine varieties of *Cajanus cajan* were evaluated for their ability to kill adult worms using the Adult Motility Assay (AMT). The AMT was conducted on mature *H. contortus* worms according to Eguale *et al*. (2006) technique. Live adult worms of *H. contortus* were collected from the abomasum of freshly slaughtered sheep at the national slaughterhouse of the city of Bouaké (Côte d'Ivoire). The worms were stored in a polypropylene bottle containing Phosphate Buffered Saline (PBS) solution. They were then immediately sent to the National Laboratory Office of Agricultural Development Support (LANADA, Côte-d'Ivoire) for anthelmintic tests. Six actively moving worms were exposed in triplicate to each of the four following treatments in separate Petri dishes at room temperature (25-30°C):

- **Treatment 1:** Hydroacetonic extracts at 60 mg/mL prepared in phosphate buffered saline (PBS) solution.
- **Treatment 2:** Hydroacetonic extract at 60 mg/mL with polyvinylpyrrolidone (PVP) at 50 mg/ml to selectively remove tannins in dissolved extracts (Williams *et al*., 2014), all prepared in PBS.
- **Treatment 3:** Levamisol at 20 mg/ml, prepared in distiller water, according to Singh *et al.* (2010).
- **Treatment 4:** PBS (Negative control).

The death time (M, minutes) of the worms was chosen as a criterion of anthelmintic activity. It represents the time elapsed between contact with the extract solution and the moment when they lost their motility, evaluated by touching the worms with a clamp for five to six seconds.

A comparison of the mean time of death of adult worms for the different varieties was performed by one-way analysis of variance (ANOVA). The post hoc statistical significance test employed was Tukey HSD, differences between the means were considered significant at $P<0.05$. The effect of PVP on the wormicidal activities of leaf extracts was assessed using t-student test at $P<0.05$. Previously, when necessary, the data was transformed into a log (x) transformation to normalize the distribution. All statistical analyzes were performed using the R software (R Development Core Team).

Table 1: Characteristics of the varieties and cultivars used.

Varieties / Cultivars	Seed color	Origin of the seed	Nature of the varieties / cultivars
White local cultivar (TCW)	White	Local market of Côte d'Ivoire	Local cultivar from Côte d'Ivoire
Red local cultivar (TCR)	Red	Local market of Côte d'Ivoire	Local cultivar from Côte d'Ivoire
Black local cultivar (TCB)	Black	Local market of Côte d'Ivoire	Local cultivar from Côte d'Ivoire
ICPL 87 119- ASHA (ICPLAS)	Red	INERA, Burkina Faso*	Improved variety
ICP 8863-MARUTI (ICPMA)	Red	INERA, Burkina Faso	Improved variety
ICP 7035-KAMICA (ICPKA)	Red	INERA, Burkina Faso	Improved variety
FKB-RED (FKBR)	Red	INERA, Burkina Faso	Local variety from Burkina Faso
ICPL 87 119 GUIMU 3 (Asha) (ICPLGA)	Red	INERA, Burkina Faso	Improved variety
ICPL 332 ABAAYA (ICPLAB)	Red	INERA, Burkina Faso	Improved variety

*INERA= Burkina National Institute for Environment and Agronomic Research.

