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ANTIMICROBIAL POTENTIALS OF LEAVES EXTRACTS OF *Thunbergia erecta* ON BACTERIA ISOLATED FROM INFANTILE DIARRHOEAL STOOL.

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ABSTRACT: The antibacterial effect of the extracts from the leaves of *Thunbergia erecta*, a medicinal plant, on bacteria isolated from infantile diarrhoeal stool was studied. Single stool samples were collected from 158 clinically diagnosed patients with infantile diarrhea aged between 2 weeks and 18 months and distributed as 82 (51.9%) males and 76 (48.1%) females. Out of the 104 micro-organisms isolated, 82 (78.9%) were *Escherichia coli*, 14 (13.5%) *Shigella* sp, 7 (6.7%) *Salmonella* sp, and 1 (0.9%) *Campylobacter sp*. Eighty one (99.0%) of the *E. coli* isolates exhibited high degrees of *in vitro* susceptibility to the plant extract at the various concentrations tested. The other clinical isolates also exhibited significant activity at concentrations of 1.5µg to the extract. This study has therefore provided scientific evidence of the antimicrobial properties of the medicinal plant, and therefore underscores the need to encourage research in this direction as new drugs are required to cope with emerging diseases and the increasing population of micro-organisms resistant to various commonly used drugs.

INTRODUCTION

Diarrhoeal diseases occur more frequently in young children than in older individuals. The immediate source of infection in most transmissible diarrhea is almost certainly faeces and infection occurs by faecal-oral route of transmission (Hattley *et al.*, 1987). There are various aetiologic agents of diarrhea but most of the works on its aetiology in clinical laboratories are limited to older established pathogens. Current reports have however incriminated newer pathogens like *Campylobacter jejuni* (Kunali and Fleming, 1979), *Yersinia enterocolitica* (Agbonlahor *et al.*, 1980), *Aeromonas hydrophilia* (Agbonlahor *et al.*, 1998 and Agbonlahor, 1989) and *Pleisiomonas* (Graevenitis, 1980) but most infections of acute infantile diarrhea in developing countries are caused by enteropathogenic *Escherichia coli* (EPEC) (Nataro and Kaper, 1998).

The use of crude extracts from plants in the treatment of various diseases dates back to antiquity even with the advent of orthodox medicine (Soforowa, 1991). Until recently, the only effective control recommended for diarrhea was the use of antibacterial agents. This was followed by the introduction of Oral Rehydration Therapy (ORT) but the campaigns to use this ORT have foundered because mothers who knew how to make effective ORT mixture did not have confidence to use it when the time comes (UNICEF, 1984). There was then need to explore other remedies for effective treatment of diarrhea. *Thunbergia erecta*, a medicinal plant, which belongs to the family Acanthaceae, has been in use by traditional medical practitioners in the treatment of infantile diarrhea. Further more, the extract from this plant has

been reported to possess a wide spectrum of antimicrobial properties against some bacteria (Umo *et al.,* 2006). This study is an attempt to investigate and therefore justify its use in the treatment of infantile diarrhea by traditional medical practitioners.

MATERIALS AND METHODS

Sources of Samples

Diarrhoeal stool samples for the isolation of microorganisms were collected from children who were within the ages of 2weeks and 18months with a clinical diagnosis of diarrhoea at St. Luke's hospitals, Uyo in Akwa Ibom State, Nigeria. The leaves of the plant were obtained from their natural habitat in Akwa Ibom State. The samples were respectively taken to Medical Microbiology laboratory of the University of Uyo Teaching Hospital and Pharmacognosy laboratory, University of Uyo, for processing and analysis.

Processing/Analysis of Sample:

The stool samples were inoculated onto Blood agar, and MacConkey agar plates respectively. The Blood agar plates were incubated at 37^0C in candle extinction jar for 18hours, while the MacConkey agar plates were incubated at 37^0C for 18hours. Macroscopic, microscopic and biochemical characterization of purified isolates were then carried out using standard identification schemes (Barrow *et al.,* 2003 and Holt *et al., 1994*).

