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ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *GONGRONEMA LATIFOLIA* STEM ON CLINICAL ISOLATE OF *ESCHERICHIA COLI* FROM DIARRHOEA PATIENTS

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ABSTRACT

This study was carried out to investigate the antimicrobial activities of *Gongronema latifolia* stem extract on clinical isolates of *Escherichia coli* from diarrhoea patients. Twenty five isolates of *E. coli* were obtained from stool samples of diarrhoea patients within the ages of 1-5 in Nsukka Health Centre, Nsukka. The antimicrobial activity of the methanolic extract was then carried out using agar cup diffusion technique. The result of the study showed that the *E. coli* was moderately sensitive to methanolic extract of *G. latifolia* stem. This shows that in the treatment of infections caused by *E. coli*, methanolic extract of *G. latifolia* stem may be used.

KEY WORDS: Antimicrobial activities, *Gongronema latifolia*, Extracts and *Escherichia coli*.

INTRODUCTION

Gongronema latifolia is a forest leafy vegetable that grows in the forests of the South-Eastern Nigeria (Akpan, 2004). The plant is highly valued for its nutritional and medicinal qualities (Agbo and Obi, 2006). Almost all the parts of *Gongronema* plant are extensively used in traditional medicinal preparation.

At present, medicinal plants are increasingly being projected as suitable alternative source of antimicrobial agents (Esimone *et al*., 2005). *G. latifolia* has been identified as a medicinal plant and can serve man as sources of drugs for the treatment of microbial infections. Thus, the challenge has been to develop effective drugs for treatment of *E. coli* infections since extensive use of antimicrobial drugs has favoured the emergence of resistant strains (Cheesbrough, 1985).

E. coli is an enteric gram-negative rod bacterium that can be found in water, soil and vegetation. It is the common cause of many infections in children and adults. The Enterotoxigenic *E. coli* (ETEC) is a common cause of diarrhoea in children in developing countries (Levine *et al*., 1993; Okeke *et al*., 2000; Oronsaye and Oziegbe, 2002). This study was undertaken to evaluate the antimicrobial activities of methanolic extract of *G. latifolia* stem on clinical isolates of *E. coli* from diarrhoea patients. The antimicrobial activity of this extract was also compared with that of a standard antibiotic. This may possibly give hope to many parents who have continued to loss their children as a result of the infant diarrhoeal episodes.

Materials and Methods

Test Organism: the test microorganism used for this experiment was *E. coli* obtained from Nsukka Health Centre, in Nsukka L.G.A, Enugu State. A total of 25 clinical isolates of *E. coli* were used.

Reagents: The following reagents were used; chloramphenicol, *G. latifolia* stem extract. The culture media used were nutrient agar and nutrient broth (Oxoid).

Sources of Samples

The plant, *G. latifolia* used for this work was obtained from Obukpa, in Nsukka local government area of Enugu State and identified in the Botany Department, University of Nigeria, Nsukka.

Method of Extraction

Sixty three grams of the powdered stem materials were weighed out using mettler sensitive balance and poured into 500 ml flat bottom flask and these were soaked in 200 ml of absolute methanol to get 300.2 mg/ml. This was stirred with magnetic stirrer for 18 hours and left to stand for 24 hours before it was filtered using a clean muslin cloth and concentrated in the oven (Gallenkamp, England) at 60 ^{0}C.

Preliminary Sensitivity Test

The preliminary sensitivity tests of the methanolic extract of *G. latifolia* stem and chloramphenicol, were evaluated by the bore plate and

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agar diffusion method as described by Agboke *et al* (2005).

Determination of the IZD of the Extract on *E. coli*

The procedure used for the determination of the IZD of the methanolic extract of the *G. latifolia* is as follows:

Sterile Petri dishes were aseptically seeded with 0.1ml of freshly prepared suspension of *E. coli* using 20 ml sterile molten nutrient agar at 45 ^{0}C. After solidifying, the agar plates were marked into four sections representing the four dilutions of the extract and lablelled 1-4 with an indelible marker. Using a sterile cork-borer (8 mm), cups were made in each of the four divisions. The two fold dilutions of the *G. latifolia* stem extracts (25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) were aseptically introduced in the cups using standard sterile dropper starting with the highest concentration of the extract to the lowest concentration.

The four plates were incubated at 37 ^{0}C for 24 hours and then, the inhibition zones were measured. This was repeated three times and the average values of the inhibition zones were determined. The graph of the inhibition zone diameter square against the logarithm of the concentrations of the dilutions used was plotted. The MIC of the methanolic extract was then determined from the graph.

