Academia Journal of Pharmacy and Pharmacology 3(1): 001-006, September 2015
DOI: 10.15413/ajpp.2015.0101
ISSN 2315-7828

Academia Publishing

Research Paper

Phytochemical Screening and Antidiarrheal Evaluation of Acetone Extract of *Acacia sieberiana* var woodii (Fabaceae) stem bark in wistar rats

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Accepted 25th April, 2015

ABSTRACT

This study is aimed at evaluating the phytochemical constituents and the antidiarrheal activities of the acetone extract of Acacia sieberiana stem bark. The phytochemical screening was carried out to identify the active constituents present in the acetone extract, and the acute toxicity test was also carried to determine the safety limit of the extract. Gastro intestinal motility and castor oil-induced diarrhea tests were evaluated in rats to determine the antidiarrheal activity of the extract. The phytochemical screening revealed the presence of saponin, tannins, cardiac glycosides, steroidal ring, resins and carbohydrates. No deaths or signs of abnormal behavior were observed in the wistar rats treated with the acetone extract of *A.sieberiana* stem bark up to 2000 mg/kg bodyweight in the toxicity test. The acetone extract of *A.sieberiana* stem bark slowed down the propulsion of charcoal meal through gastro-intestinal tract, though not in a dose-dependent manner following the administration of the extract to rats at graded doses (300, 600 or 1200 mg/kg), with the highest inhibition at 600mg/kg. This extract also exhibited a significant inhibition of castor oil-induced diarrhea in a dose-dependent manner with the highest inhibition ($p<0.001$) of 84% at 1200mg/kg dose. The results of this investigation showed that the acetone extract contains phytochemical substances with antidiarrheal properties. This provides the rationale for the use of the stem bark extract of *A. sieberiana as* an anti-diarrheal remedy by traditional healers.

Key words: Phytochemical, acute toxicity, antidiarrheal activity, *Acacia sieberiana*.

INTRODUCTION

Diarrhea is the leading cause of morbidity and mortality amongst children in developing countries (Coker et al., 1998), and is a major health problem (WHO, 1986). Moreover, it is an impaired absorption and hypersecretion syndrome of the gastro-intestinal tract (Alan, 1982) and may be brought about by viruses, bacteria, fungi, protozoa, drugs and bacteria endotoxins (Jawetz et al., 1984).

Diarrhea is characterized by an increase in the frequency of bowel movements, wet stool and abdominal pains (Ezekwesili et al., 2004). It is the world's third highest killer disease, contributing substantially to pediatric morbidity and mortality, especially in the malnourished (Havagiray et al., 2004; WHO, 2009). The incidence of diarrhea is still high (about 7.1 million per year), despite the efforts of international organizations to control its occurrence (Kouitcheu et al., 2006).

Antibiotics used as antidiarrheal drugs sometimes provoke adverse effects and microorganisms tend to develop resistance toward them (Soberon et al., 2007). Additionally, the existing antidiarrheal drugs are either not available or affordable by many rural dwellers. Therefore, the search for safe and more effective agents from plant origin has continued to be an important area of active research. Many rural dwellers in the world depend largely on medicinal herbs for the treatment of diarrheal conditions because these herbs are readily available,

affordable and are an indispensable component of traditional medicine practice.

Acacia sieberiana has its common names white thorn, umbrella thorn, flat-topped thorn, paperback thorn and paperback in English and in Hausa as Fara kaya and in Yoruba as Aluki. Its leaves, bark, pods, roots and resin have astringent properties and are used medicinally for a wide variety of ailments including stomach-ache, inflammation of the urinary tract, colds and chest problems, syphilis, gonorrhea and diarrhea (Orwa et al., 2009). *A. sieberiana* is also used by the people of plateau state, Nigeria to treat diarrhea in animals and humans (Offiah et al., 2011). Therefore, our main aim is to verify this affirmation by determining the phytochemical compounds present and evaluate the antidiarrheal activities of the acetone extract of *A.sieberiana* stem bark.