RESULTS AND DISCUSSION

Anthelmintic effect of the nine varieties of *cajanus cajan*

The extracts of the nine varieties of *Cajanus cajan* have been studied to assess their in vitro anthelmintic activity. A time-dependent response was observed with all the treatments (Fig 1). All the extracts showed an anthelmintic effect, although significantly lower compared to levamisol, as 100% of adult worms died after 60 to 200 min of exposure to the extracts while death occurred at 12 min for levamisol. Regarding PBS, no dead worm was found up to 24 hours after exposure. A significant effect ($P<0.05$) was observed on the motility of adult *H. contortus* worms in all the leaves extracts. Moreover, according to their effects, the varieties used in this study can be grouped. Group one comprising four varieties (ICPL 87 119- ASHA, ICP 8863-MARUTI, ICP 7035-KAMICA and FKB-Red) are those with a higher average time of death (156.50±23.20 mn to 177.67±15.08 mn). Group two includes four other varieties (TC-White, TC-Black, ICPL 87 119 GUIMU 3 (Asha) and ICPL 332 ABAAYA) with a medium average time of death (98.60±35.76 mn to 123.17±18.90 mn). The last group, represented by the traditional cultivar red (TC-Red), induced a low average time of death (64.83±4.73 mn). The results of this study confirm the anthelmintic properties of *Cajanus cajan*, already reported by other authors (Singh *et al*., 2010; Kaboré *et al*., 2016). However, the average times of death recorded remained greater than the 17 minutes obtained by Singh *et al*. (2010) with hydroethanolic extracts (40 mg/ml) of *C. cajan* on Indian adult earthworms *Pheretima posthuma* and much less than the six hours obtained by Kaboré *et al*. (2016) on *Haemonchus contortus* with an aqueous extract of *C. cajan* at 100 mg/ml. The anthelmintic effects of *C. cajan* are due, according to several authors, to the polyphenolic compounds present in the plant, notably tannins and flavonoids (Singh *et al.,* 2010; Kaboré *et al.,* 2016). These compounds found in the leaves are less polar and therefore are prone to dissolve in relatively less polar solvents, than water (Eguale *et al.,* 2006). The work of Mohanty *et al.* (2011) showed a concentration of flavonoids in ethanolic extracts two to three times higher than in aqueous extracts. This explains the relatively long time (6 h) obtained by Kaboré *et al.* (2016) with aqueous extracts compared to those obtained in the present study and to that obtained by Singh *et al.* (2010). These facts indicate that *in vitro* anthelmintic effects of the same plant species and the same organ are variable depending on the solvent used. Although the mechanism of action of bioactive molecules involved in anthelmintic activity is still poorly understood. It is known that anthelmintic substances can reach the target worm by oral route or by diffusion, or absorption through the cuticle of the parasite, or a combination of both routes. But, the passive transfer of the drug by the cuticle is the predominant entry mechanism (Alvarez *et al.,* 2007).

Effects of polyvinylpyrrolidone on anthelmintic effects of the extracts

Polyvinylpyrrolidone (PVP) at 50 mg/ml was used combined to hydroacetonic extract at 60 mg/mL to selectively remove tannins in dissolved extracts (Williams *et al*. 2014). The average time of death increased significantly ($p<0.05$) for the varieties ICPL 87 119 GUIMU 3 (Asha), FKB-Red, traditional red and black with the PVP addition in the hydroacetonic extracts. However, the others varieties did not experienced a significant ($p>0.05$) increase in the time of death after PVP addition (Fig 2).

This result indicates that the tannins contained in the varieties of *C. cajan* in this study are not the only ones involved in the anthelmintic activity. Other secondary metabolites in these varieties certainly play an important

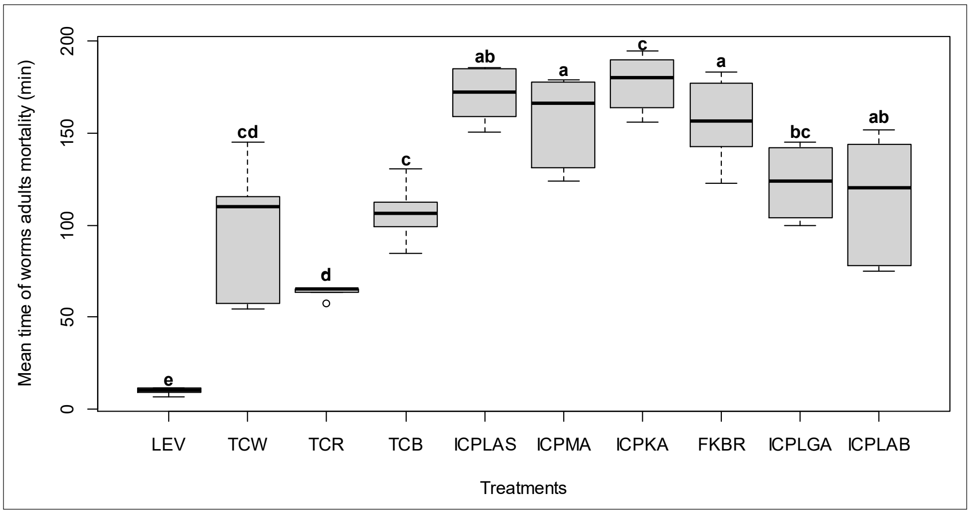

Fig 1: Graph showing in vitro anthelmintic activity (the time of death) of hydroacetonic extract at 60 mg/mL in comparison with positive control levamisol (20 mg/mL) (Lev). The time of death of the worms was used as the criterion for anthelmintic activity. Values shown are means, letter upper indicated significant difference from previous value at $P<0.05$.

