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The Assessment of the Antimicrobial Activities of *Ocimum Gratissimum* (Wild Basil) and *Vernonia Amygdalina* (Bitter Leaf) On Some Enteric Pathogen Causing Dysentery or Diarrhea in Patients

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----------ABSTRACT----------
The antimicrobial activities of leaf extract of Ocimum gratissimum and Vernonia amygdalina was investigated. 100 diarrheic stool samples were collected, 20 showed sign of growth. This means that 20 isolates were isolated and identified from 12females & 8males; these were 9 Escherichia coli, 7 Salmonella sp and 4 Shigella sp. The anti diarrheic activities of Ocimum gratissimum (efinrin) and Vernonia amygdalina (ewuro) were tested against Escherichia coli, Salmonella sp and Shigella sp .They produced different sized zones of inhibition against the growth of the organisms. The anti diarrheal effect of the extract were carried out using well diffusion method where the extracts was found to have an inhibitory effect on Salmonella sp with zones of inhibition ranging from 10-30mm, Shigella sp ranged from 17-30mm and 12-23mm for Escherichia coli at concentration ranging from 6.25-100g/ml. Statistically, there was no significant difference in the efficiency of wild basil extracts on the growth of Escherichia coli, Salmonella sp and Shigella sp also, there was a significant difference in the effect of bitter leaf extracts on the growth of Salmonella sp and Shigella sp whereas on the growth of Escherichia coli, there was no significant effect. The antibacterial agents used served as control.

KEYWORDS ; *diarrhoea/dysentery,ocimum gratissimum,vernonia amygdalina,antibiotic disc*

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I. INTRODUCTION

The use of plants and herb extract in the treatment of human ailments is a very ancient art, a practice that has been passed on for generations and Scientists in Africa and other developing countries and other are conducting research into local plants abundant in the continent for their possible use in traditional medicine (Nneamaka, 1991). Research into traditional plants and herbs received further boost due to the increasing resistance to many orthodox medicine and thus a search for new organic molecules of plants with antimicrobial properties (Sofowora, 1993). The research on the use of medicinal plants for therapy (ethnotherapy) is also assuming increasing popularity in both advanced and developing countries in clinical and biological sciences (Ukong, 1989).Cold infusion of the leaves are used for the relief of stomach upset and haemorrhoids(Essien,1982).The use of plant extracts and phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, biodegradability, low toxicity, and minimum residual toxicity in the ecosystem (Ogbo and Oyibo, 2008).). A lot of work has been carried out to prove that several plant species possess antifungal and antibacterial properties (Ficker *et al*., 2003; Erdogrul, 2002; Maji *et al*., 2005). There is new interest in the antifungal properties of plants known to have medicinal value to the people of Nigeria. The extracts of *Ocimum gratissimum* and *Acalypha* spp. have been reported to have antimicrobial properties (Amuchi, 1989; Ejechi and Akpomedaye, 1999; Owolade and Osikanlu, 1999). Secondary metabolites found in *O. gratissimum* include alkaloids, saponins and terpenoids; which are characterized by strong fragrant smell and slight pungency (Gill, 1992; Onajobi, 1986). Simply, a medicinal plant is any plant which in one or more of its organ, contains substances that can be used for therapeutic purposes or which contains substances which can be used as precursors for the synthesis of useful drugs (Sofowora, 1993). In this study, the plant *Ocimum gratissimum* is studied for its medicinal properties by assay for its antimicrobial properties on selected human pathogenic bacteria of the family enterobacteriaceae namely *Escherichia coli, Shigella flexneri* and *Salmonella typhi* .

II. THE GENUS VERNONIA

Vernonia amygdalina it is known as bitter leaf, due to its characteristic bitter taste and flavour, and may be used as an active anticancer (Izevbigie, 2003), antibacterial, anti-malaria and anti-parasitic agent (Tadesse et al., 1993). This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. The stem and root divested of the bark are used as chew-sticks in many West African countries like Cameroon, Ghana and Nigeria (Burkill, 1985; Hamowia, 1994). It is also documented that *V. amygdalina* has been used traditionally in blood clotting and has elicited a significant reduction in blood glucose levels at post-prandial time point (Uchenna et al., 2008). Fasola et al. (2010) reported that *V. amygdalina* has hypoglycemic activity. They observed a dose-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts. Yedjou et al. (2008) also demonstrated *V. amygdalina* leaf extracts as a DNA - damaging, anticancer agent in the management of breast cancer. However, much work has not been documented on the antimicrobial effectiveness of the stem extracts of this plant. This research is designed to screen the anti-microbial effectiveness of *V. amygdalina* leaf and stem extracts on selected urinary tract pathogens, compared to ciprofloxacin antibiotics.

