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Full Length Research Paper

In vitro studies of antibacterial properties of aqueous leaf extract of *chrozophora senegalensis* on sheep diarrhea

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Antibacterial studies of aqueous leaf extract of *Chrozophora senegalensis* was conducted on bacterial isolate from sheep diarrhea using 100, 200, 300, 400 and 500 mg/mL of the extract and neomycin at 10µg/mL as control. The isolates obtained were Salmonella, Shigella and Klebsiella spp. There was a dose dependent increase in sensitivity of all isolates to the extract, with the highest concentration being comparable to the neomycin at 10µg/mL. It was therefore concluded that the extract can be used as a remedy against bacterial diarrhea.

Keywords: Antibacterial, Diarrhoea, *Chrozophora senegalensis*, Sheep, pathogenic organisms

INTRODUCTION

Diarrhoea is increased frequency of defication with feacal material containing excessive fluid. The feces may vary in consistency from being soft to liquid (Susan, 2008). It may also be described as deviation from established bowel rhythm characterized by an increase in frequency and fluidity of stool (Living stone, 1987). Diarrhea results from increased fluid secretion into, or decrease resorption from, the colon. It is not a disease in itself but merely a sysmptom, which may indicate the result of an error of diet or a chill (Borden, 2002). Diarrhea can also be defined as an increased frequency, fluidity or volume of feacal excretion; the feaces may contain blood or mucous and may be smelly. However it is not possible to definitively determine the infectious organisms that may be responsible from the color, consistency or odour of the feaces (Susan, 2008).

Diarrhea can be as a result of some pathogenic organisms such as *Eschericia coli, Samonella spp, Eimeria spp, Giadia spp,* some viruses like Rota virus, fungal agents like Candida species and helminthes such as *Ostartagia spp*. *Eschericia coli* which is an important opportunistic microbe associated with sloppy environmental conditions and poor sanitation causes colibacillosis with diarrhea as a major clinical sign. It is common in lambs and kids less than 10 days of age, but is most common at 1 - 4 days of age. It usually presents itself as an outbreak in lambs and kids between 12 and 48 hours of age. (Susan, 2008).Salmonella has thousands of serotypes and all can potentially cause diarrhea in animals (Susan, 2008).

Salmonella can cause diarrhea in lambs and kids of any age. Animals less than one week of age are more likely to die without clinical signs, whereas animals older than one week are more likely to have diarrhea. Salmonella also cause outbreaks of diarrhea in late gestation and is frequently associated with abortion (Susan, 2008). Eimeria is a protozoan parasite that commonly causes diarrhea in lambs and kids. These age groups of animals are more susceptible to the problem to the problem at 1 to 4 months of age (Susan, 2008).

Giardia induced diarrhea is more common in, but not limited to, younger ones only. The diarrhea is usually transient, but infected animals can continue to shed oocysts for many weeks providing a source of infection to other animals and possibly humans.

Lambs and kids are infected with group B rotavirus, whereas most other animals and humans are infected with group A rotavirus. Rotavirus generally causes diarrhea in lambs and kids at 20 to 14 days of age. Young animals become very depressed and dehydrated

(Susan, 2008). Chemical causes of diarrhea are Arsenic, fluorine, copper, sodium chloride, mercury, molybdenum etc. Dietary causes are over feeding and simple indigestion (Susan, 2008). Before treating an animal for diarrhea it is essential to determine the cause. High rectal temperature suggests presence of systemic infection. Many of the common causes of diarrhea are self limiting and the major goals of treatment are to keep the animal physiologically stable while the diarrhea runs its course Pepoto Bismol contains bismuth which coats, soothes and relieves the irritated lining of the G I T. Kaopectate (Kaolin-pectin) can be used to treat non infectious diarrhea. Treatment with antibiotics is usually not useful when animals are infected with viruses or protozoa. However, antibiotics are useful when bacterial gents are the primary infective pathogens or where the risk of secondary bacterial infection is high. Sulfa-antibiotics of amprolium should be used in the case of infection with coccidian (Susan, 2008). Therapy for diarrhea includes fluid and electrolytes replacement, maintenance of acid/base balances and control of discomfort. Anti parasitic drugs or dietary therapy can also play an important role in the treatment of some types of diarrhea. Additional therapy may include intestinal protectants, motility modifiers, antimicrobials, anti inflammatory drugs and antitoxins (Merck, 2005).

Despite improvements in management and prevention practices and treatment strategies, diarrhea is still the most common and costly disease affecting neonatal small showed that diarrhea accounted for 46% of lamb mortality (Susan, 2008). She also reported that diarrhea in lambs and kids is complex, multi-factorial disease involving the animal, environment, nutrition and infectious agent. Resistance to antibiotics and occurrence of toxicity during prolonged treatment with present day drugs has been the reasons for extended search for newer drugs to treat microbial infections (Fostel and Lartey, 2000). In view of the fact that antibiotics are sometimes associated with adverse side effects including hypersensitivity, immunosuppressive and allergic reactions, it is of interest to develop alternative antimicrobial drugs such as medicinal plants for the treatment of infectious diseases (Clerk, 1996). The investigations of the efficacy of plant based drugs have been given great attention because of their fever side effects, cheaper cost and easy availability (Kumara *et al.,* 2001).

