PREVALENCE OF DIARRHOEA AGENTS AND THE IN-VITRO EFFECTS OF THREE PLANT EXTRACTS ON THE GROWTH OF THE ISOLATES

[+]Agu, G.C., [++]Thomas, B. T, Agu, N.C., [+++]Agbolade, O.M.

[+] Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye. [++]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University College of Helath Sciences, Sagamu. [+++]Department of Plant Science and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Ogun *State*, Nigeria.
Correspondence (e-mail): georgiaagu@yahoo.com

ABSTRACT

Isolation of bacteria associated with diarrhoea in young children below the age of five years and the antibacterial efficacy of *Dioscorea dumetorum, Oscimum suave, and Ancistracarpus densispinosus* were investigated. Leaves of these plants were dried and powdered before being soaked in 70% ethanol for 3 days. The stools were cultured and isolates were identified according to standard bacteriological methods . Agar diffusion method was employed in determining the inhibitory effects of the extracts on growth of the bacterial isolates. Out of 100 samples collected, 97% showed growth of different bacterial species. These include *Escherichia coli* (28%), *Salmonella* (21%), *Klebsiella* (16%), *Shigella* (15%), *Proteus mirabilis* (1%), and *Staphylococcus aureus* (7%). *Klebsiella, Proteus and Escherichia coli* were susceptible to the inhibitory effect of the three extracts at varying concentrations except for *Proteus mirabilis* which did not respond at all to *Ancistracarpus densispinosus.*

Statistically, *Dioscorea dumetorum* proved to be more effective than *Oscimum suave (p<o.o5).* . Hence, *Dioscorea dumetorum and Ocimum suave* could serves as an alternative to orthodox drugs in the treatment of diseases due to *Klebsiella, Escherichia coli, and Proteus mirabilis.*

Keywords: Isolation, Bacteria, diarrhea, Plant extracts, antibacterial

{**Citation:** Agu, G.C., Thomas, B. T, Agu, N.C., Agbolade, O.M. Prevalence of diarrhoea agents and the in-vitro effects of three plant extracts on the growth of the isolates. American Journal of Research Communication, 2013, 1(12): 429-444} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Infectious gastroenteritis is one of the most common diseases in humans, with particularly high morbidity (Bente *et al.*,2005) and mortality (Ani *et al.*, 1985) in children younger than 5 years of age. This infection does not only threaten the health of people, but also bring great loss to the society and economy (Zhengying *et al.*,2000). World Health Organization listed diarrhea as one of the global most important sanitation and poor quality of life problems and according to them, Diarrhea is fourth only to tumor, heart and brain vessel diseases and diabetes (WHO, 2005).This is because it results in about 13% of childhood death (WHO, 2005). In industrialized countries, such as Denmark, the associated mortality is low, but the social burden and economic costs due to care of ill children and parent's absence from work are substantial because of high incidence of the infection (Bente *et al.*, 2005; Zhengying *et al.*,2005).

The specific organisms that cause diarrhea often depend on the geographic area, its level of sanitation, economic development and also its hygienic status (Dennehy, 2005). According to him, countries like Nigeria with poor sanitation or where human wastes are used as fertilizer tend to have outbreak of diarrhea when intestinal bacteria contaminate crops or drinking water. In developed countries, including the United *State*s, outbreaks of diarrhea are more often linked to contaminated water supplies, improperly processed or preserved foods, or person to person contact especially in child care centers (Chetley, 1998). He further stressed that bacterial infections are world- wide problem and resistant bacteria have been reported to be responsible for treatment failures.

The growing resistance of multi drug resistant organisms demands new therapeutic strategies. This thus need to source for antimicrobials from the avalanche of our medicinal plants. Weibo and Boping, (1991) reported that medical plants are the only way to cure diarrhea in many countries where health services are disorganized and synthetic medicines are unavailable.

Bacterial resistance to antibacterial agent and high morbidity and mortality rate of diarrhea among children constitute the economic burden of the developing low-income nations. This has led to this present study which was aimed at investigating the the anti-diarrheal activities of three medicinal plants on the bacterial isolates of acute diarrhoea diseases isolated from three different hospitals.