III. THE GENUS OCIMUM

Most members of this family such as *Hyptis*, *Thymus*, *Origanum*, *Salvia* and *Mentha* species are considered economically useful because of their basic natural characteristics as essential oil producers. These essential oils are composed primarily of monoterpenes and sesquiterpenes and have been the subject of extensive studies due to their economic importance (Lawrence, 1993).It is widely distributed in tropical and warm temperate regions. *Ocimum gratissimum* is called 'efinrin' by the yorubas of the southwestern part of Nigeria, 'nchanwu' by the Igbos and 'Dai'doya' by the Hausas. It has been reported to contain the terpenoids, eugenol and thymol, saponins and alkaloides (Gill, 1988). *Ocimum gratissimum* is germicidal and has found wide use in toothpastes and mouth washes as well as some topical ointments (Nakamura *et al*., 1999; Holets *et al*., 2003; Pessoa *et al*., 2003). It is used as an excellent gargle for sore throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Fakae, 2000; Chitwood, 2003). In addition, *Ocimum gratissimum* carminative properties make it a good choice for stomach upset. It is used as an emetic and for hemorrhoids. The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea and mental illness (Dhawan *et al*., 1977; Oliver, 1980; Abdulrahman, 1992; Osifo, 1992; Sofowora, 1993; Sulistiarini, 1999). In addition, the plant is used as a spice and condiment in the southern part of Nigeria. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections diarrhea, headache, ophthalmic, skin diseases, pneumonia, cough fever and conjunctivitis (Onajobi, 1986). It is used by the Igbo's in southern Nigeria in the management of baby's cord. It is believed to keep the baby's cord and wound surfaces sterile (Iwu, 1986). *Ocimum gratissimum* has been reported to be active against several species of bacteria and fungi (Nwosu and Okafor, 1995; Nakaruma *et al*., 1999).Much has been documented on the antimicrobial properties of the leaf extract of this plant. This work is therefore aimed at evaluation of the antimicrobial property of the leaf extract of these plants. The whole plant and the essential oil have many applications in traditional medicine, especially in Africa and India. In folk medicine, it is used in the treatment of upper respiratory tract infection, diarrhoea, headache, ophthalmia, skin disease, pneumonia, cough, fever, conjunctivitis, gonorrhoeal, mental illness, high fever and influenza (Abdulrahman, 1992; Correa, 1932; Dhawan *et al*., 1977; Oliver, 1980; Osifo, 1992; Sofowora, 1993; Sulisfiarin, 1999).

Ocimum gratissimum leaf or the whole herbs are popular treatments for diarrhea (Dalziel, 1956). Infact, the antimicrobial activity of the water-saturated oil had been shown to be proportional to the thymol content in preparations where *Ocimum gratissimum* is used as cold infusion (El-said *et al*., 1969). Therefore, the antimicrobial effect of the extracted thymol is probably sufficient explanation for the anti-diarrheal effect. However, in certain other preparations, *Ocimum gratissimum* when boiled with water to form decoction will contain little of the steam-volatile thymol. Such aqueous decoctions were shown to be devoid of antimicrobial activity, but they do relax the guinea pig ileum and rat jejunum in-vitro (Sofowora, 1982).The current antifungal therapies used such as amphotericin B, and Fluconazole have certain limitations due to side effects and emergence of resistant strains. *Ocimum gratissimum* has been reported earlier to have in-vitro antifungal activity against some dermatophytes. Lexa *et al*., (2005) was able to demonstrate the antifungal property of *Ocimum gratissimum.* The chloroformic fraction of the extract inhibited 23 isolates (92%) of *Cryptococcus neoformans* at a concentration of 62.5µg/ml. Silva *et al*., 2005, on the other hand was able to demonstrate the effect of the essential oil of *Ocimum gratissimum* in-vitro against human pathogenic dermatophytes. In their experiment,

hexane fraction of the extract at a concentration of 125µg/ml. Eugol inhibited the growth of 80% of dermatophytes at the same concentration (Lexa *et al*., 2005).

The oil of *Ocimum gratissimum* has been formulated into creams for clinical trials where favourable results were reports for certain dermatological disorders of microbial complications (Sofowora, 1993). Mixtures for internal administration have also been formulated to utilize the anti helminthes property of the oil. The oil has been incorporated into tooth pastes on experimental basis for oral hygiene (Sofowora, 1982).Recent studies on *Ocimum gratissimum* proved the plant extract can be a source of medication for people living with Human Immunodeficiency Virus, (HIV) and Acquired Immune Deficiency Virus, AIDS (Elujoba, 2000).*Ocimum gratissimum* has been shown to possess antioxidant activity. Afolabi et al., 2007 was able to establish the antioxidant property with regards the phytochemical property.