MATERIALS AND METHODS

Plant material

The stembark of *Acacia seberiana* (Fabaceace) were harvested at Jos South Local Government, Plateau state, Nigeria in June 2012 and was identified and authenticated by Mallam Umar Gallah of the Department of Biological Sciences, Ahmadu Bello University (ABU) Zaria, Nigeria and deposited at the Herbarium with the voucher number 3575. The plant material was cut into small pieces, dried at room temperature in the laboratory, and then ground to fine powder.

Preparation of crude extracts

500g of the fine powder was macerated in acetone for 48h with regular stirring. The supernatant was filtered using Whatman filter paper No. 1 and the filtrate freeze dried. The final crude extract obtained was stored at 4°C. The yield of extraction was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of crude extracts 1'huile}}{\text{Weight of plant powder}} \times 100$$

Phytochemical screening

The protocol of Trease and Evans, 1989 was used in carrying out the phytochemical screening.

Acute toxicity

A limit dose of 2000mg/kg was administered to 5 female wistar rats daily for a period of three days using the OECD 2001 protocol.

Anti diarrheal assay

Gastrointestinal transit of activated charcoal

The extract was reconstituted with distilled water. Twenty-five rats were used for this experiment. The method of Chitme et al. (2004) was used with some modifications. The animals feed were withdrawn 16 h before commencement of the experiment, but were allowed access to drinking water. The rats were then divided into 5 groups of 5 rats each. Groups 1 and 2 received 3mg/kg of atropine sulphate and 5 ml/kg of distilled water to serve as positive and negative control respectively. The rats in groups 3, 4 and 5 were treated with graded doses of 300, 600 and 1200 mg/kg of the acetone extract of *A. sieberiana* respectively. Thirty minutes after drug and extract administrations, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of acacia powder was given orally to each rat. The rats were sacrificed 30 mins later and the abdomen opened. The distance travelled by the charcoal meal from pylorus was measured and expressed as percentage of the total length of intestine from pylorus to the cecum (Mascolo et al., 1999). All treatments in the groups were administered orally.

Castor oil induced diarrhea

The acetone extract of *A. sieberiana* stem bark were tested against castor oil induced diarrhea in wistar rats. The acetone extract was reconstituted with distilled water. Twenty-five rats were used for this study, the method of Offiah and Chikwendu, (1999) was used. The rats were fasted for 12 h before the commencement of the experiment, but were allowed access to water. They were separated into five groups. Rats in groups 1 and 2 received Loperamide at 10mg/kg body weight and 5ml/kg of distilled water to serve as positive and negative control respectively. Rats in Groups 3, 4 and 5 were given 300, 600, and 1200 mg/kg dose of the acetone extract respectively. The rats were housed singly in a cage lined with white blotting paper. One hour after treatment, all the rats in the 5 groups were given 1ml of castor oil each. The rats were observed for 4 h for watery (wet) or unformed feces. The watery feces from each rat were counted hourly, and at the end of the experiment a group mean was obtained and the percentage decrease in diarrhea was calculated. All treatments in the groups were administered orally.

Statistical analysis

The data was presented as mean ± SEM and analyzed using

Table 1: Results of phytochemical screening of *Acacia sieberiana* acetone stem bark extract.

Constituents	Acetone extract
Alkaloids	-
Saponin	+
Tannins	+
Anthraquinones	-
Flavonoids	-
Cardiac glycosides	+
Steroidal ring	+
Resins	+
Carbohydrates	+

(+): present, (-): absent

Graph Pad Prism Version 4.03. Student's t-test for unpaired data was used when comparisons were made between two groups. P values less than 0.05 were considered significant.

RESULTS

Yield of extraction

The yield obtained from the extraction is 1.61%.

Phytochemical screening

Table 1 shows the presence of saponin, tannins, cardiac glycosides, steroidal ring, resins and carbohydrates in the acetone extract.

Acute toxicity

The administration of the acetone extract of the stem bark of *A. sieberiana* at the limit dose of 2000mg/kg body weight to the rats orally did not show signs of abnormality and death of the rats. This indicates (Table 2) that the LD_{50} is above 2000mg/kg (LD_{50} > 2000mg/kg).

