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Full Length Research Paper

Antidiarrhoeal effects of the root extracts of *Guiera senegalensis* in male mice

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The antimicrobial activities of aqueous and methanol extracts of *Guiera senegalensis* were studied against some enteropathogens by agar-well diffusion method. Both extracts inhibited the growth of *Streptococcus Pneumonia, Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Salmonella* spp *and Pseudomonas euroginosa*. The methanol extract was more effective on *S. Pneumonia, E. coli, S. aureus, E. cloacae, Salmonella* spp, while the aqueous extract was more active on *Salmonella* spp and *P. euroginosa*. The methanol extract showed quicker curative action in experimentally induced diarrhoea in adult male mice when administered once or twice at a dose of 556 mg/kg daily. From this work, *G. senegalensis* root appears to be an effective measure for treating diarrhoea. Where food poisoning or metal poisoning are the causative agents. The phytochemical screening results of the aqueous extract, showed the presence of tannins, saponins, alkaloids, cardiac glycosides, coumarine and anthraquinones. However anthraquinones were not detected in the methanol extract and cardiac glycosides where not detected in the aqueous extract. Ethno medicinal use of this plant in the treatment of diarrhoea is justified by this work and is encouraged.

Keywords: Diarrhoea, root extracts, stool formation, antimicrobial activity, packed cell volume (PCV).

INTRODUCTION

Infectious disease represents a major cause of death accounting for approximately one half of all deaths in tropical countries (Iwu et al., 1999). In particular, gastro-intestinal disease is the most frequent cause of childhood morbidity and adult mortality in many parts of the developing world. The presence of enterobacteria in foodstuffs and water is a common cause of diarrhoea and dysentery among the infant population (Viera et al., 2001). The increase in antibiotic resistance causing these disease and in nosicomial and community acquired infections, has renewed interest for new strategies and incentives towards research on treatment, prevention and development of new drugs against these microbial disease (Iwu et al., 1999; Vieera et al., 2001).

Over the years, the world health organization advocated that countries should interact with traditional Medicine practitioner with a view to identifying and exploding aspects that provides safe and effective remedies for ailments of both microbial and non microbial origins (Akinyemi et al., 2005). Phytomedicine derived from plants have shown great promise in the treatment intractable infectious disease including opportunities AIDS infectious (Iwu et al., 1999). About 80% of the rural population in Nigeria depends on it as a primary health care. This represents a potential pharmaceutical market and is an incentive for research into new drugs.

Guinea senegalensis is a tropical shrub of the family Combratacea (Hutchinson and Dalziel, 1954), which is used in traditional medicine in northern Nigeria. It is used by local herdsmen in the treatment of trypanomiasis (Wueocheke and Nok, 2004). Also the plant continues to be one of the plants used by local livestock farmers, traditional healers and Fulani herdsmen in the treatment of snakebite in northern Nigeria (sallau et al., 2005). The plant is used in folkloric medicine in the treatment of dysentery, diarrhoea, stomach upset and hemorrhoids in local communities in Nigeria.

In the present study, the effectiveness of crude *G.*

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senegalensis roots extract for the treatment of experimentally induced diarrhoea in male mice was investigated. The extracts were also evaluated for antimicrobial activity against agents of gastroenteritis.

MATERIALS AND METHODS

The roots of the plant were collected from Maiduguri local government area of Borno state, Nigeria along Ngamboru Ngalla road. The plant was identified and authenticated by Dr. S. S. Sanusi a plant taxonomist, in the department of biological sciences, University of Maiduguri, Borno state, Nigeria. The roots were air dried at room temperature and pulverized into particle form and stored in polythene bags until they were needed.

Aqueous extracts

An aqueous extract was obtained by boiling 250 g of powdered plant material in 1000 cm^3 of distilled water for 1 h. The suspension obtained was allowed to cool and filtered through Whatman filter papers to obtain clear solution. The solution was evaporated to dryness at 60^0C. The dried powder representing the aqueous extract was stored in a sample bottle in a refrigerator until it was needed for use.

Methanol extract

150 g of pulverized plant material was weighed into a soxhlet extractor and extracted exhaustively with 500 ml of methanol according to the method of Olesziek (1988). The resulting extract was concentrated using a rotary evaporator maintained at 60^0C. The dried methanol extract was stored in a glass sample bottle and kept in a refrigerator until required.