IV. DESCRIPTION OF TEST ORGANISMS.

In Nigeria, shigellosis is endemic in most communities. Severe infections initially seen in children aged less than five years are now frequently occurring among adolescents and young adults (Iwalokun 2001). Most diarrheal illness among children in developing countries are self limiting, but a proportion follow a prolonged or persistent course, therefore collifer encyclopedia define diarrhea as the abnormal frequency and water state of feacal discharge and dysentery as an acute infectious diseases which involve primarily the colon and its characterized by the passage of frequent, watery, bloody stool associated with fever and prostration. Microbes also play an important role in the cause of dysentery and diarrhea. It also one of the best symptoms of water borne diseases.The condition is well known to ancient people (Ojo, 1998).Diarrhea of neonatal animal is one of the most common and economically devastating conditions encountered in the animal agriculture industry, diarrhea are second only to respiratory disease in a cause of adult death, they are the leading cause of childhood death and in some part of the world they are responsible for more years of potential life lost than all other cause, combined(Prescott et al,1999).The common organisms causing acute diarrhea are *Eschericha coli, salmonella, shigella, Vibrio cholera, campylobacter jejuni, Yersinia enterocolitica*(Prescott,1999) organisms causing dysentery are *Shigella dysentreriae Entamoeba histolytica, Acanthamoeba sp*(Thomas,1994)

Salmonella sp; These are gram negative rod shaped bacteria and they neither ferment lactose nor sucrose and with few exceptions produce abundant H2S essentially all are motile and decarboxylate lysine and omithine positive, selective media for their isolation from faeces contain brilliant green, deoxycholate, selenite, tetrathionate or citrate to suppress the growth of coliforms. *Salmonella* organism is found in virtually all animals, birds (including poultry), reptiles, rodents, domestic animals, and humans (Murray *et al*., 1998). Some serotypes of *Salmonella are virtually* species specific (Hook, 1990).

Salmonella spp. Cause many types of infections, from mild self-limiting to life-threatening typhoid fever. The infection can arise from contamination by workers in food-processing plants and restaurants, meats, poultry and dairy products are primary sources of pathogen, which results from ingestion of the organism (Prescott, 1999).The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting for seven days. Typhoid fever, the best-studied enteric fever, is characterized by fever, headache, diarrhea, and abdominal pain and can produce fatal respiratory, hepatic, spleen, and/or neurologic damage. Bacteraemia, meningitis, respiratory disease, cardiac disease, osteomyelitis, and other local infections caused by *Salmonella* spp. have been reported. *S. typhi* and *S. paratyphi* A and B cause gastroenteritis, bacteraemia, and enteric fever (Hook, 1990).

Shigellas sp; are gram negative, non motile, non spore forming rod shaped bacteria. The illness caused by shigella(shigellosis) accounts for less than 10% of the reported outbreaks of food borne illness in this country. it rarely occurs in animals, the organism is frequently found in water polluted with human faeces .other sp includes *shigella sonei, shigella boydii, shigella flexneri and shigella dysenteriae,* They are facultative rods, non lactose fermenting, non gas producing parasite of the human intestinal tract, classification is based on a combination of biochemical properties and antigenic analysis(Prescott,2004).Dysentery is usually fatal in infants and children while its often self limiting in most adults, this may be due to some immunity developed by adults in region where disease is endemic, the disease is particularly important in nurseries and homes for handicapped children(Ojo,1998)Shigellosis is an infection with shigella(a rod shaped bacteria)that normally occur in the digestive tract which is transmitted primarily from person to person by the faecal-oral route. Outbreaks can occur in day-care centre, occasionally, outbreaks can be caused by contamination of food by infected food handlers (CDC, in United States, 1983).

Escherichia coli; there are four recognized classes of entero-virulent *E. coli* (collectively referred to as the EEC group i.e. Enteropathogenic *Escherichia coli*) that causes gastro-enteritis in humans. EPEC are defined as *E. coli* belonging to serogroups epidemiologically implicated as pathogens but whose virulence mechanism is unrelated to the excretion of typical *E. coli* enterotoxin. *E. coli* are gram negative rod shaped bacteria belonging to the family Enterobacteriaceae(presscott,1999).it causes a watery or bloody diarrhea, the former associated with attachment and an acute tissue destructive process, perhaps caused by a toxin similar to that of shigella dysenteriae also called verotoxin. Occasionally, diarrhea in infants is prolonged leading to dehydration, electrolyte imbalance and death (50%) mortality rates have been reported in third world countries (CDC, 1992).

Bacillus cereus; can cause an emetic illness characterized by nausea and vomiting with an incubation time of one to six hours and a diarrhea type with an incubation of four to sixteen hours. The emetic type is often associated with boiled of fried rice, while diarrhea type is associated with a wider range of food (presscott, 1999).

Campylobacter jejuni; is considered a leading cause of acute bacterial gastroenteritis in human and can affect persons of all ages. This important pathogen is often transmitted by uncooked or poorly cooked poultry products .Contamination with as few as 10 viable campylobacter jejuni can lead to the onset of diarrhea (prescott, 1999).