Antidiarrheal assay

Effect of the extract on intestinal transit of charcoal in rats

The extracts did not significantly ($p > 0.05$) reduce the distance travelled by charcoal meal. This effect was not dose dependant; 58.64, 56.18 and 57.17% at the doses 300, 600 and 1200mg/kg respectively. The standard drug significantly ($p<0.01$) reduced the distance travelled by charcoal meal at 48.99% (Table 3).

Effect of the acetone extract on castor oil induced diarrhea

The percentage reduction of defecation was dose dependent; 28, 40 and 84% in rats treated with the acetone extract of *A. sieberiana* at the doses of 300, 600 and 1200mg/kg respectively. The highest dose of 1200mg/kg body weight was significant ($p <0.001$) in reducing diarrhea (Table 4). The percentage reduction (84%) at the dose of 1200mg/kg body weight was proportional to that of the standard drug loperamide (100%).

DISCUSSION

Acetone was used as solvent for the plant extraction because it dissolves many hydrophilic and lipophylic components present in plants, it is miscible with polar and non polar solvents, it is volatile and has a low toxicity to the bioassay used (Eloff, 1998).

The phytochemical screening revealed a number of compounds (saponin, tannins, cardiac glycosides, steroidal ring, resins and carbohydrates). Moreover, tannins have been shown to be present in leaves and pods of *A. sieberiana* tree (Mlambo et al., 2009; Nsahlai et al., 2011). Tannins, flavonoids, saponins and steroids have been shown to possess antidiarrheal properties (Ezenwali et al., 2010; Saralaya et al., 2010; Longanga et al., 2000).

In the acute toxicity, no deaths and no signs of abnormal behavior were observed in the wistar rats treated with 2000mg/kg bodyweight of the extract. This result suggests that the extract is relatively safe for practical purposes and any adverse health effects following therapy with the acetone extract from *A. sieberiana* stem bark would not be expected (OECD, 2001).

The acetone extract of *A. sieberiana* stem bark reduced the propulsion of charcoal meal through gastro-intestinal tract, though, the movement of the charcoal meal was not significant and also not in a dose-dependent manner with the highest inhibition at 600mg/kg.

Atropine was used as the standard drug and it significantly ($p<0.01$) reduced the distance moved by charcoal meal. This is because atropine is known to inhibit intestinal transit by anticholinergic effect (Izzo et al., 1999). The decrease in movement of the charcoal meal observed with the extract could be an indication of the extract having a similar mode of action with Atropine.

In the castor oil-induced diarrhea, the extract at all doses (300, 600 or 1200 mg/kg) inhibited the castor oil-induced diarrhea in a dose-dependent manner as compared to the group treated with distilled water. A significant inhibition ($p<0.001$) was observed at the dose of 1200mg/kg body weight of the acetone *A. sieberiana* stem bark. The remarkable dose-related reductions in castor oil-induced diarrhea produced by the extract is a clear evidence of the efficacy of the extract, when compared with loperamide (10

Table 2: Result of Median Lethal Dose (LD_{50}) in mg/kg body weight.

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

Table 3: Effect of the extract on intestinal transit of charcoal in rats.

Treatment	Distance travelled by charcoal meal (cm)	Total length of intestine (cm)	% Intestinal transit
Distilled water (5ml/kg)	63.00 ± 2.95	94.40 ± 1.12	66.64± 2.35
Atropine (3mg/kg)	46.20 ± 4.89	93.30 ± 2.16	48.99± 4.37**
A. sieberiana acetone extract (300mg/kg)	58.80 ± 9.14	99.20 ± 2.13	58.64± 8.38
A. sieberiana acetone extract (600mg/kg)	54.20 ± 7.62	99.00± 4.40	56.18± 9.25
A. sieberiana acetone extract (1200mg/kg)	55.80 ± 10.85	97.60 ± 2.62	57.17 ± 11.17

Values are expressed as mean + S.E.M,*Indicates the level of significance at P<0.05; using student t-test; **p<0.01.

Table 4: Effect of the acetone extract on castor oil induced diarrhea.

