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RESEARCH ARTICLE

Antidiarrheal Evaluation of Aqueous and Ethanolic Stem Bark Extracts of *Khaya senegalensis* A. Juss (Meliaceae) in Albino Rats

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ABSTRACT

The stem bark of *Khaya senegalensis* A. Juss (Meliaceae) is used traditionally in the treatment of malaria, intestinal worms, diarrhea, dysentery and venereal diseases. Despite the claim as an effective antidiarrheal remedy in both humans and animals, there is scarcity of documented scientific information of specific *in vivo* antidiarrheal test using extracts of this plant. The number of wet feces and the distance travelled by activated charcoal meal in rats orally given 300, 600 and 1200 mg/kg doses of aqueous and ethanolic extracts of the stem bark of *K. senegalensis* were evaluated in the castor oil induced diarrhea and gastrointestinal motility studies. The phytochemical constituents and acute toxicity test of the extracts were also tested using standard methods. Both extracts dose-dependently ($P<0.05$) reduced diarrhea induced by castor oil in rats, but did not significantly decrease ($P>0.05$) distance travelled by charcoal in the gastrointestinal motility test. The extracts were apparently safe at 2000 mg/kg body weight per os. Cardiac glycosides and flavonoids were present in both extracts, while tannins were present only in the ethanolic extract**.** The aqueous and ethanolic stem bark extracts of *K. senegalensis* inhibits diarrhea, at least in part, by a mechanism other than inhibition of gastrointestinal motility. The antidiarrheal activity of *K. senegalensis* may be attributed to the flavonoid and tannin constituents present in the extracts. The ability of *K. senegalensis* to significantly protect against castor oil induced diarrhea justifies its use in traditional management of human and animal diarrhea.

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INTRODUCTION

Diarrhea is a common and costly pathologic condition affecting livestock (Elhassan *et al*., 2011) and presented in most livestock diseases as a symptom with adverse effects which include anorexia, dehydration, weight loss, depression, discomfort and death (Randolph *et al*., 2007; Wyatt *et al*., 2010). Current management of diarrhea involves the use of drugs such as antibiotics, atropine sulphate, loperamide, kaolin, anthelminthics, fluid and electrolyte replacement therapy (Hall, 2011). Despite the availability of a wide range of approaches in diarrheal management, low income livestock farmers in developing countries with limited access to modern veterinary drugs and services, rely on herbal preparations for the health care needs of their animals (Offiah *et al*., 2011; Jung *et al*., 2011).

Khaya senegalensis A. Juss (Meliaceae) is commonly called the dry zone mahogany or African mahogany (Danquah *et al*., 2012) and regarded as the most popular medicinal meliaceous plant in African traditional remedies, growing up to 30 meters in height. In Nigeria, the tree has many local names; 'Madaci' in Hausa, 'Dalehi-Kahi' in Fulfulde, 'Oganwon' in Yoruba and 'Ono' in Igbo languages (Makut *et al*., 2008; Offiah *et al*., 2011). The Hausa and Fulani tribes in northern Nigeria use *K. senegalensis* as a remedy for several human and animal ailments (Makut *et al*., 2008) The stem bark of *K. senegalensis* is used traditionally in the treatment of malaria, intestinal worms, diarrhea, dysentery and venereal diseases (Adebayo and Kretti, 2011). In

traditional veterinary practice, powdered stem bark of *K. senegalensis* mixed with bran or water is administered to animals showing signs of anorexia and diarrhea (Offiah *et al*., 2011). The anti-inflammatory (Bum *et al*., 2010), antibacterial (Kubmarawa *et al*., 2008), anthelminthic (Ademola *et al*., 2004), anti-trypanosomal (Maikai *et al*., 2010), and antiplasmodial (Ahmed *et al.*, 2010), activities of *K. senegalensis* have been scientifically investigated. There is however a dearth of documented scientific information on specific *in vivo* antidiarrheal test using extracts from this plant.

Sequel to our findings in an ethnobotanical survey of medicinal plants used in the management of animal diarrhea in some selected areas of Plateau State (Offiah *et al*., 2011), this study was conducted to scientifically validate the antidiarrheal potential of crude extracts of *K. senegalensis* stem bark in adult albino rat model.

MATERIALS AND METHODS

Collection of plant material and Extraction: Fresh stem bark of *K. senegalensis* was collected in the premises of the National Veterinary Research Institute (NVRI) Vom, Jos Nigeria in February 2011. The plant was taxonomically authenticated and assigned Voucher No. 3755, in the Department of Biological Sciences, Ahmadu Bello University (ABU) Zaria, Nigeria. The stem bark of *K. senegalensis* were air-dried and ground to fine powder using a laboratory miller (Fritsch 13.1030, Germany) after crushing with pestle and mortar. Two portions of 300 g *K. senegalensis* were extracted using 3 L each of deionized water and 70% ethanol separately. The supernatant was filtered using Whatman No.1 filter paper and the filtrate was freeze-dried. The lyophilized extracts were stored in wide-mouthed screw-capped glass bottles at 4^{o}C until needed.