MATERIALS AND METHODS

Sample Collection

A total number of one hundred stool samples were collected from children between the ages of 2-7 years with the clinical signs of diarrhea between February and March, 2008. The stool samples were collected from three hospitals: University College Hospital (UCH Ibadan, Oyo *State*, Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu and General Hospital Ijebu-Ode, Ogun State. The samples contained in sterile universal bottles were taken to the Medical and Parasitology laboratory of Olabisi Onabanjo University and were processed within 6hrs of its collection.

Bacteriological Analysis

For isolation, the samples were inoculated separately onto *MacConkey* agar, Deoxycholate Citrate Agar, and Salmonella-Shigella Agar (Oxoid, Germany) plates. The plates were incubated at 37^0C for 24 hours after which the colonies were Gram stained and further confirmed using standard microbiological methods (Cheesborough,2005).

Collection and Extraction of Plants

The plants used in this study were *Dioscorea dumetorum, Oscimum suave, and Ancistracarpus densispinosus* locally known as Esuru-oko, Efinrin, and Iwaja respectively in Yoruba language. They were obtained from Oru-Ijebu in Ogun *State*, Nigeria. The plants were cut from the stem and packed in a polythene bag each and were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State.

Extraction

The method (Mother Tincture) devised by Jean-Michael (1994) was employed for the extraction. The leaves of the three plants were separately sun dried for 2 days and were finally dried in an oven at 45^0C for 3 days. The dried leaves were blended using an electric blender (Shaisho,Japan) and the powders were stored in a sterile bottle at room temperature (Akinsulire *et al.,* 2007) separately. 50 g of the powdered form of the plants was weighed and dissolved in 450ml solvent (60% ethanol and 40% distilled water) as described by Akinyemi *et al.*(2005) with slight modification. The mixture was kept in an air tight container and stored in cool dark place for 3 days after which they were individually sieved using a sterile No 1 Whatman filter paper. The liquids retrieved were concentrated by evaporation using water bath at 97^oC and were stored in sterile bottles (Atata *et al.*, 2003) until needed.

Test organisms

Three organisms *Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis* recovered from diarrheic children were maintained on nutrient agar slopes at 4^0C .

Antibacterial Activity

This was carried out using Agar well diffusion method as described by NCCLS (2002). Briefly, the extracts were double fold serially diluted using sterile distilled water to obtain the following concentrations; 100 mg/ml, 50 mg/ml, 25.0 mg/ml, and 6.25 mg/ml, 3.13 mg/ml, 1.56 mg/ml, and 0.78 mg/ml. One milliliter (1 ml) solution of each extract was added to the appropriate hole as labeled. Each of the two holes on each plate contained the extract and water (negative control) respectively. Also, an antibiotic disk Cephalexin (20µg) was placed at one end of each plate, equidistance to the holes. The plates were incubated at 37^0C for 4 hours after which the zones of inhibitions were measured using a transparent meter rule. Then the varied zones of inhibition were analyzed using ANOVA test in order to obtain the level of significance of the extracts.

RESULTS

Out of 100 stool samples analyzed, 97 (97%) had growth of bacteria while the remaining 3(3%) showed no growth. In all a total of 97 bacterial isolates with six different organisms were identified (Table 3). *Escherichia coli* (28.9%) was the most prevalent gram negative bacillus, followed by *Salmonella sp.* (21.6%), *Klebsiella* (16.4%), *Shigella* (15.5%), and lastly *Proteus mirabilis* (10.3%) with *Staphylococcus aureus* (7.2%) being the least. Table 4 shows the age distribution of diarrhea infection. It was observed that the children within the age range of 0-24 months were the most prevalent 32 (32.99), followed by 25- 36 months old 26(26.80%), and children within the months of 73-84 were the least infected 2 (2.06%).