Antidiarrhoael effects of root extract

30 adult albino mice with average body weight of 18.00 ± 0.05 g were purchased from the national research institute, Vom, Jos, Nigeria. The animals were housed in clean metal hutches and acclimatized on growers mash (ECWA FEEDS, Nigeria Ltd) for 1 week prior to commencement of the experiment. Clean drinking water was liberally provided to the animals, while feed ruminant were regularly discarded during this period. The animals were later divided into 6 groups, each having 1 animal. Members of each group were housed singly. Some of the animals were fed with epsum salt solution (20 g/20 ml) in distilled water to induce diarrhoea. The animals were monitored to observe their passing out of watery feaces. A solution of 0.2 gcm^{-3} of the extracts in distilled water was used in the treatment of diarrhoeal animals. The duration for the diarrhoea to stop after orally administering 0.5 ml solutions of 0.2 gcm^{-3} extracts to the animals as a single or bi-dose daily was recorded.

A dose was expressed as 556 mg/kg according to the concentration of the extracts and average body weight of the animals. The packed cell volume (PCV) values of the animals were also determined before, during and after treatment.

Antimicrobial activity of extracts

The bacteria samples: *Streptococus pneumoniea, escherischia coli, Staphylococus aureus, Enterbacter cloacae*, *Salmonella* spp *and Pseudominas aeruginosa* were clinical isolates obtained from the Department of Pathology, University of Maidguri teaching hospital, Maiduguri Borno, state, Nigeria. An *in vitro* test using the agar diffusion method of Bana. All the test bacteria use was incubated by introduced into nutrient agar both. A standardized inoculums (5 - 9 x 10^7 cfu/ml) of nutrients broth was introduced into petri dishes. The petri dishes were swirled to distribute the medium homogenously and allowed to dry at room temperature. After 6 mm diameter wells were bored in the agar with sterile cork borer and filled with 0.5 ml of 100 mg/l of each of the extracts. The petri dishes were allowed to stand for 1 h at room temperature for prediffusion to occur and then incubated at 37% for 24 h. At the end of this period, the petri dishes dishes were examined for growth inhibition zones formed on the media and measured with a transparent ruler in millimeter (mm). Distilled water and methanol were used as negative controls.

Table 1. Phytochemical components of crude roots extract of *G. senegalensis*

Chemical constituents'	Methanol extract	Aqueous extract
Alkaloid	+	+
Anthraquinononones	-	+
Cardiac glycoside	+	-
Coumarine	+	+
Phlabatannins	+	-
Saponins	+	+
Tannins	+	+

Determination of minimum inhibitory concentration (mic)

MIC of the extracts was evaluated by the method described by Greenwood (1989). Extracts concentration of 100 mg/l were serially diluted with distilled water to various concentration of 3.13, 6.25, 12.50, 25.00 and 50.00 mg/l. The extracts and nutrients agar broth was mixed in equal proportions in sterile test tubes. A portion of 0.5 ml of the culture medium was added to each tube respectively and incubated at 37^0C for 24 h. The least concentration of extracts showing no visible growth of bacteria was taken as the MIC.

Phytochemical screening

Test for alkaloids, anthraquinones, tannins, phlabatannins and cardiac glycosides were tested based on the procedures described by trease and Evans (1989). The method of Feigl (1960) was used to test for coumarine. The presence of saponins was ascertained according to the procedure described by Sofowora (1993).

RESULTS AND DISCUSSION

Dried methanol and aqueous crude extracts from the roots of the plants were obtained as 28.40 and 6.22% respectively. The higher yield of the methanol extract suggests a higher efficiency of the soxhlet extraction process. Alkaloids, coumarine, saponins and tannins were present in both extracts as shown in Table 1. Anthraquinones were not detected in the methanol extract while aqueous extract did not contain cardiac glycoside and phlabatannins. The findings are similar to

Table 2. Effects of root extracts on the duration of stool of formation of experimentally induced diarrhoea in male mice.

Treatment group	Dose type at 556 mgkg-1 per day	Period of stool formation (h)
A	Single dose of methanol extract	22 - 48
B	Bi-dose of methanol extract	16 - 32
C	Single dose of aqueous extract	24 - 48
D	Bi-dose aqueous extract	18 - 38
E	Untreated animals	-
F	Control animal fed adilibitum with growers mash only	ND

(-): No determination due to watery feaces and animals died: ND: Not determined (Normal feaces).

Table 3. Packed cell volume (PCV) values in the investigated mice mean PCV value.