Statement of the Problem
AIM AND OBJECTIVES
This research work is designed to;
1. Isolate and identify some of the bacterial organisms associated with dysentery or diarrhea in young children
2. Investigate the in-vitro activity of bitter leaf (*Vernonia amygdalina*) and wild basil (*Ocimum gratissimum*) against some selected pathogenic organisms causing diarrhea and dysentery.

V. MATERIALS AND METHODS

Sources of leaves (Ocimum gratissimum and Vernonia amygdalina)
The wild basil and bitter leaf were obtained from the plants growing on the premises of Olabisi Onabanjo University, Ago-Iwoye, Ogun state.

Collection of samples

Feacal sample were collected from patients between the ages of 0-20 that showed the clinical symptoms of diarrhea or dysentery at University College Hospital (U.C.H).Ibadan, Oyo State. A total number of 100 samples were collected in sterile Universal bottles and transported to the laboratory and processed immediately.

Culturing and isolation of bacterial organism

A loopful of the samples was inoculated onto an already prepared Deoxycholate citrate agar(DCA) and several loopful into selenite F- broth and inoculated at 37^0C for 24hrs.After an overnight incubation, the plates and test tubes were examined under the microscope using gram staining technique.

Gram stain procedure

Clean grease free slides were used. A drop of distilled water was dropped on the center of the slide using a sterile inoculating loop. A colony was picked from deoxycholate citrate agar plate and test tube and emulsified on the distilled water. The glass slide was passed through a flame to heat fixed the organism. The fixed organism was then flooded with crystal violet for 15 seconds and the excess was washed off with distilled water. Iodine solution s was then added and allowed to stay for about 30 seconds and excess was poured off and washed with water. The iodine served as mordant. It was now flooded with alcohol for not more than 2-5 seconds for decolorisation. A counter stain, safranin was now added for 20 seconds and washed off with water to remove the excess safranin and blotted dry. The slide was then observed under the microscope using the x10&x100 objective lens for focusing and viewing respectively.

Sub-culturing of isolates

The selenite culture was sub cultured on DCA by streaking to get a distinct colony of the organisms and incubated at 37^0c for 24hours.Organisms from the deoxycholate citrate agar were incubated on MacConkey agar by using a sterilized inoculating loop to pick one of the distinct colony and streaked it on the solidified agar medium. Organisms from this are then inoculated on agar slant to be used for biochemical test and sensitivity test.

Identification of isolates

Pure cultures of the isolates organisms were used for the biochemical testing. Biochemical tests carried out for the identification of the organisms includes Catalase test, Indole test, Oxidase test, Coagulase test, Motility test and Citrate test.

CATALASE TEST; a drop of hydrogen peroxide was dropped at the centre of a clan-grease free slide. A colony of the isolated organism was placed on the hydrogen peroxide, after 5 seconds gas bubbles were observed.

INDOLE TEST; the test organism was inoculated onto a peptone water and incubated for 48hours at 37^0C after which 0.5ml of Kovac's reagent was added and shook gently. A change in colour was observed.

OXIDASE TEST; a freshly prepared 1% solution of teramethyl-p-phenylene-diamine-dihydrocloride was poured onto a nutrient agar plate which contained the pure isolates and allowed to cover the surface of the plate while excess was decanted within some few seconds, a change in colour was observed.

COAGULASE TEST; the test organism was picked from the plate and placed on a distilled water on the slide and mixed together. Plasma was then added and there was no agglutination.

MOTILITY TEST; the test organism was transferred onto centre of a clean dry cover slip and slide with little immersion oil round its suspension edge, was inverted over the cover slip and the cover slip was carefully inverted for the culture drop to appear hanging. It was observed under oil immersion lens.

CITRATE TEST; a loopful of isolated colony were inoculated in simmons citrate medium and incubated for 24hours.There was a colour change.

After the biochemical test, the identified isolates were maintained on nutrient agar slopes at 4^0C in a refrigerator. It was then sub-cultured on MacConkey agar for 24hours at 37^0C before used.

EXTRACTION OF VERNONIA AMYGDALINA (BITTER LEAF) AND OCIMUM GRATISSIMUM (WILD BASIL).

The leaves collected were weighted on metler balance into 6.25g, 12.50g, 25.00g, 50.00g and 100.00g respectively. The leaves were thoroughly washed with distilled water placed in different containers and crushed. The crushed leaves were then extracted with 20ml of ethanol in conical flask and kept for 24 hours while the crushed leaves of 100g were extracted neither with ethanol nor distilled water for both the wild basil and bitter leaf respectively. The extracts were then filtered and the filtrate was aseptically transferred into a labeled sterile conical flask and stored in a cool dry place.

