

Exploiting antibacterial activity of *Ocimum basilicum* L. (Basil Leaf) for controlling water-borne infection due to *Vibrio cholerae* among rural communities

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Abstract

The antimicrobial activities of crude, ethanolic and methanolic extracts of *Ocimum basilicum* (OB) (Basil plant) against *Vibrio cholerae*, a bacterial pathogen responsible for cholera were examined in the present study, and their potency, were assessed by agar-streak diffusion method, to ascertain the presence or absence of inhibition zones and zone diameter. Basil leaves (*Ocimum basilicum*) were peeled off from the standing basil plant. *Vibrio* species were isolated from borehole and river water using a selective medium, Thiosulphate citrate bile salt agar after pre-enrichment in peptone water and purified in nutrient agar (NA) and used for the assay. NA plates were incubated at 37°C for 24h. Diameter of inhibition zone for sensitive cases was measured. The results showed that the ethanolic extract had slightly higher antibacterial activity compared with methanolic and crude extracts. Two-way analysis of variance indicated that there were significant differences in the zones of inhibitions (ZOI) by *V. cholerae* using different OBE [F (2,6)=6.404, p<0.05] and from different water sources [F(3,6)=54.763, p<0.05]. There was however no significant interaction in the type of OBE and source of *V. cholerae* [F (6,23)=0.114, p>0.05]. Also, chloramphenicol had significantly higher antibacterial effect on *V. cholerae* than the OBE [F(1,6)=108.571, p<0.05]. The range of ZOI reported are comparable to standard references and thus affirm that OBE can be exploited commercially and used to control diarrhoeal illnesses due to enterotoxigenic and enteropathogenic gram-negative bacteria such as *Vibrio cholerae*.

Keywords: antimicrobial activity, culturing, aseptic technique, *Ocimum basilicum* (Basil), sensitivity, zone of inhibition

Introduction

Medicinal plants are huge sources of income all over the world. It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal properties. The beneficial medicinal effects of plant materials are due active ingredients present in the plant. These compounds are mostly secondary metabolites such as alkaloids, essential oils, tannins, phenols, flavonoids, steroids and resin which are capable of producing definite physiological action on the microorganisms. Antibiotic-resistant strains of bacteria have been found to be widely inhibited by these compounds (Juliana and Simson, 2002; Kalemba and Kunika, 2003).

Ocimum plant includes annual and perennial herbal plants, as well as shrubs which belongs to the family of lamiacea and produces white-purple flowers (Daneshian *et al.*, 2009). *Ocimum* have strong antioxidant and antimicrobial properties (Yamasaki *et al.*, 1998; Wannissorn *et al.*, 2005; Kaya *et al.*, 2008) ^[10]. The most important species of *Ocimum* genus are *O. Sanctum* L. and *O. basilicum*, this latter species is usually named common basil or sweet basil (Figure 1). *Ocimum basilicum* (Basil plant) is one of the oldest and popular herbal plants with health benefiting phytonutrients. It is referred to as “holy herb” in many traditions all around the world. Basil is originally native to Iran, India and other tropical regions of Asia. Today, basil is cultivated in many Asian and Mediterranean countries. This bushy annual herb is especially grown for its medicinally useful leaves and seeds. It is

particularly planted around every home in Nigeria for the purpose of spicing pepper soup and stew.



Fig 1: Basil Plant (Scent leaf).

The leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactagogue, stomachic, and toxic agents (Falerio *et al.*, 2003). Essential oils from *Ocimum* plants are used as spices and flavours for many food products and are effective in folk medicine as well as in cosmetics and dental products

(Sacchetti *et al.*, 2004; Jirovetz *et al.*, 2003; Marwat *et al.*, 2011).

Due to random and wide usage of antibiotics in the treatment of infectious diseases, a multiple drug resistance against pathogenic microorganisms has developed in recent years (Denyer *et al.*, 2004; Kummerer, 2004; Weigand *et al.*, 2008; Dirisu *et al.*, 2017) [4, 11, 19]. The alternative is the use of therapeutic agents from plants which contain antimicrobial activities against these microbial pathogens (Kalemba and Kunicka, 2003; Faleiro *et al.*, 2003). In view of the antibiotic resistance which has become a global concern, this study was carried out to evaluate the antibacterial activity of *Ocimum basilicum* leaf extracts against one human pathogens, *Vibrio* species, a bacterium that causes severe diarrhea especially in children. The determination of the antimicrobial activity of basil plant extract is meant to ascertain whether the crude, ethanoic and methanolic leaf extract could be used in its pure form as an oral preparation to treat diarrhoea illness.

