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(RESEARCH ARTICLE)



Vermicides activities of trunk barks and leaves of *Khaya senegalensis* A. Juss (Meliaceae)

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Abstract

The use of plants for therapeutic purposes in Africa is an integral part of culture and tradition. However, as sources of new active substances, a high percentage of plant species is not yet studied. The aim of the present work was to highlight the vermicidal and antispasmodic pharmacological properties of the trunk bark and leaves of *Khaya senegalensis* A Juss (Meliaceae), which would justify its traditional use. An aqueous extraction by maceration was carried out of the trunk bark and leaves was performed and phytochemical analysis of the extracts studied by thin layer chromatography. An investigation of the cholinergic system in vitro on the earthworm and ex vivo on the isolated intestine of the rats with the extracts was carried out. NMRI strain mice were used for the acute toxicity studies. The phytochemical screening of the extracts studied revealed the presence of chemical groups such as saponosides, tannins, flavonoids, etc., with variations in levels between the two parts of the plant. *In vitro*, les effets vermicides des extraits de feuilles (CL = 8,04 mg/mL) et des écorces du tronc (CL = 3,44 mg/mL) étaient comparables au Combifrinil® (CL = 3,92 mg/mL). The lethal 50% oral dose of the extracts was greater than 5000 mg / kg. Inhibitory concentrations of aqueous macerates were 1.936 mg/mL for leaves and 0.975 mg/mL for trunk bark. These results would justify the efficacy and safety of plant extracts in the treatment of some intestinal parasitosis in traditional therapeutics.

Keywords: Medicinal plants; Khaya senegalensis; Acute toxicity; Vermicide activity; Antispasmodic

1. Introduction

In developing countries, most of the populations living in rural areas are confronted with gastrointestinal parasitosis, the most affected of which are young children, and it has been estimated that 12% of the global burden of intestinal worm disease is in children aged 5 to 14 [1]. They are faced with the public health problems of gastrointestinal parasitosis, a deficient diet associated with parasite resistance to certain modern anthelmintic substances [2] [3]. Despite the progress of pharmacology, the therapeutic use of medicinal plants is very present in developing countries [4]. According to the World Health Organization (WHO), nearly 80% of people in developing countries in the African region use traditional medicine [5].

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Thus, several strategies aim at enhancing the use of medicinal plants in human medicine [5]. The use of medicinal plants for the treatment of various diseases including gastrointestinal parasitosis is the speciality of populations in developing countries such as Burkina Faso [6]. This craze for medicinal plants is also explained by their availability, particularly in rural areas where medicines and health care are almost inaccessible for the nationals of these areas [7]. Among the plants used in traditional medicines, *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae), native to the sub-Saharan savannah, from Senegal to Uganda, is one of the most popular plants in Africa [8] for its anti-parasitic and anthelmintic properties. These different parts are used in the treatment of syphilis, jaundice, dermatitis, scorpion bites, gum infections, hookworm, mental illness, leprosy, fever, headache, bleeding sores, infertility, allergies and also as a vermifuge, tenicide, depurative, laxative and aphrodisiac [7] [9] [10].

This study was carried out with an objective to justify the traditional use of trunks bark extracts and leaves of *Khaya senegalensis* in the treatment of intestinals parasitosis in Burkina Faso from explanatory Pharmacodynamic supports.

2. Material and methods

2.1. Plant material

The plant material consisted of bark of the trunk and *Khaya senegalensis* leaves, harvested in the village of Sogdin (Kadiogo province about 20km east of Ouagadougou). The harvested samples were dried under ventilation artificial shielded from light in the laboratory of the Department of Medicine -pharmacopoeia traditional / pharmacy (MEPHATRA / PH) of the Institute for Research in Health Sciences (IRSS). After drying, the various parts harvested were reduced to powder using a grinder (East Gladiator 1931 Type BN 1 Mach 40461 1083) and store in a plastic bag for further studies.

2.2. Biological materials

- NMRI strain mice from the IRSS pet shop were used for the acute toxicity studies.

- Normotensive adult male and female rats of WISTAR strains aged 3-4 months and weighing between 150 and 300 g were used for the pharmacological study. They were provided by the pet shop of IRSS, where they are fed ad libitum to wheat cake (29% protein) and running water. They are high under air conditioning (23-25 ° C) and 60% humidity.