PREPARATION OF MEDIA

12.50g of Deoxcholate Citrate agar (DCA) was weighed on a sensitive balance and dissolved in 250ml of distilled water in a conical flask. It was allowed to disperse for 10mins before shaking vigorously to dissolve the agar and was autoclaved at 121^0C for 15minutes.

PREPARATION OF INOCULA

The pure culture of the organisms from the slant were plated out on MacConkey and incubated at 37^0C for 24 hours .after incubation, colony of the organisms was taken and inoculated into 7mls of peptone water in a bijou bottle and shook vigorously so as to obtain homogeneity of the solution.

BACTERIAL SENSITIVITY TESTING USING WELL DIFFUSION METHOD.

Inocula measured up to 1ml from the peptone water in bijou bottle were introduced on to the surface of sterile blood base agar plates. It was evenly distributed by rocking the plates and allowed to dry I air but covered to prevent atmospheric contamination. The plates were then bored in 2places where 1ml of the leaves extract at different concentration was placed in each hole. A sterile antibacterial agent (antibiotics disc) confirmed to be

sensitive to the organisms was carefully placed with a forceps on to the centre of the labeled plates of each bacterial species. The plates were incubated at 37^0C and examined growth after 24hours zones of inhibition were measured in (mm) using transparent meter rule .Antibiotics' disc was used as control.

RESULTS AND DISCUSSION

Out of the 100 samples collected, 70(70%) of the specimens were obtained from female patients while 30(30%) were from the male patients. The age distribution of the patients in relation to sex from whom diarrheic specimens were obtained and shown in the table below.

Table 1; Sample collection in relation to age and sex

Patients age(yrs)	**Patient sex**		**Total**
	Female	**male**	
0-5	**15**	**10**	**25**
6-10	**30**	**05**	**35**
11-15	**10**	**15**	**25**
16-20	**15**	**-**	**15**
Total	**70**	**30**	**100**

From the 100 samples collected 20(20%) isolates were isolated. Nine samples yielded *Escherichia coli;* seven samples as *Salmonellae* while *Shigella* were isolated from four samples in the table below,

Table 2; Incidence of the organisms according to age

Pathogens	**Numbers of isolates according to age group(yrs)**				
	0-5	**6-10**	**11-15**	**16-20**	**Total**
Escherichia coli	**2**	**4**	**2**	**1**	**9**
Salmonella sp	**3**	**2**	**-**	**2**	**7**
Shigella sp	**1**	**3**	**-**	**-**	**4**
Total	**6**	**9**	**2**	**3**	**20**

IDENTIFICATION OF ISOLATES

From the Gram stain procedure, the film was observed to have a characteristics feature of rod shape with pink colour termed as gram negative bacilli. There was no colour change after some seconds when oxidase reagent was added to the organisms. This was used for the identification of enteric bacteria which is always negative. It separate enteric from other oxidase positive bacteria. There was gas bubble production to confirm the enteric bacteria indicating a positive result. No agglutination was formed giving a negative result that the isolates are coagulase negative.

Escherichia coli and Shigella sp produces a brick red colour indicating that they are indole positive while salmonella sp does not. *Escherichia coli* and Shigella sp are motile organisms moving randomly in the hanged drop while Shigella sp is a non motile organism indicated by brownians movement.

No colour change was observed in shigella sp as shown in the table below;

Table 3; the identification of isolates.

Organisms	Gram stain	Oxidase test	Catalase test	Coagulase test	Indole test	Motility test	Citrate test	
Escherichia coli	-	-	+	-	+	+	+	
Salmonella sp	-	-	+	-	-	+	+	
Shigella sp	-	-	+	-	+	-	-	

ANTIMICROBIAL SENSITIVITY OF THE ISOLATES

After 24 hours of incubation, the plates were examined and the readings were taken. The observed zones of inhibition as shown below were measured in mm using transparent meter rule.

Table 4; the inhibitory effect of wild basil and bitter leaf against *Escherichia coli* at various concentrations.

Concentration of extracts(g/ml)	Zones of inhibition(mm)		Zones of inhibition in (%)		Control(antibiotics disc-mm)
	Wild basil	bitter leaf	Wild basil	bitter leaf	CAZ CRO CF NAL OFL PF SP TET
06.25	14.00	12.00	15	13	
12.50	16.50	13.10	18	14	
25.00	18.00	15.30	20	17	14 25 - 20 18 20 - 25
50.00	20.60	17.00	22	18	
100.00	23.00	20.00	25	22	

From the table above, it was observed that at 6.25g/ml. *Escherichia coli* showed 14mm and 12mm inhibition of growth whereas at 100g/ml, 23mm and 20mm were observed against wild basil and bitter leaf respectively. From the result it can be concluded that the zones of inhibition increased as the concentration in g/ml increases. Statistically, *Escherichia coli* could be said to be more sensitive to the inhibitory effect of wild basil than that of bitter leaf ($p<0.05$).Also the antibiotic disc used as control showed considerable zones of inhibition as shown in the table above.