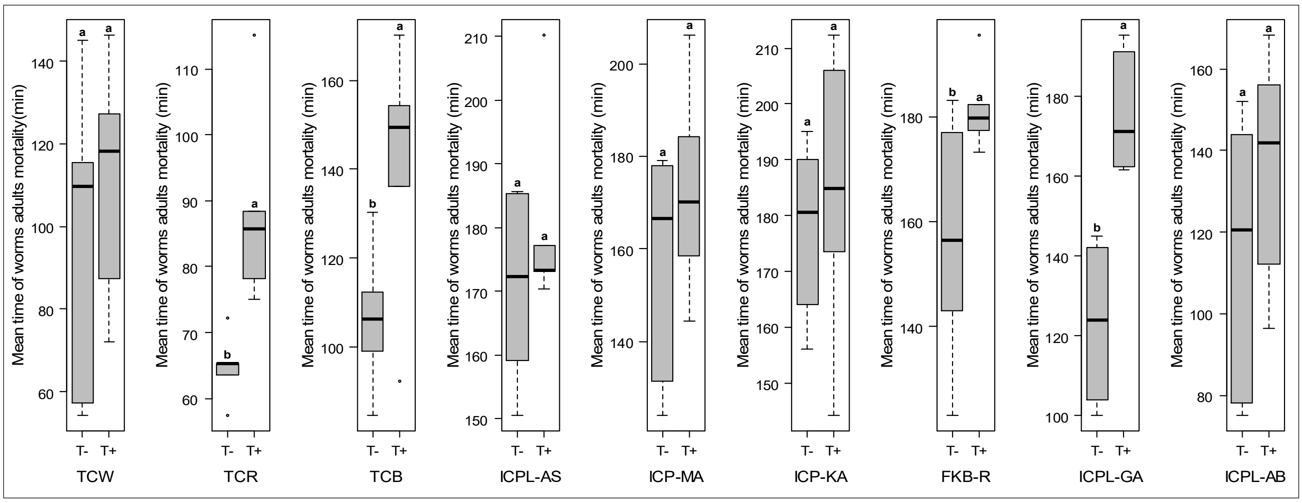

Fig 2: Graph showing *in vitro* mean anthelmintic activity (the time of death) of hydroacetonic extract at 60 mg/mL compared to combined hydroacetonic extract at 60 mg/mL with Polyvinylpyrrolidone (PVP) at 50 mg/ml on mature live *Haemonchus contortus* of sheep. The time of death of the worms was used as the criterion for anthelmintic activity. Values shown are means, letter upper indicated significant difference from previous value at P<0.05. T- = no PVP; T+ = with PVP.

role in anthelmintic actions. This could explain why the variety ICPL 332 ABAAYA showed a higher nematicidal efficacy than the varieties FKB-Red and ICPL 87 119-ASHA, while in a previous study, Koutouan *et al.* (2019) showed that the level of tannin of the first variety was very low compared to the seconds. These results confirmed the role of other secondary metabolites, apart from tannins, in the anthelmintic activity of plants, as already noted by Hoste *et al.* (2006) and Manolaraki (2011). The wormicidal activity hereby found could be due to flavonoids (Singh *et al.,* 2010; Kaboré *et al.,* 2016). Their content in the leaves of *C. cajan* is about 10 times that of tannins (Harris *et al*., 2014).

CONCLUSION

This study aimed to evaluate the anthelmintic activity of the leave extracts of nine varieties of *Cajanus cajan* a taniferous plant cultivated in West Africa. The results indicated that four of the varieties have promising wormicidal effect and that tannins were not the only ones responsible for this anthelmintic activity. However, an *in vivo* assay must be conducted in subsequent studies to confirm this result.

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