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Full Length Research Paper

The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates

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The antimicrobial efficacy of cold and hot water, hexane and methanolic extracts of fresh and dried leaf of *Ocimum gratissimum* against *Salmonella typhimurium, E. coli*, *Yersinia enterocolitica, Bacillus cereus,* and *Aeromonas hydrophila* were determined using the Agar gel diffusion method. The zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Phytochemistry of the extracts were also determined. Results obtained revealed that the cold water extracts of the fresh leaf was most potent, inhibiting all isolates with diameter zones of inhibition ranging from 5 mm to 18 mm, followed by hexane extract of the fresh leaf with zone range of 6mm to 14 mm, but *E. coli* showed no resistance to the hexane extract, methanol extract of the fresh leaf showed no inhibitory effect on all isolates. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs ranging between (12.5 - 150) mg/ml, while MBCs gave a range of (3.13 - 100) mg/ml. Phytochemical analysis of fresh and dried leaf extracts revealed the presence of antimicrobial principles such as resins, tannins, glycosides, alkaloids, flavonoids saponin, anthraquinone, cardiac glycoside, steroidal ring, steroidal terpens and carbohydrates at different concentrations. The findings from this study seem to provide the *in vitro* evidence that might justify *O. gratissimum* as a good candidate medicinal plant for further investigations, and that the active principles of the plant may be more polar in nature.

Key words: Antimicrobial, *Ocimum gratissimum*, bacterial isolates.

INTRODUCTION

The search for agents to cure infectious diseases began long before people were aware of the existence of microbes (Sofowora, 1982) . These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (Sofowora, 1982). Eating the bark of the cinchona tree for example, prevented and frequently cured malaria. Only in modern times was quinine shown to be the active ingredient in the bark. Ancient Egyptians discovered that eating bread over grown with blue-green mould helped persons afflicted with certain diseases (Conway, 1973).

It is estimated that of the about 250,000 - 500,000 species of plants on earth, a relatively small percentage (1 - 10%) of these are used as foods by both humans and other animal species (Borris, 1996). For many years, medicine depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredient is extracted, refined and made ready for consumption while in traditional medicine a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

Traditional medicine is widespread throughout the

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world, and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease, and which may rely exclusively on past experience and observation handed down from generation, verbally or written (Sofowora, 1982). Medicinal plant, has been defined by WHO consultative group as any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Andrews, 1982).

The genus *Ocimum* (Family *Lamiaceae* formerly *Labiatae*) collectively called Basil, consist of about 160 species and is spread over the tropical, sub tropical and parts of the temperate regions of both the hemispheres ranging from sea level to 180 of attitude (Balyan and Pushpanga, 1988). Spice basil, scientifically called *Ocimum* is commonly called, sweet basil. To the Igbos it is called Nchuanwu, Effirin in Yoruba, Basillic in French, Tulsi in Indian and Basilica in Italian (Elujoba, 2000). The plant is extensively cultivated in and around town and villages in Nigeria, tropical regions of Asia, Africa, central and South America (Bailey, 1924). It is commonly used in folk medicine to treat different diseases of upper respiretory tract infections, diarrhea, headache, ophthalmia, skin disease, pneumonia, also as a treatment for cough, fever, and conjunctivitis (Correa, 1932). Recent studies on *Ocimum gratissimum* proved to be a useful medication for people living with Human Immuno deficiency virus (HIV), and Acquired Immune Deficiency virus AIDs (Elu-joba, 2000). Spice or sweet basil is also thought to be an antispasmodic, carminative stimulant and insect repellant.

It is said to have numerous properties, such as the tannins and sweet smelling volatile oil known to have antibacterial agent (Elujoba, 2000). The volatile oil also stops spasm, the hyperactivity of the gastrointestinal tract, by combining with the antibacterial activity and thus lowers the amount of times the muscle of the stomach and gastrointestinal tracts contracts stopping the diarrhea (Elujoba, 2000). The terpene, Camphor in basil and other members of the *Lamiaceae*, have been suggested as agents in allelopathic reactions (Chukwuma, 2004) . Gastroenteritis is an inflammation of the stomach and intestine (Larry and Greenberg, 1996). Important bacteria that may cause gastroenteritis include *Salmonella, Shigella, Vibrios, Campylobacter jejuni, Aeromonas hydrophila, Yersinia enterocolitica, Clostridium perfringens, Bacillus cereus,* and *Escherichia coli* (Janda, 1992; Agbonlahor, et al., 1983).

This study was designed to evaluate the antibacterial efficacy of *O. gratissimum* on bacterial pathogens associated with bacterial gastroenteritis and to determine the active principles in the plant extract, so as to offer informed recommendation on its use for the treatment of diarrhoea especially for those with increasing problem of antibiotic resistance.

MATERIALS AND METHODS

Source of *Ocimum gratissimum*

O. gratissimum is widely planted by most people on nearby farmlands around NVRI Vom, Nigeria as vegetables mostly for stews. A branch of this plant was obtained, confirmation and botanical naming, was done at Federal College of Forestry, Jos Plateau State, Nigeria.