Table 5; the inhibitory effect of wild basil and bitter leaf against *salmonella sp* at various concentrations.

Concentration of extracts(g/ml)	Zones of inhibition(mm)		Zones of inhibition in (%)		Control(antibiotics disc-mm)
	Wild basil	bitter leaf	Wild basil	bitter leaf	CAZ CRO CF NAL OFL PF SP TET
06.25	12.00	10.00	13	11	05 10 36 15 - 27 40 -
12.50	20.00	13.00	22	14	
25.00	23.10	16.00	25	17	
50.00	28.00	17.00	31	18	
100.00	30.00	20.50	33	33	

The inhibitory effect of *salmonella sp* by wild basil (efinrin) and bitter leaf (ewuro) was obtained to be 30mm and 20.5mm at 100g/ml respectively. At 6.25g/ml, the inhibitory effect by wild basil was showed to be 12mm whereas for bitter leaf it was 10mm from the table, it was deduced that zone of inhibition of growth increased as the concentration of extracts increases showing that the test organism becomes more sensitive to the plant extract as the concentration increases. The organisms was also sensitive to some antibiotics disc e.g. ciprofloxacin, sparfloxacin, and perfloxacin that served as control. Statistically, it can be concluded that both the extracts efinrin and ewuro were effective in the treatment of diarrhea or dysentery caused by *salmonella sp* ($p<0.05$) and as such can be used in the treatment of infection caused by this organism.

Table 6; the inhibitory effect of wild basil and bitter leaf against *shigella sp* at different concentrations with their corresponding percentage zones of inhibition and controls.

Concentration of extracts(g/ml)	Zones of inhibition(mm)		Zones of inhibition in (%)		Control(antibiotics disc-mm)
	Wild basil	bitter leaf	Wild basil	bitter leaf	CAZ CRO CF NAL OFL PF SP TET
06.25	18.20	17.00	20	18	32 35 25 - 27 - 30 15
12.50	21.00	20.00	23	22	
25.00	22.00	22.80	24	25	
50.00	28.00	24.00	31	26	
100.00	30.00	26.00	33	28	

The table above shows that zones of inhibition of wild basil and bitter leaf on the growth of *shigella sp*. This revealed that at 6.25g/ml wild basil extract 18.20mm was obtained as the zones of inhibition while bitter leaf extract showed 17mm inhibition of growth. It was also observed that zones of inhibition increased as the

concentration of extract increased. Also at 100g/ml of wild basil extract 30mm inhibition of growth was observed while the bitter leaf extract showed 26mm.The antibiotics used served as control to compare the effectiveness between the extracts and the disc. It was deduced that Ceftrianzone(CRO) is more active followed by Ceftazidine, Sparfloxacin(SP), Ofloxacin(OFL) and Ciprofloxacin(CF) with the following zones of inhibition 35mm,32mm,30mm,27mm and 25mm respectively. Statistically, *shigella sp* could be said to be sensitive to both the inhibitory effect of wild basil and bitter leaf respectively ($P<0.05$).

Table 7; the overall table showing the inhibitory effect of wild basil and bitter leaf against *Escherichia coli; Salmonella sp and Shigella sp* at various concentrations with their corresponding percentage zones of inhibition and control.

organisms	Concentration of extracts in g/ml	Zones of inhibition(mm)		Percentage of zone of inhibition (%)		Control-antibiotic disc(mm)							
		Wild basil	Bitter leaf	Wild basil	Bitter leaf	CAZ	CRO	CF	NAL	OFL	PF	SP	TET
Escherichia coli	06.25	14.00	12.00	15	13	14.00	25.00	-	20.00	18.00	20.00	-	25.00
	12.50	16.50	13.10	18	14								
	25.00	18.00	15.39	20	17								
	50.00	20.60	17.00	22	18								
	100.00	23.00	20.00	25	22								
Salmonella sp	06.25	12.00	10.00	13	11	5.00	10.00	36.00	15.00	-	27.00	40.00	-
	12.50	20.00	13.00	22	14								
	25.00	23.10	16.00	25	17								
	50.00	28.00	17.00	31	18								
	100.00	30.00	20.50	33	33								
Shigella sp	06.25	18.20	17.00	20	18	32.00	35.00	25.00	-	27.00	-	30.00	15.00
	12.50	21.00	20.00	23	22								
	25.00	22.00	22.80	24	25								
	50.00	28.00	24.00	31	26								
	100.00	30.00	26.00	33	28								

From the table above, it can be concluded that the zones of inhibition increased as the concentration in g/ml increases. Statistically, *shigella sp* could be said to be more sensitive to the inhibitory effect of wild basil and bitter leaf (P0.05) on comparing *Escherichia coli* and *salmonella sp.* Also the antibiotic disc used as control showed considerable zones of inhibition as shown in the table above.