Treatment groups	Animals before treatment	Animal during treatment	Animal after treatment
A	50.60 ± 2.30	48.20 ± 2.17[a]	48.40 ± 1.14
B	50.10 ± 2.00	48.00 ± 2.15a	48.40 ± 1.14
C	52.00 ± 1.87	50.40 ± 2.07[a]	48.40 ± 1.14
D	51.00 ± 1.50	50.20 ± 0.05	48.40 ± 1.14
E	51.67 ± 0.58	54.50 ± 0.55	ND
F	50.17 ± 1.83	54.00 ± 0.63[a]	48.40 ± 1.14

previous results obtained in the various fractions of organic solvents used for extracting decoction of *G. senegalensis* (Lamer et al., 2005).

The effect of root extract on the treatment of epsum salt induced diarrhoea in adult male mice is shown in Table 2. The administration of the root extract at a dose of 556 mg/kg daily reduced the duration of the induced diarrhoea in all treatment groups (A - D). The least period of group B and D for the bi-dose treatments using methanol and aqueous extracts were found to be between 16 - 32 h and 18 - 38 h respectively. The methanol extract had the lesser duration of stool formation than the aqueous extract both at the single and bi-dose levels daily. The methanol extract is therefore more effective in reducing the amount of wet feaces due to diarrhoea. This result showed that the methanol extract might contain more of the bioactive compounds with strong antienteobacteria effects.

It is observed from Table 2 that all the extracts decreased the amount of wet feaces and that all animal could be diarrhoea free before 48 h after treatment. Obviously the phytochemical constituents detected in this work should be responsible for these quick antidiarrhoea effects of the extracts. Furthermore, the root extracts might elicit antidiarrhoea effects as a consequence or as a result of alkaloids on transit time in the small intestine that could inhibit intestinal motility (Cowan, 1999) and antimicrobial properties of the other constituent compounds in the extracts.

The mean packed cell volume (PCV) value of the animals when treated with root extracts of G. senegalensis is recorded in Table 3. There was no significant difference in groups A - D ($p < 0.05$) in the PCV values of the animals before and after treatment relative to the control group (F). However, during treatment, there is a statistical significant difference ($p < 0.05$) between the PCV values of the treated animals and the control. The effects of diarrhoea may result to mucosal to the small or large intestine, or may be associated with the exudation of serum protein, blood and mucus and sloughed cells due to infections or inflammatory disorders. These types of pathophysiologic mechanism may account for the differences in PCV values during treatment compared to control. Overall, this result was an indication that the animals maintained their physiological conditions.

All the extracts are water soluble and are therefore physiologically compatible during and after treatment. Probably, this could be considered as a positive preliminary safety result for the use of these extracts in pharmaceutical formulation but further *in vivo* evaluation will be required to prove non toxicity of the extract.

Results are expressed as mean ± S.D; ND: Not determined (Animal died): A: Animal treated with single dose methanol extract; B: animals treated with bi-dose methanol

Table 4. Antimicrobial activities of crude extract tested against some pathogens.

Test bacteria	Methanol extract	Aqueous extract	Distilled water	Methanol
S. pneumonia	30	20	-	-
E. coli	34	20	-	-
S. aureus	28	20	-	-
E. cloacae	35	20	-	-
S. spp	13	19	-	-
P. euroginosa	36	39	-	-

(-): No activity; minimum inhibitory concentration (MIC) of extracts on all the tested bacteria was found to be 3.13 mg/l

Table 5. Some antimicrobial compound in *G. senegalensis* plant.

Bioactive	Reference(s)
Phenolic acids	
Gallic acid	Keindrebeogo, et al. (2006) and Bouchet et al. (2000)
Vanillia acid	Kiendrebeogo et al., 2006
Cinnamic acid	Kiendrebeogo et al., 2006
Tannins	
Polyphenols	Lamien et al. 2005
Phlobatannins	Present work
Flavonoids	
Mycricetin and derivatives	Males et al. 1998
Quercitin and derivatives	Males et al. 1998
Rhamnetin	Males et al. 1998; Bucar et al. 1998
Tiliroside	Males et al. 1998
Steroids	Lamien et al. 2005.

extracts; C: Animals treated with aqueous extracts; D: Animals treated with bi-dose aqueous extract; E: Animals not treated; F: Control group fed adilibitum with growers mash only; [a]PCV values on vertical level are statically significantly different ($p < 0.05$) compared to control.

The bioactive compound in the root extracts of *G. senegalensis* were evidence in the antidiarrhoea effects recorded in the extracts. Table 4 shows the *in vitro* anti-microbial activities of solvents (negative control), metha-nol and aqueous extracts. Both methanol and aqueous extracts showed various antimicrobial activities ranging from 13 - 36 mm and 19 - 39 inhibition zones at un... respectively. No inhibition was observed for the control using solvents only. Hence all the crude extracts possess potentially effective antimicrobial activities towards the 6 bacteria tested for. However, the methanol extract is weakly effective on *salmonella* spp. The absence or lack of anthraquinones in this extract may be responsible for this observation.