Bacterial isolates

The clinical isolates of *S. typhimurium, A. hydrophila*, *E. coli, B. cereus* and *Y. enterocolitica,* were obtained from the diagnostic and bacteriology laboratory of Federal College of Veterinary and Medical Laboratory Technology Vom, Nigeria. Purity plate of each of the bacterial isolates was obtained by culturing the bacterial isolates on their selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates.

Processing of plant samples

The fresh leaves were harvested and properly washed in tap water (H_2O), and then rinsed in sterile distilled H_2 O. The leaf was divided into two equal parts; one part was dried in the hot air oven at 40°C for 3 days, while the second portion was blended fresh using electric blender. The dried leaf was pulverized using sterile laboratory mortar and pestle to obtain the powered form. These were stored in airtight glass containers protected from sunlight until required for analysis.

Extraction of plant material

Cold and hot extraction with H_2O and soxhlet extractions with methanol (99%) and hexane as described in AOAC (1980) were adopted for the study. 20 g of each sample was weighed into 100 ml of the solvent (water, methanol and hexane). For cold extraction the samples and solvent were stirred every 30 min for 3 h and allowed to stand for 24 h, while for hot extraction the samples and solvent were heated for 30 min and stirred every 30 min for 3 h and allowed to stand 24 h.

After preparation of the crude extract as described, the hexane extracts were diluted using 50% Dimethylsulphoxide (DMSO), while the aqueous and the methanolic extracts were reconstituted using sterile distilled H_2O to obtain concentrations of 200, 150, 100 and 50 mg/ml.

Phytochemical screening

This was done on the different extracts to ascertain the presence of bioactive components present in *O. gratissimum* leaf. The presence of alkaloids, resins, saponins, glycoside, tannins, flavonoids, cardiac glycoside, steroidal ring, steroidal terpenes, anthraquinone and carbohydrates were determined, as described by Trease and Evans (1989), Sofowora (1982), Yen (1971) and Wall et al. (1952).

Preparation of the test bacterial isolates

Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Colonies of Fresh cultures of the different bacterial isolates were picked and suspended in 5 ml nutrient broth in well-labeled sterile bijou bottles. They were incuba-

Table 1. Antibacterial activity of the fresh and dried leaf extracts of *Ocimum gratissimum* on *Aeromonas* and *Bacillus cereus.*

Isolates	Mean zone diameter of inhibition (MM)						Extracts
	200 mg/ml	150 mg/ml	100 mg/ml	50 mg/ml	Distilledwater	Gentamycin	
A.h	12	10	7	5	0	14	CH_2O F
A.h	0	0	0	0	0	14	CH_2O D
A.h	0	0	0	0	0	14	HH_2O F
A.h	0	0	0	0	0	13	HH_2O D
A.h	0	0	0	0	0	10	MET F
A.h	0	0	0	0	0	14	MET D
A.h	10	8	7	6	0	14	HEX F
A.h	0	0	0	0	0	14	HEX D
B.c	14	10	8	7	0	16	CH_2O F
B.c	0	0	0	0	0	16	CH_2O D
B.c	0	0	0	0	0	16	HH_2O F
B.c	0	0	0	0	0	14	HH_2O D
B.c	0	0	0	0	0	15	MET F
B.c	0	0	0	0	0	16	MET D
B.c	14	10	8	6	0	16	HEX F
B.c	0	0	0	0	0	15	HEX D

A.h, - *Aeromonas hydrophila;* B.c - *Bacillus cereus;* CH_2OF - Cold Water (Fresh leaf); HH_2OF - Hot Water (Fresh leaf); METF - Methanol (Fresh leaf), HEXF - Hexane (Fresh leaf); CH_2D - Cold Water Dried leaf); HH_2OD - Hot Water Dried leaf); METD - Methanol (Dried leaf); HEXD - Hexane (Dried leaf).

ted for 24 h at 37ºC, except for *Y. enterocolitica* isolate which was incubated at 28°C. Using ten-fold dilution, 1 ml each of the broth cultures of the isolates were diluted in 9 ml sterile normal saline. For all the bacteria except *Yersinia*, 0.02 ml of 10^{-4} to 10^{-9} dilutions were picked using 0.5 ml syringe and plated out on nutrient agar (NA) to obtain the population density. A 0.02 ml (10^{-1} to 10^{-7}) dilution was done likewise for *Y. enterocolitica*; this is because *Yersinia* is a slow growing organism. After incubation, different numbers of colonies were obtained at different dilutions for different bacteria isolates (Miles and Misra, 1938).

Antimicrobial activity (agar diffusion test)

Semi-solid nutrient agar plates were seeded with 1 ml of the standard inoculum dilution of the test bacterial isolates. The plates were swirled, allowing the inoculum to spread on the surface of the agar, and the excess discarded in a disinfectant Jar. The plates were allowed on the bench for about 20 min to set, and dried in the incubator for 30 min at 37ºC. With the aid of the sterile standard *Cork borer,* 6 wells were bored at equal distance around the plates. The bottoms of the wells were sealed with one drop of the sterile nutrient agar, to prevent diffusion of the extracts under the agar.

