

Effect of Aqueous Extract of *Acacia nilotica* on Microbial and Castor Oil Induced Diarrhoea

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(Received 30:06:15; Accepted 15:07:15)

Abstract

The seeds of *Acacia nilotica* were evaluated for *in vitro* bioactivity against *Escherichia coli* and *Salmonella typhi* using agar dilution method. The aqueous seeds extract exhibited bactericidal effects against *E. coli* and *S. typhi* at 1mg/ml. The minimum inhibitory concentration (MIC) of aqueous extract of *A. nilotica* was 0.5mg/ml for both organisms. Five (5) Albino rats of both sexes weighing between (85-125g) were used for each experiment and diarrhoea was induced using Castor oil. The extract showed remarkable anti-diarrhoea activity which was dose dependent at 100, 200 and 400mg evidenced by decrease in volume of intestinal content significantly different at $P < 0.05$ among the treatment. Anti-diarrhoea activity of the extract was comparable to 2mg/kgbw loperamide (standard drug) at 400mg/kgbw. The results suggest that aqueous extract of *A. nilotica* could be useful in the treatment of diarrhoea.

Key words: *Acacia nilotica*, aqueous extract, Loperamide, Diarrhoea.

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Introduction

Diarrhoea refers to a familiar phenomenon of unusually passage of loose or liquid stools about three times or more per day (WHO, 2013). The severity of diarrhoea is determined by the size and number of stools passed within a period of time, severe diarrhoea could pose risk of dehydration which may be a feature of chronic disease as body lose large amount of fluid quickly (Webmd, 2012). Drugs such as Loperamide hydrochloride, Chloramphenicol, Metronidazole and Kaopectate useful in the treatment of diarrhoea are associated with depression of bone marrow in iron utilization, anaemia, leucopenia, unpleasant mouth taste, nausea, vomiting, constipation, dizziness and loss of appetite amongst other (Mohammed *et al.*, 2009; Sanders *et al.*, 2007). Since diarrhoea chemotherapy is beset with multitude of problems such as drug toxicity, adulteration and increase in drug resistance, there is an urgent need to search for new drugs that are devoid of these problems. Plants are sources of

these new drugs, therefore it is necessary to carry out preliminary investigation on a medicinal plant such as *A. nilotica* with anti-diarrhoea activity on microbial and castor oil induced diarrhoea. *A. nilotica* has been used as medicinal plants in parts of Northern Nigeria, West Africa and other parts of the world generally (Okoro *et al.*, 2014). Bark of *A. nilotica* had been reported useful in the treatment of haemorrhage, tuberculosis and diarrhoea (New 1984). The root had been reported useful as an aphrodisiac and the flower used for treating syphilis lesions (New 1984). The ethyl acetyl fraction of *A. nilotica* pods was reported to contain anti-diarrhoea property (Sanni *et al.* 2010). In South Africa, bark of *A. nilotica* is useful to treat cough locally, the leave is believed to have cured potency while the bark, leaves, gum and pods are useful together as astringent because of the tannin they possess (Krause, 2005). Thus, *A. nilotica* seeds were selected in this research for study of its anti-diarrhoea activity in *in vivo* and *in vitro* models.

Materials and Methods

Plant Collection and Extract

Preparation: The seeds of *Acacia nilotica* were collected from National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The plant was authenticated by ethno botanists in Herbarium Department of NIPRD. NIPRD (2009) Protocol for extraction of plant materials was used for extraction of seeds of *Acacia nilotica*. The seeds were washed, rinsed and air dried at a room temperature ($28\pm 2^{\circ}\text{C}$) in a shade for a period of two weeks. The seeds were blended into powder using blender and the powdery form was used for herbal preparation. One hundred gram (100g) of the powdered seeds of the plant sample was extracted in 800ml of sterile distilled water by refluxing for six hours. The resulting mixture was then filtered with muslin cloth and the filtrate was then evaporated to dryness using a rotary evaporated at 50°C . The dried extract was kept in a sterile film plastic and stored in a refrigerator until required for antimicrobial and anti-diarrhoea assay.