VI. DISCUSSION

From this project work, it was observed that bacteria play an important role in infants and adults diarrhea or dysentery. The high occurrence of these organisms could be attributed to poor sanitation on the part of the parents or wards of such infants.The effect of wild basil (efinrin) and bitter leaf (ewuro) on Escherichia coli, Salmonellae and Shigellae isolated from diarrheic patients (0-20years) were investigated, a total number of 100 samples were collected and 20 isolates were isolated.Out of the 20 isolates, 9 were Escherichia coli, 7 isolates for salmonella sp and 4 isolates for Shigella sp respectively. The high occurrence of Escherichia coli isolated confirmed the report given by Ojo,1998 that Escherichia coli causes dirrhoea in human particularly in infant and young children in which it is fatal.It was also reported by Black et al,1984 that children who suffered a persistent episode of dirrhoea experienced a significantly higher diarrheal burden over one year study. Ojo 1998, in his study stressed that persistent diarrhea was an important problem for children during the first 2years of life and that Escherichia coli was the major causative agent. Wild basil showed the highest inhibitory effect against salmonella sp and shigella sp with 30.00mm inhibition of growth followed by Escherichia coli 23.00mm whereas bitter leaf extract showed the highest inhibitory effect against shigella sp with inhibition zone of 26.00 followed by salmonella sp and Escherichia coli with 20.50mm and 20.00mm as the inhibition at 100g/ml respectively.At 06.25g/ml, wild basil extract shoed the highest inhibitory effect against shigella sp with 18.20mm and subsequently followed by Escherichia coli with 14.00mm and salmonella sp with 12.00mm, while shigella sp was observed to has the highest zone of inhibition of the three isolates with 17.00mm, followed by Escherichia coli with 12.00 and salmonella sp with the least value of 10.00mm.Statistically , this research work established the fact that wild basil(efinrin) extract could be used in the treatment of dysentery or diarrhea infection caused by Escherichia coli and salmonella sp and shigella sp while bitter leaf extract was observed to be significantly effective in the treatment of diarrheic infection caused by salmonella sp and shigella sp respectively Recently, there has been an increase in the number of people in Nigeria depending on herbal drugs. Herbal drugs are cheap, rapidly available and unadulterated. Their antibacterial activity could be increased by partial purification and subsequent concentration of the active ingredients.

VII. conclusion

The antimicrobial activities of Ocimum gratissimum and Vernonia amygdalina on Escherichia coli, salmonella and shigella sp have been experimented. The result revealed that the organisms were active against wild basil extract even at 6.25 concentration, but the organism may develop resistance while bitter leaf, the organisms were not as active except for shigella sp which showed considerable sensitivity.

Since this is just an in vitro test, it is yet to be established if bitter leaf extract and wild basil extract will be as efficient in an in vivo test. Therefore, the socio political justification for the research of pharmacologically useful agents to relieve pains suffering is more logical in a concise society on the basis for better management and utilization of natural resources (Mahandra 1996).

Appendix

Table 1; statistical table showing the goodness of fit of the extracts (efinrin&ewuro) in the treatment of *Escherichia coli*

Observed frequency				**Expected frequency**	
g/ml	**Wild basil**	**Bitter leaf**	**Total**	**Wild basil**	**Bitter leaf**
6.25	**14.00**	**12.00**	**26.00**	**14.12**	**11.87**
12.50	**16.50**	**13.10**	**29.60**	**16.08**	**13.51**
25.00	**18.00**	**15.30**	**33.30**	**18.09**	**15.20**
50.00	**20.60**	**17.00**	**37.60**	**20.43**	**17.16**

100.00	**23.00**	**20.00**	**43.00**	**23.36**	**19.63**
Total	**92.10**	**77.40**	**169.50**	**91.98**	**77.37**

To calculate expected value for *Escherichia coli using* the formula below;

Total of both extracts x overall total of wild basil

Overall total of both extracts

To calculate the chi square using the formula below

X^2 = (Observed frequency-Expected frequency)^2 = (O-E)^2

Expected E

X^2 wild basil $=\frac{(14-14.12)^2+(16.50-16.08)^2+(18-18.09)^2+(20.60-20.43)^2+(23-23.36)^2}{14.12 \quad 16.08 \quad 18.09 \quad 20.43 \quad 23.36}$

= 0.001+0.010+0.0004+0.001+0.005 = 0.0174(F_{cal})

d.f = 5-1 = 4 at x^2 0.05= 9.488(F_{tab})

x^2 bitter leaf $=\frac{(12-11.87)^2+(29.6-13.51)^2+(33.3-15.20)^2+(37.6-17.6)^2+(43-19.63)^2}{11.87 \quad 13.51 \quad 15.20 \quad 17.6 \quad 19.63}$