Determination of IZD of Chloramphenicol

Five hundred milligrams of chloramphenicol was dissolved in 10 ml of sterile distilled water to make 50 mg/ml solution of drug. Then, three two-fold serial dilutions were made from the 1 in 10 dilution and their concentrations noted.

The IZD was therefore determined as described in the extract above only that the extract was substituted by the chloramphenicol solutions.

Statistical Analysis

Means of triplicates were measured. Data were analyzed by analysis of variance to determine if the effects of the extract on the test organism were statistically significant (Genstat, 2003). Student's t-test was used for comparison between the two treatments A difference was considered to be statistically significant when $P<0.05$.

RESULTS

The results of the preliminary sensitivity tests of *G. latifolia* stem extract and chloramphenciol are presented below. The Preliminary test showed that *E. coli* was moderately sensitive to *G. latifolia* stem extract and highly sensitive to chloramphenicol.

Sensitivity of *E. coli* to the Antimicrobial Agents Used

Antimicrobial Agent	***E. coli***
G. latifolia stem extract	++
Chloramphenicol	+++

++ *E. coli* was moderately sensitive to the *G. latifolia* stem extract.

+++ *E. coli* was highly sensitive to chloramphenicol.

The minimum inhibitory concentrations (MICs) were calculated from the graph of inhibition zone diameter square against the log concentration of the agents (extracts and chloramphenicol).

The minimum inhibitory concentrations (MICs) of the two antimicrobial agents used were shown below. The result showed that the MIC of chloramphenicol is 0.26 mg/ml while that of the extract is 3.09mg/ml.

Minimum inhibitory concentrations (MICs) of *G. latifolia* stem extract and chloramphenicol on *E. coli*.

Agents	**Organism**	**MIC (mg/ml)**
G. latifolia	*E. coli*	3.09
Chloramphenicol	*E. coli*	0.26

The MIC of the *G. latifolia* stem extract is higher than the MIC of chloramphenicol.

Table 1: Effects of different concentrations of *G. latifolia* stem extracts on inhibition zone diameters of *E. coli*

Con (mg/ml	**Zones of inhibition (mm)**	**Log of Conc (mg/mgl)**	**IZD^2 (mm^2)**
200.00	25.00	2.30	1625
100.00	21.00	2.00	441
50.00	15.00	1.70	225
25.00	8.00	0.70	64

Values are means of three replicates from three trials after 24 hours of incubation.

Table 2: Effects of different concentrations of chloramphenicol on inhibition zone diameters (IZD) of *E. coli*

Con (mg/ml)	**Zones of inhibition (mm)**	**Log of Conc. (mg/ml)**	**IZD^2 (mm^2)**
5.00	23.00	0.699	529.00
2.50	22.00	0.378	484.00
1.25	20.00	0.097	400.00
0.625	14.00	-0.204	196.00

Values are means of three replicates from three trials after 24 hours of incubation.

Table 3: Analysis of Variance

***** Analysis of variance *****
Variate: IZD

Source of variation	d.f.	s.s.	m.s	v.r.	F pr.
TRT	1	40.04	40.04	1.34	0.259
Residual	22	655.92	29.81		
Total	23	695.96			

***** Tables of means*****
Variate: IZD
Grand mean 18.5

TRT	CRUDE	STD
	17.2	19.8

*** Least significant differences of means (5% level)***

Table	TRT
Rep.	12
d.f.	22
l.s.d.	4.62

Table 4: Student's t-test table for comparing IZD of *G. latifolia* and chloramphenicol

Group Statistics

chromamph	N	Mean	Std. Deviation	Std. Error Mean
glat 1.00	12	17.2500	6.75715	1.95062
2.00	12	19.8333	3.73761	1.07895

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
glat Equal variances assumed	6.746	.016	-1.159	22	.259	-2.58333	2.22914	-7.20629	2.03962
Equal variances not assumed			-1.159	17.155	.262	-2.58333	2.22914	-7.28318	2.11651

The result of the analysis of variance of the inhibition zone diameter (IZD) of the extract (crude) and chloramphenicol (std) showed that differences in the IZDs of *G. latifolia* stem extract were statistically not significant.

DISCUSSION AND CONCLUSION

In many parts of the world, the use of plant products in treating various infections and disorders have been well documented (Ivoke, 2005). At present, natural products either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs because of the matchedless availabilities of their chemical diversity (Abad *et al*., 2007).