- Earthworms belonging to the family of oligochaetes and the branching of annelids. They were collected at the dam No. 2 Ouagadougou (Burkina Faso) and adapted to the survival tank in the laboratory. These worms were used in this study for their biology close to helminths, usually digestive parasites.

2.3. Extraction

A mass of 150 g of each powder (leaves and bark of trunk) was mixed with 800 ml of distilled water and allowed macerate for 24 hours with magnetic stirring at room temperature. The mixture was then filtered on hydrophilic cotton and the filtrates obtained were centrifuged at 2000 rpm for 5 minutes and the extraction yield was calculated. The supernatants were concentrated in an oven at constant temperature (60°) until dry extract and a part of each extract was used to determine the extraction yields (n = 3). After extraction, the yield of the macerates obtained with the leaves (16.6%) the trunk barks (15.7%) were used for the experimental studies.

2.4. Phytochemical screening

Phytochemical screening was carried out on chromatoplates (60 F250, 20x20 glass support, Fluka-Silica gel) according to the methods described in the literature [11]. This involved searching for large chemical groups by thin layer chromatography (TLC) such as steroid compounds, terpene compounds, phenolic compounds, nitrogen compounds.

Several specific reagents have been used to reveal these groups of compounds: Sulfuric vanillin reagent and Libermann Burchard reagent for terpenes and sterols; methanolic 5% (V / V) KOH reagent for coumarins; Dragendor'ff reagent for alkaloids; Neu reagent for flavonoids; FeCl₃ reagent for tannins and phenolic compounds and sulfuric anisaldehyde reagent for saponosides.

2.5. Toxicological studies

Test are using according to the method described by Trevan modified par Lichtfield and Wilcoxon [12] [13]. Animals aged three 03 months and weighing between 20 and 40 g, previously fasted for 18 hours are divided into 05 lots and a control batch of 06 mice each. The extracts dissolved in distilled water were administered orally and intraperitoneally, the control mice receiving the solvent. The solutions were prepared so as to administer on average to each mouse only between 0.2 and 0.8 ml of extract. For the determination of the lethal dose 50% (LD₅₀) by the oral route, six (06) lots respectively received the doses of 500; 1000; 2000; 3000; 4000 and 5000 mg / kg of body weight of the aqueous macerates, after a pre-test of 3 lots which received the macerated doses of 500; 750 and 1000 mg / kg. The search for the 50% lethal dose (LD₅₀) by the intraperitoneal route necessitated the administration to six (06) batches of increasing doses: 750; 900; 1000; 1125 and 1500 mg / kg body weight of water-evaporated dry macerates. The animals once treated were observed during the 2 hours following the administration of the extracts at the end of which they are fed. They are then observed for 24 h, then 48 h and 72 h during which the dead mice in each batch were counted for the determination of the LD₅₀.

2.6. Study of vermicide activity

The study of the earthworm, Lombricus terrestris (Oligochaetes) was done in two complementary steps: the toxicity on the earthworm (method developed by the laboratory because of the similarity between the earthworm and the helminths) and the implementation evidence of the mechanism of vermicidal action by tests on isolated gut. Toxicity on earthworms was determined using the method developed by Guissou et al. [14]. Six earthworms were incubated in bowls filled with 400 g clean sand (removal of all traces of dust) and neutralized with 0.1% H2SO4 while hot. The sand of each bowl is impregnated with a known dose of the extract of the plant: 1 mg/mL; 3.75 mg/mL; 5 mg/mL; 7.5 mg/mL and 10 mg/mL. The behavior of earthworms was noted as was the number of deaths per batch at the following time intervals: 30 min, 1 h, 2h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 18 h, 20 h, 24 h, 48 h, and 72 h.