= 0.001+19.16+21.55+22.72+27.82 =91.25(F_{cal})

d.f = 5-1 =4 at x^2 0.05 = 9.488(F_{tab})

$F_{cal} > F_{tab}$ (Hp is not significant) P>0.05).Therefore the hypothesis is not accepted and the goodness of fit is not good

Table 2; statistical table showing the goodness of fit of the extracts (efinrin&ewuro) in the treatment of *salmonella sp*

Observed frequency				**Expected frequency**	
g/ml	**Wild basil**	**Bitter leaf**	**Total**	**Wild basil**	**Bitter leaf**
6.25	**12.00**	**10.00**	**22.00**	**13.12**	**8.87**
12.50	**20.00**	**13.00**	**23.00**	**19.68**	**13.31**
25.00	**23.10**	**16.00**	**39.10**	**23.32**	**15.77**
50.00	**28.00**	**17.00**	**45.00**	**26.84**	**18.15**
100.00	**30.00**	**20.50**	**50.50**	**30.12**	**20.37**
Total	**113.10**	**76.50**	**189.60**	**113.08**	**76.47**

To calculate expected value for *salmonella sp using* the formula below;

Total of both extracts x overall total of wild basil

Overall total of both extracts

To calculate the chi square using the formula below

$X^2 = \frac{(\text{Observed frequency-Expected frequency})^2}{\text{Expected}} = \frac{(O-E)^2}{E}$

X^2 wild basil $= \frac{(12-13.12)^2+(20-19.68)^2+(23.10-23.32)^2+(28-26.84)^2+(30-30.12)^2}{13.12 \quad 19.68 \quad 23.32 \quad 26.84 \quad 30.12}$

$= 0.095+0.005+0.002+0.050+0.0004 = 0.1524(F_{cal})$

d.f = 5-1 =4 at x^2 0.05 = $9.488(F_{tab})$

$F_{cal} < F_{tab}$ Hp is significant (P<0.005)

X^2 bitter leaf $= \frac{(10-8.87)^2+(13-13.31)^2+(16-15.77)^2+(17-18.15)^2+(20.5-20.37)^2}{8.87 \quad 13.31 \quad 15.77 \quad 18.15 \quad 20.37}$

$= 0.1439+0.007+0.003+0.072+0.0008 = 0.2267(F_{cal})$

d.f.. = 5-1 =4 at x^2 0.05 $= 9.488(F_{tab})$

$F_{cal} < F_{tab}$ Hp is significant ($p<0.05$), the hypothesis is accepted and the goodness of fit is good

Table 3; statistical table showing the goodness of fit of the extracts (efinrin&ewuro) in the treatment of *shigella sp*

Observed frequency				**Expected frequency**	
g/ml	**Wild basil**	**Bitter leaf**	**Total**	**Wild basil**	**Bitter leaf**
6.25	**18.20**	**17.00**	**35.20**	**18.32**	**16.87**
12.50	**21.00**	**20.00**	**41.00**	**21.34**	**19.65**
25.00	**22.00**	**22.80**	**44.80**	**23.31**	**21.48**
50.00	**28.00**	**24.00**	**52.00**	**27.06**	**24.93**
100.00	**30.00**	**26.00**	**56.00**	**29.14**	**26.85**
Total	**119.20**	**109.80**	**229.00**	**119.17**	**109.78**

To calculate expected value for *shigella sp using* the formula below;

Total of both extracts x overall total of wild basil

Overall total of both extracts

To calculate the chi square using the formula below

$X^2 = \frac{(\text{Observed frequency-Expected frequency})^2}{\text{Expected}} = \frac{(O-E)^2}{E}$

X^2 wild basil $= \frac{(18.20-18.32)^2+(21-21.34)^2+(22-23.31)^2+(28-27.06)^2+(30-29.14)^2}{18.32 \quad 21.34 \quad 23.31 \quad 27.06 \quad 29.14}$

$= 0.0007+0.005+0.073+0.032+0.025 = 0.1257(F_{cal})$

d.f = 5-1 =4 at x^2 0.05 = $9.488(F_{tab})$

$F_{cal} < F_{tab}$, Hp is significant($P<0.005$) the hypothesis is accepted and the fitness is good

X^2 bitter leaf $=\frac{(17-16.87)^2+(20-19.65)^2+(22.8-21.48)^2+(24-24.93)^2+(26-26.55)^2}{16.87 \quad 19.65 \quad 21.48 \quad 24.93 \quad 26.55}$

$= 0.001+0.006+0.081+0.034+0.011 =0.1330(F_{cal})$

d.f.. = 5-1 =4 at x^2 0.05 = $9.488(F_{tab})$

$F_{cal} < F_{tab}$ Hp is significant ($p<0.05$)

Hypothesis is accepted and the goodness of fit is good

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