Materials and Methods

Sample collection and treatment

Sample collection

Leaves of Basil plant (*Ocimum basilicum*) (OB) were randomly harvested from a garden along Omoku-Ahoada road Omoku by peeling them off from the standing basil plant, which is the normal method of collecting it when used for food spicing.

Water samples were collected in sterile plastic bottles from borehole located in Federal College of Education (Technical) and Omoku River by Iyasara road and Obohia, all in Omoku, [N: 14° N S: 4° N W: 2° E E: 15° E] Rivers State, Nigeria and designated as BHW, RW1 and RW2 respectively.

Culture Media

Culture media used for the study were Thiosulphate citrate bile salt agar. (TCBA) (Biotech) and nutrient agar (Fluka) and were prepared according to manufacturer's guidelines. Ethanol and Methanol were obtained from Chemistry Lab.

Sterilization

All glass wares were sterilized in a hot oven at 105°C for 1hour. Media were sterilized by autoclaving at 121°C for 15 minutes. Bench surface was sterilized by cleaning with 95% ethanol

Preparation of extracts

Basil leaves were cleaned of debris and placed into watch glass containing 25 cm³ of 95% ethanol and methanol respectively and allowed to stand for 2hours at room temperature. Thereafter, each extract was filtered using Whatman filter paper™, No.1. Crude extract was also

obtained by blending the leaves with 20ml of distilled water and filtering through Whatman Filter paper™ No. 1. The residue was discarded and the filtrate stored in a refrigerator until when used

Bacteria

Vibrio species was used for the assay. This bacterium was isolated from the water samples – BHW, RW1 and RW2 using standard method and selective media- TCBSA (APHA, 2005) [2]. It was identified as yellow colonies in Thiosulphate citrate bile salt agar (Holt *et al.*, 1994) [7]. The organism was purified in nutrient agar (NA) and incubated at 35°C for 24 hours.

Screening of antimicrobial activities

Inoculums containing 10³ bacterial cells mL⁻¹ were spread on NA. Antimicrobial activity test was carried out by agar-streak diffusion Method. Briefly, sterile Whitman filter paper was cut into smaller circular shape and soaked into the plant extracts. Using a sterile forceps, three extracts- impregnated filter paper was placed on the NA to which the bacterial suspension had been spread. The inoculated agar plates were incubated at 37°C for 24 h. After the incubation period, the diameter of inhibition zone for sensitive cases was measured in duplicates and expressed in millimeter (mm). The inhibition zone is the area surrounding the growth of the inoculated microorganism. Standard antibiotic, chloramphenicol (30mg) was also used to check the level of sensitivity against the leave extract. Negative controls had no bacteria on the agar plates. Based on the scheme given by Forbes *et al.* (2007) [6], tested organisms can be described as sensitive, intermediate or resistant if the diameter of zone of inhibitions are >12mm, 4-12mm and <4mm respectively.

Instrumentation

Instruments used were autoclave (Shenan, model D5X-280), incubator, Oven (N/C-101 EDI), Metre rule

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to ascertain whether there was significant difference between the antibacterial activity of leaves extracts against the test organism and the interactions between the source of the *V. cholera* and antibacterial agents investigated. Analysis was carried out using Microsoft Excel Data analysis Pak.

Result and Discussion

Antibacterial activity of basil plant extract

The zones of inhibition (mm) of Basil plant extract by *Vibrio* species is shown in Tables 1 with ANOVA statistics in Tables 2 and 3 respectively.