2.7. Study of antispasmodic activity

The use of the rat for the test was in accordance with internationally accepted principles for the use and care of laboratory animals as defined by the European Community Directives (1986 EEC Directive, 86/609 / EEC). This study carried out according to the method described by Magnus5 [15]. It consists in evaluating the relaxing effect of the extract, on a contraction phase of contraction of the isolated rat duodenum, induced by a contracting agent. To evaluate the relaxing effect of the aqueous extract, cumulative concentrations of each extract (260 μ g / mL, 560 μ g / mL, 1160 μ g / mL, 1960 μ g / mL, 2960 μ g / mL) injected into the isolated organ the isotonic phase and then in contraction tonic phase of the duodenum induced with acetylcholine (1 mM) or BaCl₂ (160 μ g / mL). The peak obtained at the recorder level with each extract compared with that previously created by acetylcholine (Ach). The references used were atropine. The percentage inhibition of contraction (PI) calculated according to the formula:

 $PI = (h1 - h2 / h1) \times 100$. h1: height of the peaks due to the contractor alone; h2: heights of the peaks due to the contractor in the presence of the extract.

2.8. Statistical treatment of results

The statistical data were processed with the PCS software, one-way analysis of variance. The results were expressed in average form \pm Standard Mean Error (E.S.M.). The t-student test allowed the comparison of averages. The different figures were plotted using GraphPad Software Prism version 2.01. The series are considered significant when the probability of error (p) is lower than the agreed risk: 0.05 (p <0.05).

3. Results and discussion

3.1. Phytochemical study

Thin layer chromatography (TLC) analysis revealed the presence in the two extracts of the plant studied, the chemical groups represented in Table I. The compounds of interest were compounds known for their vermicidal and anthelmintic properties. TLC revealed the presence of compounds such as flavonoids, tannins, terpenes and sterols, saponosides, coumarins and the absence of alkaloids in both extracts (Figure 1). Several other studies have highlighted the presence of flavonoids and coumarins [9], tannins [16], terpenes and sterols [17], saponins [16] [18]. The absence of alkaloids has also been noted by many authors, including Fagbohoun and Atto et al., in the different parts of the plant [16] [17].

Extracts	Leaves	Trunk bark		
Flavonoïds	++	+		
Tannins	++	+++		
Terpenes and sterols	+	+		
Alcaloïdes	-	-		
Saponosides	+++	++		
Coumarines	++	++		

Table 1 Chemical groups characterized in the aqueous extract of K. senegalensis

а

Figure 1 Chromatogram of the main chemical groups revealed in daylight or UV; Tannins (a), Saponosides (b) and Flavonoïds (c)

b

с

3.2. Acute general toxicity

Assessment of the acute toxicity of the studied extracts of Khaya senegalensis is very important for setting the limit doses for the use of the plant. The oral LD_{50} of the two extracts was greater than 5000 mg / Kg, which allowed them to be classified according to the Hodge and Sterner and WHO toxicity scales as extracts with virtually no toxicity per os [19] [20]. The LD₅₀ of the various extracts administered intraperitoneally are shown in Table II and the regression of the LD_{50} curves was validated by the equality of the ratios LD₉₅ / LD₅₀ and LD₅₀ / LD₅. The LD₅₀ of the leaf extracts is much lower than that of the extracts of the trunk bark. Indeed, the LD₅₀ obtained by *i.p.* aqueous leaf macerate was 903.414 mg / kg which would classify them as slightly toxic extracts according to the Hodge and Sterner (1943) and WHO (2002) toxicity scales. That of the trunk bark was 316 mg / kg which would classify them as moderately toxic extracts according to the toxicity scale of Hodge and Sterner (1943) and that of WHO (2002). The very high LD_{50} of the various oral extracts, unlike that obtained by the intraperitoneal route, could be explained by the difference in the routes of administration. Indeed, the digestive tract can play a role of barrier to the passage of toxic substances in contrast to the intraperitoneal route which offers a better bioavailability of the toxic substance. The safety indices of the two extracts were less than five (5), which indicates that the handling of the extracts is difficult because easily the toxic doses are reached since the difference between the non-toxic dose (LD₅) and the surely fatal dose (LD₉₅) was weak.