The plant leaves were thoroughly sun dried, and ground to obtain 200g of the fine powder before extraction with 2.5 litres of 96% ethanol using soxhlet apparatus. The resulting extracts were filtered using Whatman No. 1 filter paper and the filtrate was evaporated at 60^0C to yield 4.0% dry weight residue.

Phytochemical Screening

The dried, pulverized leaves were subjected to phytochemical analysis to determine the presence of secondary metabolites such as alkaloids, saponins, anthraquinones, and tannins. The phytochemical screening was carried out using standard procedures (Culei, 1982, Soforowa, 1993 and Trease and Evans, 1989) as briefly presented:

Alkaloids: 70ml of 10% Hydrochloric acid was added to 4g of each sample in appropriately labelled conical flasks and boiled for 10 minutes. Each boiled sample was filtered and allowed to cool. The filtrates were poured into four labelled test tubes. Few drops of Dragendoff's, Mayer's, Wagner's reagents were added to each test tube separately. Alkaloids were recorded as present in the sample if turbidity or a brownish precipitate was observed.

Saponins: 4g of the sample was dissolved in distilled water and heated for 2-5 minutes. The mixtures were filtered, allowed to cool and shaken continuously for 2 minutes to induce the production of froth. They were then left to stand for 15 minutes. The observation of frothing was indicative of presence of saponin.

Tannins: 1g of the sample was heated with 20ml of water for 5 minutes in appropriately labelled test tubes. The solution was allowed to cool and then filtered. 1m of the filtrate was diluted with 5ml distilled water in a test tube; few drops of 0.1% ferric chloride solution were added. A characteristic blue, blue-black, or blue-green colour and precipitate indicate the presence of tannin.

Anthraquinones: 1g of sample was shaken with 10ml of ferric chloride solution mixed with 5ml of hydrochloric acid. The mixture was heated in a water bath for 10-15 minutes, filtered and allowed to cool. The filtrate was extracted with chloroform and shaken gently. The clear layers at the base were pipette into test tubes and 2ml of ammonia solution was added. An observation of a delicate pink rose colour indicated the presence of anthraquinones.

Preliminary Sensitivity Testing
The preliminary sensitivity testing that helped in determining the comparative potency of the extract with a standard antibacterial agent against standard microorganism was earlier carried out using agar diffusion method (Umo *et al*., 2006).

Minimum Inhibitory Concentration
The minimum inhibitory concentration of the extract to clinical bacterial isolates and standard organisms were also previously determined (Umo *et al*., 2006).

Susceptibility Testing
The agar well diffusion method as earlier described by Cruickshank *et al.,* (1975), was used to test the plant extracts for antimicrobial activity. Briefly, 15ml of melted and cooled nutrient agar (Difco Laboratories, USA) was added to 0.2ml of 1 in 100 dilutions of the bacterial culture in sterile Petri dishes, and the contents properly mixed. After the agar in each plate had solidified, 6 wells of 5mm each were bored in each plate using a sterile cork borer. 0.1ml of the plant extract at varying concentrations of 1.5µg/ml, 3.0µg/ml, 6.0µg/ml, 12.5µg/ml, 25.0µg/ml, and 50.0µg/ml as well as standard antibiotic solutions prepared from Gentamycin(10µg), Ampicillin(20µg), Chloramphenicol(30µg), Ampiclox(20µg), Augmentin(30µg) and Septrin(30µg) were seeded into wells. Control experiments were set up using erythromycin (2µg/ml).

The plates were incubated at 37^0C for 24h. All inoculation procedures were undertaken under aseptic conditions. According to pharmacological and biometric specifications, the antimicrobial studies were done in triplicates. With the aid of a transparent ruler the diameters of zones of inhibition around the wells were measured in millimetres for all the three replicates and the average of the three measurements was calculated as an indication of activity.