Table 1: Mean Zone of Inhibition (mm) of *V.cholerae* from various water by agar-streak diffusion method

Source of bacterial Isolate	Diameter of inhibition zone (mm)			
	EE	ME	CE	Chloramphenicol
CBH	13.75 ±3.04	8.65 ±3.04	9.4 ±0.85	22.5 ±3.54
RW1	17.25 ±2.33	11.05 ±2.47	13.13±5 1.51	25.5 ±0.99
RW2	16.55±1.34	11.05 ±2.47	13.135 ±1.51	24.95 ±0.64

Results shows mean± standard deviation of measured ZOI

Key: CBH=College borehole water, RW1=River water1, RW2=River water 2; EE=ethanol extract, ME=methanol extract; CE=crude extract

Table 2: Two-way analysis of Variance on sensitivity of *Ocimum basilicum* extracts (OBE) on *V. cholerae* from different water sources in Omoku

Source of Variation	SS	df	MS	F	P-value	F crit
Bacterial source	55.65216	2	27.82608	6.403928	0.01281	3.885294
OBE	713.8669	3	237.9556	54.7634	2.83E-07	3.490295
Interaction	2.974808	6	0.495801	0.114104	0.99283	2.99612
Within	52.1419	12	4.345158			
Total	824.6357	23				

There were significant differences in the zones of inhibitions (ZOI) by *V. cholerae* using different OBE (basil extracts), $F(2,6)=6.404, p<0.05$ and from different water sources, $F(3,6)=54.763, p<0.05$. There was however no significant interaction in the type of OBE and source of *V. cholerae*, $F(6,23)=0.114, p>0.05$.

Comparative analysis of the Basil leaf extracts and Chloramphenicol gave mean ZOI as indicated in Figure 2 below. ANOVA statistic in Table 3 indicated that chloramphenicol had significantly higher antibacterial effect on *V. cholerae* than the OBE $F(1, 6) = 108.571, p<0.05$.

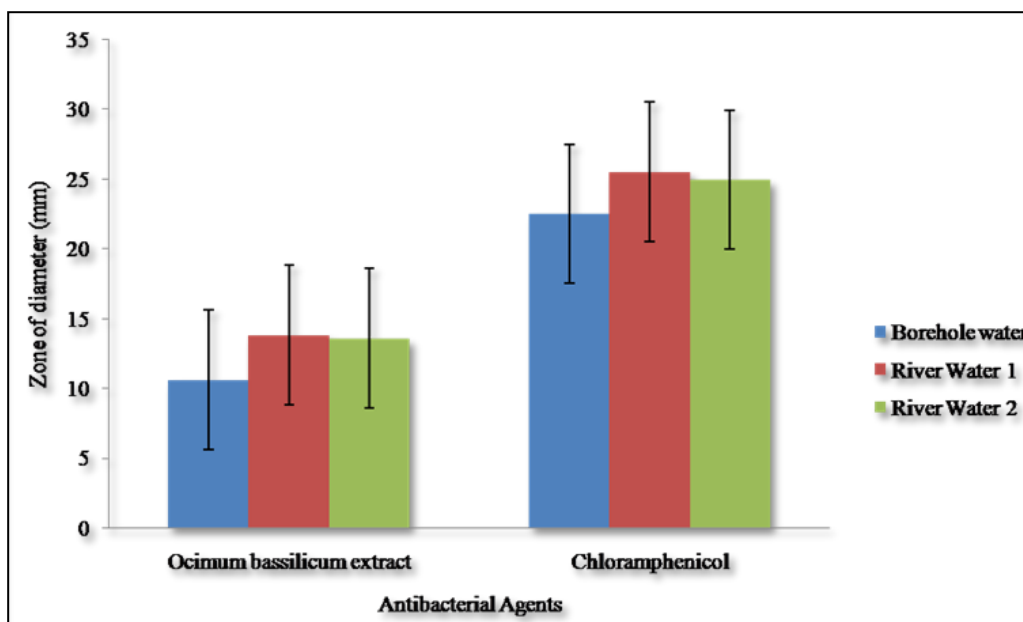


Fig 2: Comparative sensitivity of *V. cholera* isolated from water to Basil leaves extract and Chloramphenicol

Table 3: ANOVA statistic on comparative antibacterial activities of *O. basilicum* and Chloramphenicol

Source of Variation	SS	df	MS	F	P-value	F crit
Vibrio source	26.07876	2	13.03938	3.896833	0.082303	5.143253
Antibacterial agent	363.2934	1	363.2934	108.5706	4.58E-05	5.987378
Interaction	1.205842	2	0.602921	0.180184	0.839474	5.143253
Within	20.07689	6	3.346148			
Total	410.6549	11				