Laboratory Animals: Albino rats (85-125) of either sex were obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Vom, Plateau State. The animals were maintained in a well ventilated room in appropriately design metallic cages bedded with dry clean wood shavings. The animals were fed with standard NIPRD formulated feed and had access to clean water from Abuja municipal waste water supply. The experimental rooms were clean and disinfected regularly. Soiled wood shavings were replaced often. The feed, water containers and animal cages were washed regularly.

Experimental designs: Tijani *et al.* (2009) method for experimental designs of animals was used. The experimental animals were twenty five albino rats of either sex, the animals were randomized into five groups, A, B, C, D and E with each group consisting of five animals. Individual animals were weighed and randomized into cages and code was used to differentiate each. The randomization is to ensure an average distribution of weight. The animals were fasted for 24 hours and were allowed free access to water. One gram (1g) of the extract was weighed and dissolved into 10ml of distilled water to obtain 100mg of the aqueous extract. Group A was set as a negative control and receive no treatment, group E was set as a positive control and was given 2mg loperamide (a standard drug for treatment of diarrhoea) While group B, C and D were given

100mg, 200mg and 400mg of the aqueous extract two hours before castor oil was given to them.

Induction of Diarrhoea: Diarrhoea was induced into the animals using castor oil after the animals have fasted for 24hours but allowed free access to water using Abdullahi *et al.* (2003) method. 1ml of castor oil was given to each animal in each group (A-E).

Standardization of Organisms: A loopful of *Escherichia coli* and *Salmonella typhi*, pure culture obtained from NIPRD was inoculated in 5ml of sterile broth and then incubated for 24hours. After incubation, 0.2ml of the inoculums was inoculated into 20ml of fresh nutrient broth. The incubation was done for 3-5 hours to obtain approximately 10^6 cfu/ml of the organism. A loopful of the standardized culture was used for antimicrobial assay (Babayi *et al.*, 2004).

Screening of the extract for antimicrobial activity (MIC): Mueller Hilton Agar was prepared according to manufacturer's direction. One mill (1ml) of the standardized test organisms was inoculated into the molten agar poured into sterile Petri-dishes which was allowed to get at room temperature. Eight Millimetres (8mm) diameter sterile cork borer was used to make wells which base was covered with molten agar. Different concentration of the prepared extract was introduced into the wells and was allowed to pre diffuse for 30minutes. The plates were then incubated at 37°C for 24 hours, after which the inhibition zone was measured and recorded in millimetre (Mm).

Screening of the extract for Minimum Inhibitor Concentration (MIC): Minimum Inhibitory Concentration (MIC) of the seeds aqueous extract of *Acacia nilotica* was determined using agar dilution method of NIPRD (2006). 200mg, 100mg, 50mg, 25mg, 12.5mg and 6.25mg of the extract was reconstituted in 5ml of sterile distilled water and vortexed for homogeneity. One mill (1ml) each of the reconstituted extract was added to Petri-dishes containing 19ml of sterile molten agar, these plates were prepared in triplicates, allowed to cool and were labelled with appropriate test organisms. The plates were streaked with a loopful of standardized test organisms (*Salmonella typhi* and *Escherichia coli*). Control plates included organisms' viability control (OVC), Extract sterility control (ESC) and medium sterility control (MSC). The plates were then incubated at 37°C for 24hours.

Screening of the extract for Minimum Bacteriocidal Concentration (MBC): Minimum bacteriocidal Concentration was tested using agar dilution method of NIPRD, (2006). Fresh 19ml of nutrient agar containing no extracts were prepared and allowed to solidify. These plates without incorporated extract were subsequently inoculated with MIC plates of aqueous extract of *A. nilotica* showing no visible growth using sterile swab stick. Control plates were included and the plates were incubated at 37°C for 24hours.

Statistical Analysis: All experiments were carried out in triplicate. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan Multiple Range Test (SPSS 20.0 version). Differences were considered significant at $P \leq 0.05$.