G. latifolia plant could represent a lead source of these natural products since it has provided many developing countries with invaluable products for medicare. The plant is used in curing some diseases such as *malaria*, *Diabetes mellitus* including those caused by bacteria and helminthes (Agbo *et al*., 2005).

The antibacterial compounds such as tetracyclines and aminoglycosides provided the most effective treatment for *E. coli* infection despite their

potential toxicity on humans. Besides, the emergence of chloramphenicol whose antibacterial activities are rather bacteriostatic than bacteriocidal gave hope for better treatment option in managing bacterial infections at large and *E. coli* infections in particular. However, the overuse and misuse of these antimicrobials have led to the death of sensitive strains leaving resistant strains to survive, multiply, and infect new hosts (Cheesbrough, 1985).

The challenge has been to discover effective strategies for the treatment of *E. coli* infections; considering the increase in the incidence of life threatening *E. coli* caused diarrhoea cases among children in our society today. There is therefore, a great demand for novel antibiotics belonging to a natural product with fewer side effects. One approach might be the testing of the antimicrobial activities of this bacterial isolate to *G. latifolia* stem extract done in this work.

In this study, the *G. latifolia* stem extract was found to have antimicrobial property but not as that of the chloramphenicol which is a standard agent. However, the differences between the zones of inhibition of the extract and that of standard agent (chloramphenicol) were statistically not significant (P> 0.05) in all the concentrations used. This can be seen from the result of student's t-test analysis. From the results obtained, the value of MIC of *G. latifolia* stem extract above shows that *G. latifolia* can give reliable therapeutic effect in the treatment of diarrhea caused by *E. coli* in children. However, it is recommended that more studies should be done on *G. latifolia* stem extract for suitable human application in clinical settings.

REFERENCES

Abad, M.J., Ansuategui, M. and Bermejo, P., 2007. Active antifungal substances from natural sources. ARKIVOC (vii) 116-145.

A4bo, C.U. and I.U. Obi., 2006. Macropagation techniques for different physiological ages of *Gongronema latifolia* Benth Cuttings. *Africa Journal of Biotechnology* 5 (13). 1254-1258.

Agbo, C.U., Baiyeri, K.P. and Obi, I.U., 2005. Indigenous knowledge and utilization of *Gongronema latifolia* Benth: A case study of women in University of Nigeria, Nsukka. *Journal of Bio-Research* 3 (2): 66-69.

Agboke, A. A., Eze, E.I. and Adikwu, M.U., 2005. Combined activities of colloidal silver concentrate and cephalexin on *Staphylococcus aureus* using the agar diffusion technique: *Journal of. Bio-Research* 3(2): 7-10.

Akpan, P.A., 2004. Food from the Nigeria forest. African farming September/October. P. 23.

Cheesbrough, M., 1985. Medical Laboratory Manual Butterworth and Co. Publishers Ltd. London. Pp. 196-249.

Esimone, C.O., Grunwald, T., Wildner, O., Nchinda, G., Tippler, B., Porkschk, P. and Uberl, K., 2005. *In vitro* Pharmacodynamic evaluation of antiviral medicinal plant using a vector based assay techniques. *J. Appl. Microbiol*., 99(6): 1346-55.

Genstat Release 4.23DE Copyright 2003. Lawes Agricultural Trust (Rothamsted Experimental Station).

Ivoke, N., 2005. Preliminary studies on the efficacy of *Aloe vera* (*Aloe barbadensis*) extracts in experimental *Trypanosome brucei* infection of Mice. Journal of Bio-Research 3 (1): 21-25.

Levine, M.M., Ferreccio, C., Prado, V., Cayzzo, M., Abrego, P., Martinez, J., Maggi, L., Baldini, M.M., Martin, W. and Maneval, D., 1993. Epidemiologic studies of *Escherichia coli* diarrhoeal infections in a low socioeconomic level peri-urban community in santiago chile. *AMJ, Epidemiol* 138:849-69.

Okeke, I. N., Lamikarira, A., Steinruck, H and Kapar, J. B., 2000. Characterization of *Escherichia coli* strains from cases of Childhood diarrhea in provincial Southwestern Nigeria. *J. Clin. Microbiol*. 38:7-12.

Oronsaye, F. E. and Oziegbe, E. I., 2002. Prevalence of enteropathogenic *E. coli* in purely breastfed babies in Benin City. Unpublished paper presented at International Biotechnology (FADIB) genetic engineering workshop, Enugu, Nigeria.