The morphology of the colonies and the biochemical characteristics of the isolates were shown in Tables 1 and 2. The sensitivity pattern of the isolates to the extracts varied according to the

concentration. The *in-vitro* activities of the extracts against all the isolates tested are shown in tables 5-7. The three organisms tested were sensitive especially at higher concentrations of 100 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.13 mg/ml with zones of inhibition of 61 mm, 63 mm, 34 mm for *Klebsiella*, 62 mm, 41 mm, 40 mm for *Escherichia coli* and 65 mm, 42 mm, 33 mm, and 22 mm for *Proteus mirabilis* as shown in Table 5. The sensitivity of these three organisms to *Dioscorea dumetorum* was significant ($P<0.05$) (Table5-7). The sensitivity of these organisms to *Oscimum suave* and *Ancistracarpus densispinosus* were shown in Tables 6 and 7. Comparatively, *Dioscorea dumetorum* seemed to be more effective against the three organisms than *Oscimum suave and Ancistracarpus densispinosus*. Similarly, *Klebsiella* seemed to be more sensitive to the inhibitory effect of *Dioscorea dumetorum and Oscimum suave* followed by *Proteus mirabilis* than *Ancistracarpus densispinosus* in which *Escherichia coli* responded most (Tables 5-7).

Table 1:Characteristics of the isolates on different Media

Colonies	Morphological Appearance	Media	Probable Organisms
A	Non-lactose fermenting pale colour	MA	*Salmonella, Shigella*
B	Non-lactose fermenting pale colour	MA	*Salmonella, Shigella*
C	Lactose fermenting pale colour	MA	*Klebsiella*
D	Swarming, Non-lactose fermenting	MA, CLED	*Proteus mirabilis*
E	Lactose fermenting, smooth pink colonies	MA	*Escherichia coli*
F	Lactose fermenting, smaller colonies	MA	*Staphylococcus aureus*

Table 2: Biochemical characterization of bacteria obtained from the excreta

Colonies Isolates	Gram Staining	Cell shape	Indole test	KIA test	Citrate test	Methyl red test	Catalase test	
A	-	rod	--	+	+	--	-	*Salmonella*
B	-	rod	--	+	-	--	--	*Shigella*
C	-	rod	-	--	+	+	--	*Klebsiella*
D	-	rod	-	--	--	--	--	*Proteus*
E	-	rod	+	+	-	+	--	*E. Coli*
F	-	cocci	--	--	--	--	+	*Staph. spp*

Key

+ Positive

- Negative

-- No reaction was observed

Table 3: Number and percentage of bacteria isolated

Isolated bacteria (%)	Frequency	Percentage
Salmonella species	21	21.7
Shigella species	15	15.5
Klebsiella pneumoniae	16	16.5
Proteus mirabilis	10	10.3
Escherichia coli	28	28.9
Staphylococcus aureus	7	7.2
Total	97	97

Table 4 : Occurrence of diarrhea within age groups

Age range (Months)	Number examined	Number positive	Percentage (%)
0-24	32	32	32.99
25-36	27	26	26.80
37-48	20	20	20.62
49-60	14	12	12.37
61-72	05	05	5.15
73-84	02	02	2.06
Total	100	97	97

Table 5: The inhibitory effect of *Dioscorea dumetorum* extract on the growth of *Klebsiella*, *Escherichia coli* and *Proteus*

Concentration	Zone of inhibition (mm)		
Concentration of *Dioscorea Dumetorum*(mg/ml)	*Klebsiella*	*E.coli*	*Proteus*
100	61	62	64
12.5	63	41	42
6.25	51	40	33
3.13	34	32	22
1.56	00	00	10
0.78	00	00	00

Table 6: The inhibitory effect of *Oscimum suave extract* on *Klebsiella, Escherichia coli* and *Proteus*

Concentration	Zone of inhibition (mm)		
Concentration of *Oscimum suave* (mg/ml)	*Klebsiella*	*E.coli*	*Proteus*
100	64	41	52
12.5	62	22	33
6.25	54	00	21
3.13	34	00	20
1.56	20	00	00
0.78	00	00	00

Table 7: The inhibitory effect of *Ancistracarpus densispinosus* extract on *Klebsiella, Escherichia coli* and *Proteus*

Concentration	Zone of inhibition (mm)		
Concentration of *Oscimum suave* (mg/ml)	*Klebsiella*	*E.coli*	*Proteus*
100	44	51	00
12.5	00	33	00
6.25	00	20	00
3.13	00	00	00
1.56	00	00	00
0.78	00	00	00

DISCUSSION

Diarrhea infections have been described by several authors as the most common cause of morbidity and mortality in children world wide (WHO, 2005; Bente *et al*.,2005).