Experimental animals: Adult albino rats of both sexes, weighing 130-200 g were obtained from the Small Animal Section of the Diagnostic Division, NVRI Vom. The rats were kept in cages and allowed to acclimatize to the laboratory environment for two weeks before the commencement of the experiment. Freshly dried wood shavings were used as beddings and changed three times per week. The animals were fed standard pelleted rat feed from Dagwom Farm Mill, NVRI Vom with drinking water provided *ad libitum*. All experiments were conducted in accordance with the principles and guide for the care and use of laboratory animals (NRC, 1996), and approved by the animal welfare and ethics committee of the NVRI, Vom.

Phytochemical screening of extracts: The aqueous and ethanolic extracts of *K. senegalensis* stem bark were evaluated for the presence of phytochemicals using the methods described by Trease and Evans (1989), and briefly explained here. To 0.5 g of extract, 5 ml of ethanol was added and filtered. 4 ml of 1% HCl was added to the filtrate, and formation of white resinous precipitate confirms the presence of resins. To test for flavonoids, 2 g of the powdered plant material was detanned with acetone and placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample and the mixture was filtered while hot. The filtrate was allowed to cool, and then 5 ml of 20% sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids. About 0.5 g of the extract was stirred with 1 ml of distilled water and filtered, followed by the addition of ferric chloride solution to test for tannins. A blue-black, green or blue–green precipitate was taken as evidence for the presence of tannins.

To test for alkaloids, 5 g of the extract was stirred with 3 ml of 1% aqueous hydrochloric acid on a steam bath and filtered; 1 ml of the filtrate was treated with a few drops of picric acid solution and Mayer's reagent. Precipitation in each case was taken as preliminary evidence for the presence of alkaloids. The Keller Killani test was used to test for cardiac glycosides. Here 0.1 g of the extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. Concentrated sulphuric acid (1 ml) was added gently by the side of the test tube. A brown ring at the interphase indicates the presence of deoxy sugar, which is characteristic of cardenolides. Testing for anthraquinones involved placing 0.5 g of the extract in a dry test tube and adding 5 ml of chloroform, then shaken for 5 minutes and filtered. The filtrate is shaken with equal volume of 100% ammonia solution and observed for pink violet or red color in the ammoniacal layer (lower layer), indicative of the presence of free anthraquinones. About 5 g of the extract was shaken with water in a test tube to test for saponins, and frothing which persists on warming was taken as positive preliminary evidence.

Acute toxicity study: The mean lethal dose (LD_{50}) of the extracts was determined using standard protocol of the Organization of Economic Co-operation and Development (OECD, 2001).

Castor oil induced diarrhea in rats: The aqueous and ethanolic extracts were reconstituted in deionized water and 0.2% Dimethyl sulfoxide (DMSO), respectively. The method described by Offiah and Chikwendu (1999), was used. The rats were fasted for 12 hours prior to commencement of the experiment, but allowed access to drinking water *ad libitum*. They were separated into 9 groups of five rats each. Rats in groups I, II and III received 300, 600, and 1200 mg/kg dose of aqueous extract respectively. Groups IV, V, and VI rats were given 300, 600, and 1200 mg/kg dose of the ethanolic extract respectively. Those in groups VII and VIII received 5 ml/kg deionized water and 5 ml/kg 0.2% DMSO respectively to serve as negative control while animals in group IX were given standard antidiarrheal drug Loperamide (Imodium®) 10 mg/kg as positive control. All treatments in the groups were administered orally using a gastric tube. The rats were housed singly in cages with wire meshed floors and white blotting paper placed underneath to collect the feces. One hour after treatment, all rats in the 9 groups were given 1 ml of castor oil orally and observed for 4 hours. At the end of the experiment the number of wet or unformed feces from each rat was counted, group means obtained, and the percentage decrease in diarrhea was calculated.