Treatment	Mean of defecation	% Inhibition of defecation
Distilled Water (5ml/kg)	5.00 ± 0.77	-
Loperamide (10mg/kg)	0.00 ±0.00***	100
A.sieberiana acetone extract (300mg/kg)	3.60 ± 0.40	28
A. sieberiana acetone extract (600mg/kg)	3.00 ± 0.84	40
A. sieberiana acetone extract (1200mg/kg)	0.80 ± 0.49***	84

Values are expressed as mean + S.E.M, *Indicates the level of significance at P<0.05; using student t- test; ***p< 0.001.

mg/kg body weight) a standard anti-diarrheal drug. At 1200mg/kg body weight, the extract inhibited defecation by 84% while Loperamide inhibited defecation by 100%. This could be an indication that the extract has a similar action as loperamide, the standard drug.

In the present study, *A. sieberiana* stem bark inhibits diarrhea at least in part by a mechanism other than inhibition of gastro intestinal motility and this observed mechanism is similar to that reported with the aqueous and ethanolic extract of *Kaya senegalensis* (Elisha et al., 2012). Castor oil was used to induce diarrhea since its active component ricinoleic acid produces an irritating and inflammatory action on the intestinal mucosa, leading to the release of prostaglandins (Etuk et al., 2006; Etuk et al., 2009; Chaulya et al., 2011). This condition increased the permeability of the mucosal cells and provokes changes in electrolyte transport thus, causing diarrhea (Vieira et al., 2000; Kumar et al., 2010). Therefore, inhibitors of prostaglandin synthesis are known to delay diarrhea induced with castor oil (Sunil et al., 2001). The observation suggests that the antidiarrheal effect of the extract may be due to inhibition of prostaglandin synthesis (Okokon et al., 2010; Teke et al., 2010; Okokon et al., 2011). Moreover, the mechanism of castor oil is associated with dual effects of the gastrointestinal motility as well as, water and electrolyte transport (decreasing Na^+ and K^+ absorption) across the intestinal mucosa (Rouf et al., 2003). This phenomenon of secretions could be responsible for early production of stools in the distilled water treated animals (Ayinde and Owolabi, 2009).

The observed antidiarrheal activity of the acetone extract of *A. sieberiana* could be explained by the presence of tannins and steroids (Galvez et al., 1993; Havagiray et al., 2004; Ezenwali et al., 2010; Saralaya et al., 2010). These constituents each acts through different mechanisms thus, tannins have been reported to possess astringent property and acts by denaturing proteins in the intestinal mucosa by forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray et al.,2004; Chaulya et al.,2011). Medicinal plants possessing astringent property are recommended for the management of diarrhea because they are thought

to exert this effect on the mucosal lining of the small intestine (Akuodor et al., 2011).

In addition, steroids are useful for the treatment of diarrhea and also may enhance intestinal absorption of Na^+ and water (Longanga et al., 2000; Goodman et al.,1996; Maiti et al.,2007). Hence, steroid may also be responsible for the antidiarrheal activity. The presence of these compounds in the acetone extract could act singly or in combination with other constituents to exhibit the observed activity (Khadem et al., 2011).

Conclusion

The results of this investigation revealed that acetone extract contains pharmacologically active substance(s) with antidiarrheal properties. This provides the rationale for the use of the stembark extract of *A. sieberiana as* an anti-diarrheal remedy by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anti-diarrheal activity observed.

ACKNOWLEDGEMENT

The authors are grateful to the Africa Education Initiative and the National Veterinary Research Institute (NVRI), Vom for funding this project.

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Cite this article as:

Zeuko´o ME, Jurbe GG, Ntim PS, Ajayi TA, Chuwkuka JU, Dawurung CJ, Makoshi MS, Elisha IL, Oladipo OO, Lohlum AS (2015). Phytochemical Screening and Antidiarrheal Evaluation of Acetone Extract of Acacia sieberiana var woodii (Fabaceae) stem bark in wistar rats. Acad. J. Pham. Pharmacol. 3(1): 1-6.

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