RESULTS

A total of 158 clinically diagnosed patients with infantile diarrhoea were studied. They comprised of 82 males (51.9%) and 76 females (48.1%). From the 158 stool samples, 104 microorganisms were isolated, characterized and identified as aetiologic agents of either single or mixed infections. Cultures from 54 samples yielded no growth of incriminating bacteria.
The results showed predominance of *Escherichia coli* (78.9%) over other isolates. This was followed by *Shigella sp.*13.5%, *Salmonella* sp 6.7% and *Campylobacter* sp 0.9%, (Table 1).

Table 1: Organisms Isolated from Infantile Diarrhoea cases

Organism	No. (%)
Escherichia coli	82 (78.9)
Shigella sp	14 (13.5)
Salmonella sp	7 (6.7)
Campylobacter sp	1 (0.9)
Total	104(100)

Preliminary Sensitivity Test
The comparative potency test result had showed that when the extract was challenged with standard *Staph. aureus* NCTC 6571 and *E.coli* NCTC 10418, it was potent.

Minimum Inhibitory Concentration
The minimum inhibitory concentration (MIC) of the extract against standard *Staph. aureus* NCTC 6571 and *E.coli* NCTC 10418 were 1.8µg/ml and 1.6µg/ml respectively.

Susceptibility Pattern
Ninety nine per cent of the *E. coli* exhibited high susceptibility to the plant extracts, while multiple resistance were common among other bacterial isolates. However, all the organisms

exhibited various degrees of susceptibility to other antimicrobial agents tested. The observed high activity at a low concentration of 1.5µg was generally comparable with the commercially obtained antibiotics (Table 2).

Table 2: Susceptibility Pattern of Isolates against Different Extract Concentrations of *Thunbergia erecta*

Extract conc. (µg/ml) Organisms	Susceptibility *pattern(%)*	1.5	3.0	6.0	12.5	25	50
		(Average zone in mm)					
Escherichia coli (n = 82)	81 (99.0%)	10	18	22	26	28	30
Shigella sp (n = 14)	4 (26.6%)	8.0	12	16	22	24	26
Salmonella sp (n = 7)	2 (28.6%)	9.6	14	18	24	26	28
Campylobacter sp (n = 1)	1(100.0%)	9.4	16.4	18.5	24.7	26	29

DISCUSSION

The result obtained from this study has identified *E.coli* as a leading causative agent of infantile diarrhoea, having been isolated from 78.9% of the total samples. Other organisms like *Salmonella, Shigella* and *Campylobacter sp* had low frequency of occurrence. This result is in agreement with Nataro and Kaper (1998) and Itah (1999) who reported *E.coli* as a leading cause of infantile diarrhoea. Extracts of *Thunbergia erecta* has been known to contain pharmacologically active principles as earlier reported by Umo *et al.,* (2006). The results of this work have revealed an impressive 99.0% pattern in the *in vitro* susceptibilty by *E.coli* to the extracts. Results of the antimicrobial susceptibility testing show that the *E. coli* isolates encountered in this study were resistant to most of the antibiotics commonly used in therapy. This also agrees with earlier report by Itah (1999) where the *E. coli* isolated from infantile diarrhoeal stools in Calabar were 100% resistant to common drugs like erythromycin and cloxacillin. The high degree of resistance to co-trimoxazole and chloramphenicol in this study is a cause for concern. It is hereby speculated that if there is no control over antibiotic administration, there would be wide-spread R-plasmid mediated resistance to most commonly used antimicrobial drugs in Nigeria.

However, the impressive level of antimicrobial property of the plant extract underscores the need to encourage researchers in this direction as new drugs are required to cope with the emerging diseases and increasing population of microorganisms resistant to various commonly used drugs. The minimum inhibitory concentration (MIC) of the plant extract yielded promising results that are worthy of note with a low MIC of 1.6 – 1.8µg/ml. This suggests that they can be gainfully employed in the production of antibiotics, as low MICs mean that only a small quantity of the extract will be required to impair microbial growth as earlier reported (Umo *et al*., 2006).

The presence of antimicrobial constituents in the plant extract also provides baseline information that most of the important raw materials could be sourced locally from our indigenous medicinal plants if efforts are geared towards it. This will help to make our pharmaceutical companies self-reliant.

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