Also from Table 4, it is observed that the antibacterial activity of the methanol extract was high compared to the water extract against *S. pneumonia, E. coli, S. aureus and E. Cloacae.* The stronger extraction capacity of methanol may have produced a greater number and concentration of bioactive components that may have contributed to this antibacterial activity. The result justifies the choice of this plant for its traditional use to treat gastrointestinal disease. Perhaps, *G. senegalensis* could be used in the treatment of dysentery and diarrhea caused by *E. coli and S. aureus*. The high antibacterial activity against *P. euroginosa* is equally a support for the local use of the herb as a remedy for stomach disorders. Judging from the inhibition zones of *Salmonella* spp, the aqueous extract could show better anti-typhoid activity than the methanol extract anthraquinones content of these extracts could be responsible for this observation.

Generally, the antimicrobial properties of plant extracts are attributed to secondary metabolites such as alkaloids, tannins, saponins etc. (Okoli and Iroegbu, 2005; Akinyemi et al., 2005). Previous studies on *G. senegalensis* extract in organic solvent system indicated the presence of some phonemics as presented in Table 5. These chemical constituents could provide useful basis for the antidiarrhoea effects of the extracts in the investigated mice.

In the present study, it is interesting to observe growth inhibiting effect on *S. aureus* by both root extracts in Table 4. This could be attributed to the presence of

phenolic substances such as phenolic acids, tannins, flavonoids (Table 5), anthraquinones and coumarine as shown in (Table 1). Phenolic acids are highly hydroxylated phenols. Scientific evidence show that increased hydroxylation on phenols result to increased toxicity to pathogens. For example, gallic acid was found to show relative toxicity of 1.5 against *S. Aureus* while cinnamic acid exhibited haemagglutination activity on influenza virus (Cowan, 1999)). Many authors have also reported the efficacy of some phenolic compounds against other microorganism (Kaur et al., 1997; Loarca-Pina et al., 1998; Fernandez et al., 1998; Fredman et al., 2003).

The likely mechanism thought to be responsible for phenolic toxicity to microorganism includes enzymes inhibition by oxidized compound possibly through reaction with the sulphydryl groups or through more non-specific interaction with proteins in the organism (Cowan, 1999). The mixture of the phenolic compounds in Table 5 which are found in *G. senegalensis* extract might exhibit synergistic effects that could enhance the potency of the extracts against bacteria.

Flavonoids are also hydroxylated phenols that are found to be effective antimicrobial agents (Dixon et al., 1983; Tsuchiya et al., 1996). Their activities are probably due to their ability to complex with intracellular and soluble proteins and to complex with bacteria cell walls (Cowan, 1999). The multiple composition and structural differences of flavonoids and other phenolic compound in *G. senegalensis* may have produced a complex mix of bioactive that may be effective against multiple organism as well as offer therapeutic benefits such as in the treatment of diarrhea. Typically, phenolic acid and rhamnetin are known to show potent ant oxidative properties besides their antibacterial effects (Bucar et al., 1998; Keindregeogo et al., 2006).

Lamien et al. (2005) reported the present. Alkaloids, steroids, saponins and polyphenols in this work various fractions of the organic solvents used in extracting decoction from *G. senegalensis*. This finding is similar and significant to this work. These compounds are known to be potentially effective against *S. aureus* and *Salmonella* spp (Vieira et al., 2001). Polyphenols and other tannins are attributable to the reduction in enzyme activities of bacteria (Sallau et al., 2005). Alkaloids may be particularly useful against HIV infections, intestinal infections associated with AIDS and show antidiarrhoea effects (Cowan, 1999). Saponins and alkaloids have been shown to be most efficient against gram positive and negative gram bacteria (Iwu et al., 1999) and such *streptococcus* spp, *P. euroginosa* and *Salmonella* spp (Deeni and Hussain, 1991) used in this work.

Conclusion

The results of this work show that the methanol and aqueous extract derived from *G. senegalinsis* demonstrated effective antimicrobial activities against pathogenic enterobacetria such as *E. coli, Samonella* spp, *P. aeuginosa, E. cloacae* and antibiotic resistant bacteria such as *S. aureus.* These extracts therefore could be used as alternatives for commercial antibiotics for treatment and prevention of diarrhoea in rural communities where the plant is readily available avoided their toxicities are research within the tolerable limits.

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