Gastrointestinal transit: Both extracts were tested for effects on gastrointestinal motility using the method described by Chitme *et al*. (2004) with some modifications. Forty-five animals were fasted for 18 hours prior to treatment, with free access to drinking water, and thereafter divided into 9 groups of 5 rats each. Groups I and II received 5 ml/kg of deionized water and 5 ml/kg of 0.2% DMSO respectively to serve as negative controls. The rats in groups III received 3 mg/kg atropine sulphate and served as positive control. Groups IV, V and VI were treated with 300, 600 and 1200 mg/kg doses of the aqueous extract respectively, while groups VII, VIII and IX received equivalent doses of ethanolic extract. Thirty minutes after drug and extract administration, each rat received 1 ml of 5% activated charcoal suspension in 10% aqueous solution of acacia powder by oral gavage. The rats were sacrificed 30 minutes later following light ether anesthesia and a median abdominal incision was made for laparotomy. The small intestine was carefully separated from the mesentery avoiding being over-stretched. The distance travelled by the charcoal meal from the pylorus was measured and expressed as percentage of the total length of intestine from pylorus to the cecum (Mascolo *et al*., 1999).

Statistical analysis: The data is presented as mean±SEM and analyzed by one way ANOVA with Tukey's post test performed using GraphPad Prism Version 4.03 for Windows, GraphPad software 119 San Diego, California, USA www.graphpad.com. P values less than 0.05 were considered significant.

RESULTS

Extraction and phytochemical screening of *K. senegalensis*: The yield of the plant was 4.15% and 5.19% of the dried bark for the aqueous and ethanolic extracts respectively. The result of phytochemical screening shows that the aqueous extract contains glycosides and flavonoids while the ethanolic extract contains tannins, glycosides and flavonoids. Alkaloids, saponins and resins were not present in both extracts (Table 1).

Acute toxicity (LD_{50}): The oral LD_{50} in rats for both ethanolic and aqueous extracts was above 2000 mg/kg body weight (Table 2).

Castor oil induced diarrhea: The percentage reduction in number of unformed feces was statistically significant (P<0.05) reaching 57.35, 81.08 and 76.35% in rats treated with aqueous extracts of *K. senegalensis* at 300, 600 and 1200 mg/kg, respectively. For the ethanolic extract, there was statistically significant (P<0.05) reduction of 64.87, 67.56 and 86.49% in numbers of unformed feces for rats treated with 300, 600 and 1200 mg/kg of the extract, respectively (Table 3).

Gastrointestinal transit of activated charcoal meal: The extracts did not significantly (P>0.05) decrease the distance travelled by the charcoal meal. Atropine (3 mg/kg) on the other hand significantly decreased the distance travelled by the charcoal plug (Table 4).

Table 1: Result of phytochemical screening of aqueous and ethanolic extracts of *Khaya senegalensis* bark.

Phytochemical	Aqueous	Ethanolic
Alkaloid	-	-
Saponins	-	-
Tannins	-	+
Cardiac glycosides	+	+
Flavonoids	+	+
Resins	-	-
Anthraquinones	-	-

+ Detected; – Not detected

Table 2: Result of acute oral toxicity (LD_{50}) of *Khaya senegalensis* extracts in rats

Set of Rats	No of rats	Dose (mg/kg)	Mortality	LD_{50} (mg/kg)
Set 1	3	2000	0/3	>2000
Set 2	3	2000	0/3	>2000
Set 3	3	2000	0/3	>2000

The same procedure was used for both aqueous and ethanolic extracts.

Table 3: Effects of aqueous and ethanolic extracts of *Khaya senegalensis* stem bark on castor oil induced diarrhea in albino rats (data are mean±SEM, n=5 in each group).

Treatment Aqueous Extract	Mean defecation in 4 hours	% Inhibition of defecation
	Aqueous Extract	
Castor oil + Water	7.40±0.10	-
Castor oil + Loperamide	-	100
Castor oil + KS (300 mg/kg)	3.60±0.67**	51.35
Castor oil + KS (600 mg/kg)	1.40±0.75***	81.08
Castor oil + KS (1200 mg/kg)	1.75±0.43***	76.35
Ethanolic Extract		
Castor oil + Water	7.40±0.10	-
Castor oil + DMSO	6.60±1.30	10.81
Castor oil + Loperamide	-	100
Castor oil + KS (300 mg/kg)	2.60±0.81*	64.87
Castor oil + KS (600 mg/kg)	2.40±1.50**	67.56
Castor oil + KS (1200 mg/kg)	1.00±0.63***	86.49

DMSO: Dimethylsulfoxide; KS: *Khaya senegalensis;* *=P<0.05; **=P<0.01; ***=P<0.001 versus control, one way ANOVA.

Table 4: Effects of aqueous and ethanolic extracts of *Khaya senegalensis* stem bark on gastrointestinal transit (data are mean±SEM, n=5 in each group).