The standard antibiotic used, chloramphenicol had significantly higher antibacterial effect on *V. cholerae* than the plant extract at 5% alpha level. The mean zone of diameter reported in this study is lower than 12.5mm, 18.5mm and 20.0mm of Basil crude extract, hexane soluble and streptomycin soluble extract (Mann, 2012) [12]. The result of the present study indicated that active ingredients extracted from basil leaves (result not shown) had antibacterial activity against *Vibrio* to a small extent. Mann (2012) [12] reported that crude 95% ethanol extract of *O. gratissimum* demonstrated antimicrobial activity against *N. gonorrhoeae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *K. pneumoniae*, and *V. cholera* at a concentration of 1000 µg/ml. In an earlier study,

Semeniuc *et al* (2016) [17] demonstrated that essential oil of basil plant had mild antibacterial effect against *Escherchia coli* and *Bacillus cereus* but did not inhibit *Salmonella typhimurium*, *P. aeruginosa*, and *Staphylococcus aureus*) Also, Derakhshn *et al.* (2011) reported that essential oil of *C. cyminum* exhibited antimicrobial activities against *V. cholerae*, *S. aureus* and *E. coli*. These pathogens are implicated in gastroenteritis of various clinical presentations. Furthermore, Penduka *et al.* (2011) showed that the methanolic and aqueous extracts of *G. kola* seeds had antibacterial activities against fifty *Vibrio* isolates obtained from wastewater. Basil is widely used in systems of folk medicines and

traditional Chinese medicine for treating digestive system disorder, such as stomach ache and diarrhea, kidney complaints and infections (Davis 1994; Service, 1995). In Africa, for example, it is used for treating whooping cough and various types of fever. The leaves are pulped in water to make ear and eye drops in parts of West Africa and a leaf decoction used for treating coughs (Kalemba and Kunika 2003, Juliana and Simson, 2002; Falerio *et al*, 2003). The leaves are also used to make an insecticide that can protect stored crops from beetle damage (Martin and Ernst, 2004) and mosquito (Oparaocha *et al*, 2010) ^[15].

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Kates, 2001, Ndukwa *et al*, 2010) ^[9, 14]. Iwalokun *et al* (2011) ^[8] have equally reported the antibacterial activity of *O. gratissimum* and other plants against *Shigella spp*, another enteric pathogen. Methanol-soluble fraction of *O.gratissimum* has phenolic and flavonoids, which accounts for its antimicrobial activity. Some studies claim that the phenolic compounds present in spices and herbs play a major role in their antimicrobial effects (Hara-Kudo *et al.*, 2004). According to Moghadam *et al* (2011), Mann (2012) ^[12] and Stefan *et al* (2013) ^[18], the active ingredients in Basil plant are essential oil (EO), which belongs to the class of compounds known as terpenoid. They have been implicated in antimicrobial activity against *Salmonella* and other organisms that cause gastroenteritis or enteric fever such as *E.coli* (Chukwuka *et al*, 2010). The result of this study lends support to the fact that local medicinal plants have antibacterial activity against *Vibrio* species as also reported by various authors (Pirbalouti *et al*, 2011) ^[16]. Moghaddam *et al* (2011) ^[13] reported that EO from Basil leaf showed antibacterial activity against both Gram positive and Gram negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive ones including *Bacillus cereus*, and *Staphylococcus aureus* although Adiguzel *et al* (2005) ^[1] noted that *O. basilicum* EOs have better activity on Gram negative bacteria than on Gram positive bacteria.

From the result of this study using extracts only, it does appear that the type and concentrations of the active ingredients in the leaf may account for the level of sensitivity to *V. cholerae* tested. Based on the standard stipulated by Forbes *et al* (2007) ^[6], the range of ZOI reported all fall within the sensitive values and thus indicates that OBE can be used as antimicrobial remedy for controlling diarrhoeal illnesses in rural communities without access to chemotherapy.

Conclusion

The result of this study showed that leaves of *O. basilicum* (basil) can be used as a first aid remedy for controlling diarrhoeal illness due to drinking water contaminated water by enterotoxigenic bacteria such as *V.cholerae*.

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