The result of this study indicated that the Gram negative bacilli were more common in diarrhea than Gram positive bacteria. The high number of enteric pathogens (97.6%) isolated could mean that these bacteria play an important role in children diarrhea. The incidence was highest in age group below 24 months. This supports the work done by Parashur *et al. (*2003) and Patricia *et al.* (2001) and could mean that the incidence of diarrhea among children decline with increase in age (Wang, 2000).This reflects the combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria and direct contact with human or animal faeces when the infants start to crawl (WHO, 2005), or when the child attends day care centres (Shane *et al.*, 2003). Bennish *et al.* (2002) and Madkour *et al.*, (1995), attributed high occurrence of diarrhoea to poor sanitation especially on the part of the parents.

The incidence of *Escherichia coli* as the most predominant isolate was in accordance with the work done by Patricia *et al.*, (2001). *Escherichia coli* being the most common organism associated with diarrhea in this environment agrees with the work of Onabakin (2004), who observed nine isolates of *Escherichia coli* out of twenty enteric pathogens isolated from diarrheagenic stools. However, this disagrees with that of Ogbe (2004), and Morten *et al.*, (2003), who reported Salmonella sp. (20%) and (55.2%) respectively as the mot prevalent in their works. The occurrence of *E.coli* as the highest enteric pathogen in this study confirms the *State*ment of Ojo (1993), who reported that persistent diarrhea was an important problem for children during the first two years of life and that *Escherichia coli* was the major causative agent. It is a common knowledge that *Escherichia coli* is one of the normal flora of the human

intestine. According to Mead and Griffiths (1998), disease by *Escherichia coli* has been 0n the increase during the past two decades. The Centers for Disease Control and Prevention estimates that *E.coli* 0157:H7 causes an average of 500 outbreaks that affect more than 73, 000 persons and results in more than 61 deaths each year in the United *State*s (Mead *et al.*, 1999). The epidemiology of organisms associated with diarrhea has become an important research topic as manure harbouring enteric organism is dispersed, and soil, food and water are cross-contaminated with feces containing enteric organisms. In Denmark, surveillance data indicated that *Thermo tolerant Campylobacter* is most common bacteria gastrointestinal pathogens for all ages (Bente *et al.*, 2005).

The ethanolic extracts of these plants (*Dioscorea dumetorum*, *Oscimum suave*, and *Ancistracarpus densispinosus*) showed varying degrees of inhibitory effects on the growth of three organisms especially at higher concentrations. The effects of these extracts on the growth of the isolates could be said to be organism specific with *Klebsiella* and *Proteus* being more sensitive to the inhibitory effect of *A. densispinosus* than *Klebsiella* and *Proteus* (Tables 5-7). However, there was a significant diffrerence in the effectiveness of *D. dumetorum* to the three organisms than *O. suave* ($P>0.05$). The antibacterial activities of the ethanolic extracts of these plants could be attributed to the presence of their active ingredients.

Several species and varieties of plants of the genus *Oscimum* have been reported to yield oil of diverse nature known as basilic oils. Some chemical compounds and active ingredients like Eugenol, Linaol, Methyl cinnamate, Camphor and Thymol have been observed and it has been demonstrated that Eugenol isolated from *Oscimum gratissimum* had antimicrobial activity (Janine de Aquino *et al.*, 2005). Ntezurubanza *et al.* (1984); Nakamura *et al.* (1999); Iwalokun *et al.* (2003); Janine de Aquino *et al.* (2005), reported that the ethanolic extracts of the fresh leaf of the *Oscimum gratissimum* inhibited the growth of *Listeria monocytogenes* at varying

concentrations and that the plant contained alkaloids, tannins, glycosides, Saponin, resins, cardiac glycosides, steroidal terpens and flavonoids. These are believed to be responsible for the observed antibacterial effects observed in this work. Nweze *et al.* (2004), attributed the antimicrobial effect of species of *Oscimum* to the presence of these secondary metabolites.