Treatment	Total Length of Intestine (cm)	Distance travelled by charcoal meal (cm)	% Intestinal Transit
Aqueous Extract			
Water	98.38±5.14	82.00±6.12	83.04±2.31
DMSO	100.5±1.72	74.10±3.19	73.71±2.71
Atropine (3 mg/kg)	96.20±0.80	50.20±5.07	52.32±5.61**
KS (300 mg/kg)	91.80±2.65	60.00±5.60	66.20±7.47
KS (600 mg/kg)	99.80±2.71	72.80±2.08	73.27±3.43
KS (1200 mg/kg)	103.40±1.57	68.20±7.21	65.82±6.71
Ethanolic Extract			
Water	98.38±5.14	82.00±6.12	83.04±2.31
DMSO	100.5±1.72	74.10±3.19	73.71±2.71
Atropine (3 mg/kg)	96.20±0.80	50.20±5.07	52.32±5.61**
KS (300 mg/kg)	93.60±1.75	73.20±1.83	78.38±2.95
KS (600 mg/kg)	94.80±2.00	69.92±4.72	73.83±4.96
KS (1200 mg/kg)	102.80±2.35	73.60±3.82	71.51±2.80

DMSO: Dimethylsulfoxide; KS: *Khaya senegalensis;* **=P<0.01 versus control.

DISCUSSION

The popularity of plants as credible remedies for diseases is on the increase. This is made possible by the presence of bioactive phytochemicals in them. The presence of some phytochemicals in aqueous and ethanolic extracts of *K. senegalensis* is an indication that it will possess some form of pharmacological activity. Our findings show that the ethanolic extracts of *K.*

senegalensis stem bark in addition to glycosides and flavonoids contains tannins and this is in agreement with the findings of Makut *et al*. (2008) and Kubmarawa *et al*. (2008). Use of different solvents in extraction process has been shown to determine the phytochemical composition of extracts (Makut *et al*., 2008) and consequently influence the resulting biological activity.

In the acute toxicity studies, dose of up to 2000 mg/kg *per os* of ethanolic and aqueous extract did not result in any obvious signs of toxicity thus signifying that both extracts are apparently safe for all practical purposes (OECD, 2001).

The significant reduction of diarrhea in rats treated with *K. senegalensis* (Table 2) shows that the extracts possess antidiarrheal activity. This activity may be due to the flavonoid content of both extracts and in addition the tannin content of the ethanolic extract. A study of phytochemicals of plants found along the Mediterranean coast and used to treat diarrhea reports the constituents probably responsible for the antidiarrheal activity to be tannins and flavonoids since they were found in all plants tested (Atta and Mouneir, 2005). Tannins are astringent, bitter plant phenolic compounds that complex and precipitate proteins making the intestinal mucosa more resistant to the action of castor oil and also reduce intestinal fluid secretion (Chaulya *et al*., 2011). Plants with astringent property are usually recommended for diarrhea management because they are thought to exert this effect on the mucosal lining of the small intestine (Akuodor *et al*., 2011).

In the motility studies using charcoal meal marker, both aqueous and ethanolic extracts did not significantly reduce gastrointestinal motility. The ability of *K. senegalensis* to inhibit diarrhea therefore is likely to be at least in part by a mechanism other than inhibition of GIT motility. This assumption is supported by the phytochemical composition since alkaloids, tannins, flavonoids and phenolics are thought to be responsible for antidiarrheal activity of plants (Palombo, 2006). Mechanistic investigation shows that tannins and flavonoids increase water and electrolyte reabsorption in the colon while other phytochemicals act by inhibiting intestinal motility (Palombo, 2006). Black tea extract containing flavonoids and tannins has also been shown to increase upper gastrointestinal tract transit but inhibited castor oil induced diarrhea and intestinal fluid accumulation (Besrea *et al*., 2003). When ingested, castor oil is broken down into recinoleic acid which causes irritation and inflammation of the intestinal mucosa and release of prostaglandins (Chaulya *et al*., 2011). This acid also alters permeability of the intestinal mucosa to electrolytes and water thereby causing diarrhea (Kumar *et al*., 2010). The antidiarrheal activity of *K. senegalensis* may in part be mediated via its anti-inflammatory action since it has been shown to influence biosynthesis of prostaglandins (Teke *et al*., 2010). *K. senegalensis* has also been shown to have antibacterial (Makut *et al*., 2008; Kubmarawa *et al*., 2008) and anticoccidial (Nwosu *et al*., 2011) activity. These actions will further increase the antidiarrheal activity of *K. senegalensis* in diarrhea caused by susceptible agents.

The ability of *K. senegalensis* to significantly protect against castor oil induced diarrhea justifies its use in diarrhea management.

Conclusion: The results of this investigation revealed that aqueous and ethanolic extracts of *K. senegalensis* stem bark contains pharmacologically active substance(s) with antidiarrheal properties. Further research is to be carried out to fractionate and purify the extract, in order to find out the compounds responsible for the antidiarrheal activities evaluated.

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