However, because of the immense socio-economic demand for adequate pharmaceutical supply in the rural areas, transportation difficulties, the need for expertise for the rational use of drugs, the availability and cost of these products, herbal medicine remains the most viable and cheap way to bridge the gap in medical care (Charles, 1998). According to the WHO, (1991), because of poverty and lack of access to modern (orthodox) medicine about 65 – 85% of the world population which leaves in developing countries depends essentially on plants for primary health care. Currently the major pharmaceutical companies have demonstrated recent interest in investigating higher plants as sources of new lead structures and also for the development of standardized phytotherapeutic agents with proved efficacy, safety and quality (Desmet *et al.,* 1997). The plant chrozophora senegalensis is claimed to have antidiarrhoeic activity in both human and animals and it is widely used in most of the northern states of Nigeria especially among Hausa/Fulani for this and other related purposes. It is chiefly employed as a remedy for syphilis, also as an ingredient in a mixture called “Rigakafi”, some other ingredients of which are root and leaves of *Crotalaria spp, Portulaca oleracea, Fertia canthiothes* taken as a reputed preventive and curative concoction for syphilis (Dalziel, 1995). In some districts, it is used as black dye and as a remedy for intestinal pain, for conjunctivitis as well as cicatrizant (Tignokpa *et al*., 1986, Etkin, 1997). Other phytotherapeutic agents used in the treatment of diarrhea include *Jatropha curcus, Pacderia factida Xylocarpus oluccensis,* etc. The aims and objectives of this research work include: To establish some bacterial causes of diarrhea in sheep, to determine the sensitivity of the bacterial isolates of sheep diarrhea to some graded concentrations of the extract of *Chrozophora senegalensis* and to compare the antibacterial activity of the various concentrations of the extract with that of neomycin.

MATERIALS AND METHOD

Plant collection and identification

The plant material was collected at Shuni in Sokoto state with the help of a traditional medical practitioner. The collected plant material was authenticated to be *Chrozophora senegalensis* by a taxonomist in the Botany unit, Faculty of Science, Usmanu Danfodiyo University, Sokoto as recommended by Kumar *et al*. (2000). A sample with voucher no. 1 has been deposited in the botany unit for reference.

Extraction of plant material

The air-dried plant material was reduced to coarse powder by pounding in a mortal by a wooden pestle. 100 g of the powder was weighed using a Metler Balance and placed in a two liter beaker containing one liter of distilled water. The mixture was shaken vigorously for 6 h at a regular interval of 5 min of shaking and 10 min of rest, then mixture was allowed to stand for 18 h, and then shaken again for 5 min and it was then filtered using whatman® filter paper size no 1 into a conical flask. The filtrate was gradually evaporated at 50°C in an electric drier to dry. As earlier recommended by Edwardo *et al*. (2000). The dried extract was then weighed and the

percentage yield was determined as follows:

$$\%\text{Yield} = \frac{\text{Weight of dried filtrate} \times 100}{\text{Weight of pulverized material used}}$$

Sample collection

10 diarrhoea samples were collected from 10 different sheep at State Veterinary Clinic Sokoto in the sample bottle and transported to the lab and refrigerated immediately until the analyses started.

Media preparation

MacConkey agar

This media was prepared by dissolving 7.275g in 150mL of distilled water in a conical flask, the media was then sterilize in an autoclave at 121°C for 15 min the media was allowed to cool and then dispense in 10 sterile Petri dishes and allowed to solidified, the samples were inoculated using sterile inculcating wire loop and incubated at 37°C for 24 h in the incubator as earlier described by Cheesbrough,(1985).

Salanite Broth Media

This media was prepared by dissolving 19.0 g of formula A and 4 g of formula B in one litre of distilled water, the media was then sterilize using water bath at 100°C for 10 min the sterilized media was then dispense in 10 sterile test tubes and the samples were inoculated and labeled A – J, the test tubes were inoculated at 37°C for 24 h as describe by Cheesbrough, (1985).

Sub culturing

Some specific colonies on the MacConkey agar were selected from 10 plates and subcultured on the same media and then incubated at 37°C for 24 h. Specific colonies were selected and Gram staining and biochemical test were conducted on them as describe by Cheesbrough, (1985).

Biochemical test

Catalase and triple sugar iron tests were conducted result in (Table 2).

Preparation of paper disc

Paper discs measuring 5mm in diameter were cut out from absorbent whattman® filter paper size 1 using paper puncher. The discs were transferred into a clean dry petri dish and sterilized in hot air oven at 160°C for 90 min. The crude extract was concentrated to 5mL volume. Sufficient sterile papers were added to completely absorb the 5 mL volume. The impregnated paper dishes were dried in an oven 70°C for 12 h and kept ready for use.
Freshly prepared sterile nutrient agar was inoculated by placing a sterile wire loop containing the culture organisms in the middle of each Petri dish and cross streaked. Paper discs impregnated with the extract were placed aseptically and pressed firmly on the surface of the agar. All the plates were incubated aerobically at 37°C for 24 h. The plates were examined for evidence of inhibition of growth of organisms which is usually indicated by an area around the disc completely devoid of bacterial growth. The diameters of such zones of inhibition were determined using a transparent plastic ruler.