This study shows that *Escherichia coli* is the most prevalent organism associating with diarrhea in the areas studied. The observed antibacterial effects of the extracts, though *in-vitro* appears interesting and promising. The result obtained in this study revealed that Dioscorea dumetorum and Oscimum suave could be used as an alternative treatment to orthodox antibiotics in the treatment of diseases due to *E. coli, Klebsiella* and *Proteus* especially as they frequently develop resistance to known antibiotics.

REFERENCES

Akinsulire, O.R., Aibinu, I.E., Adenipekun, T., Adelowotan, T., and Odugbemi, T. (2007): *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllium pinmatum* and *Kalanchoe crenata, African Journal of Traditional, Complimentary and Alternative medicine*. 4 (3): 338 – 344.

Akinyemi, K.O., Oladapo, O, O., Okwara, C.E., Ibe, C.C., and Fasure, K.A (2005): Screening of crude extracts of six medicinal plants used in south – west Nigerian unorthodox medicine for antimethicillin resistant Staphylococcus aureus activity. *BMC Complementary and Alternative medicine*. 5:6.

Atata, R., Sani, A., and Ajewole, S.M. (2003): Effect of stem back extracts of *Enantia chloranta* on some clinical isolates, *Biokemistri.* 15 (2): 84 – 92.

Ani, A., Takahashi, M., Saida, H., Kozak, W., Kumar, V., Shonekan, R.A., and Agbolahon. (2003): Aetiological Studies of Infantile Diarrhea seen in Jos Teaching Hospital, Jos, Nigeria: *A Preliminary report*. 23: 1-5.

Bennish, M., Griffiths, J., Salam, A., and Bhan, M. (2002): *Shigellosis:* Clinical Update: A supplement to issue no. 44. Dialogue on diarrhea online, the international newsletter on the control of diarrhea diseases. Vol. 2: 1-4.

Bente, O., Jacob,N., Blenda Bottiger. (2005): Etiology of Diarrhea in young children in Denmark: a case study. 43:3636-3641.

Chetley, A. (1998): The Antibiotic crisis. *The Nigerian journal pharmacy*. 29 (4): 183-188.

Dennehy, P.H.(2005):Acute diarrheal disease in children: Epidemiology, Prevention, and Treatment. *Infect. Dis. North Am*. 19(3): 585-602.

Iwalokun, R.A., Gbenie, G.O., Adewole, T.A., Smith, S.I., Akinsinde, K.A., and Omonogbehin, E.O. (2003): Effect of *Oscimum gratissimum L.* essential oil at sub-inhibitory concentration on virulent and multi-drug resistant *Shigellosis strain* from Lagos, Nigeria. *APMIS*. 3(4): 477-482.

Janine de Aquino L., Xisto, S.P., Orionaide de Fatima L.F., Relinoi de Paula, J., Pedro, H.F., Hasimooto de Suza, L.K., Aline de Aquion L., and Maria de Rosario, R.S. (2005) : Antifungal activity from *Oscimum gratissimum* L. Towards *Cryptococcus neoformans. Mem. Inst.Oswaldo Cruz.* 100 (1) : 55-

Madkour, A.A., Mohammed, N.Z., and Omar, E.F. (1995): Nutritional Outcome of appropriate feeding during and after acute diarrhea disease Program of world Health Organization. 1(20): 162-175.

Mbata, T.I., Saika, A. (2008): Antibacterial activity and Phytochemical screening of Crude oil Ethanolic extract of leaves of *Oscimum gratissimum L.* on *Listeria Monocytogenes*. *The Internet Journal of Microbiology*. 4:2.