Extracts	LD95 mg / kg	LD50 mg/kg	LD5 mg / kg	LD95 / LD50	LD50 / LD5	LD95 / LD5
Leaves	1366.9	935.6	640.4	1.46	1.46	2.13
Trunk bark	700	316	146	2.2	2.2	4.8

Table 2 Acute toxicity of the aqueous extract from trunk bark and leaves of *K. senegalensis* in the mice (intra peritoneal way)

3.3. Vermicide activity

The earthworm was used because it shows anatomical and physiological resemblance with intestinal round worms and therefore serves as a suitable model for screening of anthelmintic drugs and because of their easy availability they are used as suitable models for screening of anthelmintic drugs [21] [22]. The tests showed that the two extracts used had vermicidal activities, an $LC_{50} = 3.37$ mg/mL for the trunk bark and $LC_{50} = 8.25$ mg/mL for the leaves under the conditions compared to the reference product, Combifrinil® (pyrantel pamoate pharmaceutical product, anthelmintic used in human health) with an LC₅₀ = 3.92 mg/mL (Fig 2). The effect appears earlier (30 minutes) for Combifrinil® and is at 100% mortality after 5 hours, whereas those of the extracts of the bark of the trunk and the leaves appear respectively after 2 hours and 5 hours after the implementation, contact and is 100% mortality after 16 h and 22 h of contact (Table III). These results are in agreement with those of Lompo [9] which showed an LC₅₀ (5.76 mg/mL) of the trunk bark more active than that of the reference compound of Combantrin® (12.9 mg/mL). He added that these manifestations were also observed in the nematode worm Caenorhabditis elegans. Which suggested to him the research of the interaction of the aqueous extract with the cholinergic system [9]. The anthelmintic properties of Khaya senegalensis have been demonstrated in some studies [23] [9] and are thought to be due to the phytochemicals contained in the various extracts of the plant. According to Minaflinou and al., Medicinal plants owe their anthelmintic properties to their chemical composition [24] so the vermicidal action of *Khava senegalensis* extracts is due to these phytochemical constituents. Poalin and al., have shown that tannins and flavonoids play an essential role in the anthelmintic activity of plants [25] and according to Ademola and al., saponosides have an anthelmintic effect which would be manifested by destabilization of membranes and increase of permeability of the cells causing the bursting of the cells by turgescence [26].

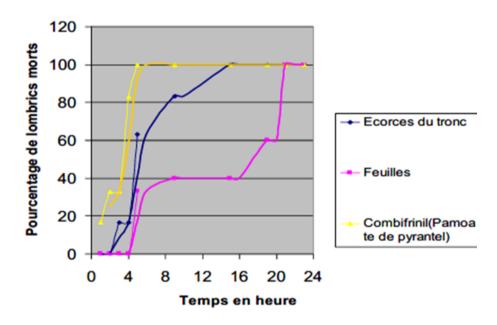


Figure 2 Kinetics of toxicity induced in earthworms by aqueous macerates and Combifrinil (pyrantel pamoate) at an equal concentration of 7.5 mg/mL

Dose (mg/mL)	Leaves			Trunk b	Trunk bark		Combifrinil		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
1	0	0	0	0	0	0			
3,75	0	0	40	20	40	60	16,324	70	100
5	0	0	60	80	100	100	66,66	100	100
7,5	20	60	100	100	100	100	100	100	100
8,75	60	100	100						
10	100	100	100						

Table 3 Vermicidal activity of the aqueous macerate of trunk bark, leaves of Khaya senegalensis and Combifrinil

3.4. Antispasmodic activity

The results observed in this study reveal a spasmolytic effect of the aqueous macerates of the plant on spontaneous contractions or caused by Ach at the submaximal concentration of 0.35 μ M. Indeed, the injection of macerated and atropine (anticholinergic reference) causes relaxation (inhibition of spontaneous contractions of the intestine) induced by acetylcholine on the smooth muscle of duodenum rats. The aqueous extracts of both parts of *Khaya senegalensis* inhibited the contractions (isolated gut) caused by acetylcholine in curative administration (Figure 3). The concentration of trunk bark extracts capable of inhibiting acetylcholine contractions by 50% was 0.97 mg/mL and 1.93 mg/mL for leaf extracts. (Figure 4). Statistical analysis of these results indicated that there was no significant difference between these different extracts (p < 0.05). These results corroborate those of Lompo and al., who had shown that these effects were of the anticholinergic type, therefore of the neurotropic type [10].