Results

Antimicrobial activity of aqueous extract of *Acacia nilotica*: The *in-vitro* bioactivity of *Acacia nilotica* aqueous extract against *Escherichia coli* and *Salmonella typhi* are shown in Table 1. The crude seeds extract exhibited bacteriocidal effects against *E. coli* and *S. typhi* at 100mg/ml. The MIC of *A. nilotica* was 50mg/ml for both organisms

Effects of aqueous extract of *Acacia nilotica* on castor oil induced diarrhoea: The aqueous extract of *A. nilotica* seeds was found to be effective against castor oil induced diarrhoea on experimental rats at various doses 100, 200 and 400mg/kg Table 2. The extract produced a profound decrease in intestinal transit at 100 and 200mg/kg when compared with control and anti-diarrhoeal activity was more evident at 400mg/kg when compared with control. The activity at 400mg/kg of the extract was compared with 2mg/kgbw loperamide (Positive control).

Table 1: Antimicrobial activity of aqueous extract of *A. nilotica*

Concentration of extract mg/ml	Bacterial isolates <i>Escherichia coli</i>	<i>Salmonella typhi</i>
200	+	+
100	+ ^o	+ ^o
50	+ *	+*
25	-	-
12.5	-	-
6.25	-	-

Key: + = Activity - = No activity, * = MIC, ^o = MBC

Table 2: Effect of Aqueous extract of *A. nilotica* on castor oil induced diarrhoea.

Treatment (mg/kgbw)	Volume of Intestinal Content	% inhibition of volume of intestinal content
Control	0.5±0.12	-
100	0.42±0.00*	16
200	0.4±0.10*	20
400	0.28±0.12**	44
2	0.26±0.12	48

Values are expressed as means ± S.E.M for n=5

*Significantly $P < 0.05$ lower than control, ** $P < 0.05$ significant when compared with control.

Discussion

The extraction of bioactive components from medicinal plants facilitates pharmacological study leading to synthesis of more active drugs with reduced toxicity (Gandhamathi et al., 2009). Seeds aqueous extract of *A. nilotica* exhibited a significant antimicrobial activity against *E. coli* and *S. typhi*. The inhibition of the growth demonstrated by the extract may be due to the presence of phytochemical constituents which

produce definite physiological actions. Phytochemical studies of *A. nilotica* had revealed the presence of tannins, flavanoids, glycosides and carbohydrates (New, 1984). The sensitivity of *E. coli* and *S. typhi* to stem bark and root methanolic extract of *A. nilotica* had been reported (Okoro et al., 2010). The leaves and the bark methanolic extract of *A. nilotica* were reported to contain potent antimicrobial property against *E. coli* (Kshipra et al., 2012). Diarrhoea can occur when a poorly absorbed,

osmotically active substance is ingested (WHO, 06). Castor oil (1ml) treatment in rats was accompanied with watery and frequent defecation because Castor oil prevents re-absorption of water, thus making up the intestinal content and causing diarrhoea (Mekeon 1999). Oral administration of seeds aqueous extract of *A. nilotica* exhibited a significant dose dependent anti-diarrhoea activity as the result at 400mg/kgbw was comparable to that of the standard drug loperamide (2mg/kg). The extract significantly reduced intestinal transit as observed by marked reduction in volume of intestinal contents (16-44%) of castor oil infected rats. One of the probable mechanisms of mode of action of *A. nilotica* may be its ability to enhance fluid and electrolyte absorption through the gastrointestinal tract or GI motility. Besides these possibilities, the extract under investigation may contain certain components having affinity with micro receptor, which is an opioid receptor located on GI mucosa and relieves diarrhoea when activated by an agonist (Mohammed *et al.*, 2008).

Conclusion

The result of this investigation revealed that the seeds extract of the *A. nilotica* exhibited significant *in-vitro* and *in-vivo* anti-diarrhoea activity. These results therefore support the ethno-botanical uses of *A. nilotica* as anti-diarrhoea herb.

Acknowledgement

The authors are thankful to Dr. Tijani A. Y., Pharmacology and Toxicology Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria for providing basic research facilities.

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