RESULTS AND DISCUSSION

PERCENTAGE YIELD

Percentage yield of the extract is calculated below.

$$\%\text{Yield} = \frac{\text{Weight of evaporated extract} \times 100}{\text{Weight of pulverized material used}}$$

$$= \frac{14.5 \times 100}{100}$$

= 0.145 x 100

= 14.5%

Gram staining

The results obtained following culturing of the organisms showed that the Gram negative organisms were in higher concentration than Gram positive once as shown in the (Table 1).

Antibiotic sensitivity test

The antibiotic screening express the zone of inhibition of bacterial organisms, the crude extract showed considerable amount of inhibition against salmonella organisms than shigella spp and klebsiella spp. The standard antibiotic disc (neomycin) used in this study inhibition the growth of test bacteria, the zones of inhibition produced by the standard antibiotic disc were found to be greater than those produced by the crude extract. From the results of zones of inhibition presented in (Tables 3 and 4) it can be summarized that the active ingredient responsible for the antibacterial activity is

Tables 1. Gram staining appearance.

Number of samples	Gram staining appearance
1	Gram – ve rods
2	Gram – ve rods
3	Gram + ve cocci
4	Gram – ve rods
5	Gram – ve rods
6	Gram + ve cocci
7	Gram – ve rods
8	Gram – ve rods
9	Gram – ve rods
10	Gram – ve rods

Total of Gram negative organisms = 8
Total of Gram positive organisms = 2

Table 2. Triple sugar iron test

S/No.	But	Slope	Gas	H_2S	Mortility	Remark
1	Y	R	-ve	-ve	-ve	*Shigella spp*
4	Y	R	-ve	-ve	-ve	*Klebsiella spp*
5	Y	R	-ve	-ve	-ve	*Shigella spp*
8	Y	R	-ve	-ve	-ve	*Salmonella spp*
9	Y	R	-ve	-ve	-ve	*Salmonella spp*
10	Y	R	-ve	-ve	-ve	*Salmonella spp*

Table 3. Effect of various concentrations of the extract on isolated organisms

Conc. (mg/mL)	100	200	300	400	500	10ug Neomycin
Salmonella spp	1.2mm	1.3mm	1.7mm	2.5mm	2.9mm	3.4mm
Shigella spp	0.9mm	1.1mm	1.4mm	1.9mm	2.3mm	3.1mm
Klebsiella spp	1.0mm	1.2mm	1.5mm	1.8mm	2.2mm	3.2mm

Table 4. Analysis of variance

100mg	1.33 $\pm$ 0.152
200mg	1.20 $\pm$ 0.10
300mg	1.533 $\pm$ 0.152
400mg	2.066 $\pm$ 0. 378
500mg	2.466 $\pm$ 0.378

present in the crude extract. The effect of various concentrations of the extract on the isolated organisms produced no significant difference statistically ($P>0.05$). The percentage yield (14.5%) obtained following extraction of the leafy part of the plant suggests the presence of large quantity of fiber. The activity of the extract against salmonella organisms suggest that the extract is more effective on salmonella organisms when compared to other organisms used in this study. The ability of the extract to produce inhibitory activities against all the test bacterial organisms provide some scientific basis for some of its uses in traditional medicine against some of the claims mentioned earlier. This is in line with the fact that the plant has been used to treat infectious diseases this is due to the presence of various kind of phytochemicals including phenolic compounds, alkaloids, terpenoids and essential oils (Lewis and Elvin-Lewis, 1997). The plant is claimed to have antidiarrhoeic activity in both human and animals, and it is widely used in most of the northern states of Nigeria especially among Hausa/Fulani for this and other related purposes it is chiefly employed as a remedy for syphilis (Dalziel, 1995). It is also of interest to report here that extract from this plant could serve as remedy for typoid fever. This finding lends credence to the traditional use of the plant as remedy for boils; diarrhea and syphilis the plant could be potent antibacterial preparation (Dalziel, 1995). The control agent (neomycin) used in this research produce more zones of inhibition than the crude extract may be due to the fact that the active substance responsible for antibacterial activity has not been isolated.

Conclusion

Our findings in this study shows that the crude extract

has high potential as source of remedy against bacterial infection, however, more general and species of pathogenic bacteria need to be tested in order to ascertain the spectrum of activity of the antibacterial substances present in this plant.

Recommendation

Since the crude extract had some degree of inhibition on the organisms used in this study, it is suggest that, to increase the spectrum of activity of this preparation as an effective and reliable antibacterial agent, more study is however, need to be done with the aims of identifying the actual chemical nature of the active substances as well as their mode of action on bacterial cells.

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