Mead, P.S. Griffiths, P.M. (1998): *Escherichia coli* O157: H7. Lancet 352: 1207-1212.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C *et. ai.,* (1999): Food-related illness and death in the United *State*s.

Morten, H., Pernile, V., Peter, G., and Kare, M. (2002): Short and long term mortality associated with food borne bacteria gastrointestinal infection: Registry based study. *BMJ.COM*. 326: 357-360.

Nakamura, C.V., Nakamura, T.V., Bando, E., Melo,A.F.N., Cortez, D.A.G. and Dias Filho, B.P. (1999): Antibacterial activity of *Oscimum gratissimum L.* essential oil. *Mem. Inst. Oswaldo cruz*. 94: 675-678.

Ntezurubanze, L.I., Scheffer, J.J.C., Looman, A., and Baerhiem Svends. (1984); Composition of essential oil of *Oscimum kilimandscharicum* grown in Rwanda. Plant Medica.PP. 385-388.

Nweze, E.I., Okafor,J.I. and Njoku, O. (2004); Antimicrobial activities of Methanolic extracts of *Trema guinensis* (Schumm and Thorn) and Morinda lucida Benth used in Nigeria herbal medicinal practice. *J. Biol. Res. Biotech.*, 291: 39-46.

Ogbe, A.A. (2004): The antibacterial activity of *Allium sativum and Aloe vera* on enteric organisms isolated from dysentery and diarrheal patients (children). Unpublished B.Sc thesis, Department of Microbiology, Olabisi Onabanjo University, AgoIwoye, Ogun *State,* Nigeria. Pp. 45-47.

Ojo, M.O. (1993): Diarrhea in manual of pathogenic bacteria. 1st edition, Shaneson Limited, Ibadan. Pp. 350-357.

Olayemi, J.O., and Ajaiyeoba, E.O. (2007): Anit-inflammatory studies of yam (*Dioscorea esculenta)* extract on Wistar rats. *African Journal of Biotechnology*. Vol. 6(16: 1913-1915.

Onabakin, O.O. (2004): Assessment of the antibacterial activities of *Oscimum gratssimum and Vernonia* amygdalina on some enteric pathogens causing dysentery and diarrhea in children. Unpublished B.Sc thesis, Department of Microbiology, Olabisi Onabanjo University, AgoIwoye, Ogun *State*, Nigeria. Pp 31-44.

Parashurr, U.D., Hummelman, E.G. and Bresee, J.S. (2003): Global illness and Death caused by rotavirus disease in children. *Emerg. Inf. Dis*. 9 (5): 547-562.

Patricia, P. O., Tatiane, S. and Gleiciene, F. M., (2001): Enteropathogens Associated with Diarrhea Disease in Infants of poor urban Areas of Porto Velho, Rondonnia; a preliminary study. 96(5): 621625.

Saveedra, J. (2000): Probiotics and Infectious diarrhea. *AM Journal of Gastroenterol.* 95: 516-518.

Shane, A.l., Tucker, N.A., and Crump, J.A. (2003); Sharing of *Shigella*. Risk factors for a multi-community outbreak of Shigellosis. *Arch. Pediatric Adolesc. Med*. 157: 601-603.

Wang, Z. and Guo, Y. (2000): Analysis of Infectious and causes of Acute Respiratory Tract Infections and Diarrhea among Children below 5 years old in some area of Shanxi pronvince. Pp. 252-257.

Weibo, L.U., and Boping, W.U., (1991): Various Clinical Uses of Medical Plants. In: Proceedings of international conferences on Traditional Medicinal Plants. The United Republic of Tanzania. Dar Es Salam University Press Ministry of Health – Tanzania. Pp 391.

WHO, (2005): Reading on Diarrhea, Student Manual, Geneva pp 1-121.

Xia, Y., Gong, Y. and Gu, X.Y. (1998): Diarrhea Burden Survey Target – DALY. *China Hygienic Statistic*, 15 (4): 54-57.

Zhengying, F., He, W. and Chen, C. (2000): The situation and the economical loss caused by respiratory system disease and the diarrhea of child below 6 years old n China. *Hygienic study*. 2 (5): 283-287