The contraction of the ACh-induced rat duodenum $(0.35 \ \mu\text{g} / \text{mL})$ is thought to be the activation of muscarinic (M3) receptors located at the smooth muscle cell membrane [27]. This contractile response was inhibited in the presence of increasing cumulative concentrations of the extracts in a concentration-dependent manner [28]. The effect of the extracts on the contraction induced by ACh is similar to that of atropine which is a neurotropic spasmolytic. The results of these anticholinergic activities of the aqueous extracts could be explained by the fact that the latter contain antagonistic substances (direct or indirect) of acetylcholine responsible for these activities by acting on the M3 receptors [29]. These anticholinergic properties could be related to the presence of chemical groups including alkaloids, coumarins and derivatives, saponosides and tannins [25]. The antispasmodic effect of triterpenic saponins in anesthetized rats was evidenced by Pommel, B [30]. Quinone compounds such as anthracenosides have an action in the regulation of digestive activity, laxative and purgative depending on the dose [9]. Terpene compounds such as monoterpenes and sesquiterpenes would have spasmolytic, anthelmintic and antifungal properties [31]. The presence of terpene and triterpenic compounds and their antispasmodic and spasmolytic activities may constitute an explanatory pharmacodynamic support for the use of this plant as a traditional therapy in the treatment of gastroenterological pathologies.

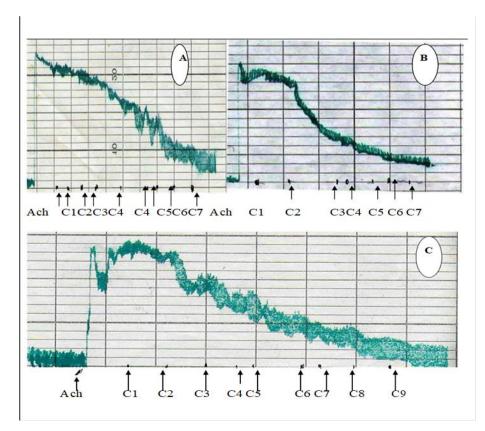


Figure 3 Recording cumulative effect of trunk bark (A), leaf (B) and atropine (C) extract on ACh-induced duodenum contraction. Paper unwinding speed: 0.1 mm / s. ACh= 0,35 µg / mL

C1= 500 μ g / ml, C2= 1000 μ g / ml, C3= 1500 μ g / ml, C4= 2000 μ g / ml et C5= 2500 μ g / ml C6= 3000 μ g / ml, C7= 3500 μ g / ml of the two extracts (A and B) in the isolated organ.

C1= $10^{-4} \mu g / ml$, C2= $3,16.10^{-4} \mu g / ml$, C3= $5,01.10^{-4} \mu g / ml$, C4= $1,00 \ 10^{-3} \mu g / ml$, C5= $1,99.10^{-3} \mu g / ml$, C6= $3,98.10^{-3} \mu g / ml$, C7= $1,00.10^{-2} \mu g / ml$, C8= $1,99.10^{-2} \mu g / ml$, C9= $3,98.10^{-2} \mu g / ml$ of atropine (C) in the organ.

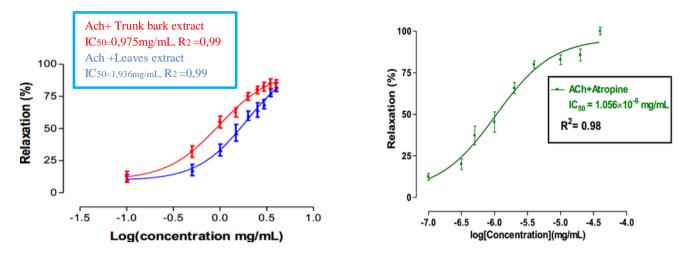


Figure 4 Curve Doses-effects of cumulative trunk bark extract, leaf extract and atropine on ACh-induced duodenum contraction.

4. Conclusion

This study revealed the vermicidal and antispasmodic properties of aqueous macerates in the trunk bark and *Khaya senegalensis* leaves through an explanatory pharmacodynamic support. Phytochemical screenings show that samples of harvested trunk bark and leaf contain similar chemical groups with variations in levels from one extract to another. Harvested leaf samples were richer in saponosides and flavonoids than those in the trunk bark harvested at the same time. On the other hand, the bark of the trunk was richer in tannins and anthocyanosides than the leaves harvested at the same time. The results of phytochemical, pharmacological and toxicological studies carried out in vitro and ex vivo have made it possible to justify the use of the extracts of this plant in traditional therapy in the treatment of some intestinal worms. Therefore, a more in-depth study is necessary to lead to the development and development of new molecules with anthelmintic potential from the plant.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest in this study.

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