The 5^{th} and 6^{th} wells served as positive and negative controls. The negative control well was filled with sterile distilled H_2O, however, for hexane extracts, DMSO, served as the negative control. Gentamycin was used as the positive control. 0.2 ml of each prepared concentration of the extracts was aseptically introduced into Wells, 1-4. The plates were allowed on the bench for 40 min, for pre-diffusion and then incubated at 37ºC overnight. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the bacterial isolates in question at that particular concentration (Abayomi, 1982).

Minimum inhibitory concentration (MIC)

The MIC of the potent extracts was determined according to the macro broth dilution technique (Baron and Finegold, 1990; Tilton and Howard, 1987). Standardized suspensions of the test organisms were inoculated into a series of sterile tubes of nutrient broth containing two- fold dilutions of leaf extracts, and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Minimum bactericidal concentration (MBC)

MBCs were determined by first selecting tubes that showed no growth during MIC determination, a loopful from each tube was subcultured onto extract free agar plates, incubated for further 24 h at 37°C. The least concentration, at which no growth was observed, was noted as the MBC.

Mode of action of the extracts

All plates showing no visible growth on the NA indicated bactericidal effect of the concentration of the extracts used. Plates showing light growth indicated the bacteriostatic effect of the extract concentration. Concentrations of the extracts showing moderate and heavy growth were considered to have no inhibitory effect on the organism (Puyveld, 1986).

RESULTS

The antimicrobial properties of the different extracts of the fresh and dried leaf of the plant on the test isolates were revealed. *A. hydrophila* was inhibited by both cold H_20 and hexane extract of the fresh leaf with zones inhibitory ranged from 5–12 mm and 6–10 mm, respect-tively. Similarly, *B. cereus* was inhibited by same extra-cts; cold H_2O fresh leaf, 7–14 mm and hexane fresh leaf, 6–14 mm (Table 1). *E. coli* was susceptible to all the extracts used except cold H_2O and hexane extracts of dried leaf as well as methanol extract of fresh leaf. *S. typhimurium* was susceptible to cold H_2O and hexane extract of the fresh leaf with zones ranging from 5–10 mm and 6 – 12 mm, respectively (Table 2). *Y. enterocolitica*

Table 2. Antibacterial activity of the fresh and dried leaf extracts of *Ocimum gratissimum* on *E.* Coli and *Salmonella typhimurium.*

Isolates	Mean zone diameter of inhibition (MM)						EXTRACTS
E.c	18	15	11	7	0	18	CH_2O F
E.c	0	0	0	0	0	18	CH_2O D
E.c	10	8	6	0	0	18	HH_2O F
E.c	10	8	6	0	0	18	HH_2O D
E.c	0	0	0	0	0	18	MET F
E.c	14	10	8	6	0	18	MET D
E.c	0	0	0	0	0	18	HEX F
E.c	0	0	0	0	0	18	HEX D
STM	10	8	7	5	0	16	CH_2O F
STM	0	0	0	0	0	16	CH_2O D
STM	0	0	0	0	0	15	HH_2O F
STM	0	0	0	0	0	16	HH_2O D
STM	0	0	0	0	0	16	MET F
STM	0	0	0	0	0	16	MET D
STM	12	10	8	6	0	16	HEX F
STM	0	0	0	0	0	16	HEX D
Concentration of the Extracts mg/ml	**200**	**150**	**100**	**50**	**-C**	**+C**	

E.c - *Escherichia coli,* STM - *Salmonella typhimurium,* -C - Distill water and 50% DMSO, +C – Gentamycin, CH_2OF - Cold Water (Fresh leaf), HH_2OF - Hot Water (Fresh leaf); METF - Methanol (Fresh leaf); HXF - Hexane (Fresh leaf); CH_2D - Cold Water Dried leaf); HH_2OD- Hot Water Dried leaf); METD - Methanol (Dried leaf); HEXD - Hexane (Dried leaf).

Table 3. Antibacterial activity of the fresh and dried leaf extracts of *Ocimum gratissimum* on *yersinia enterocolitica.*

Isolates	Mean zone diameter of inhibition (MM)						EXTRACTS
Y.e	14	10	8	7	0	16	CH_2O F
Y.e	0	0	0	0	0	16	CH_2O D
Y.e	0	0	0	0	0	16	HH_2O F
Y.e	0	0	0	0	0	16	HH_2O D
Y.e	0	0	0	0	0	16	MET F
Y.e	0	0	0	0	0	16	MET D
Y.e	12	10	9	7	0	16	HEX F
Y.e	0	0	0	0	0	16	HEX D
Concentration of the Extracts mg/ml	**200**	**150**	**100**	**50**	**-C**	**+C**	

Controls
Negative: For Aqueous and methanolic extracts was Sterile D/W; Negative: For hexane extract was 50% DMSO Diethyl sulphoxide; Positive: For all extracts was Gentamycin; Y.e -*Yersinia enterocolitica;* -C - Distill water and 50% DMSO; +c – Gentamycin; CH_2O F - Cold H_2O Fresh; CH_2O D - Cold H_2O dried; HH_2O F - Hot H_2O fresh; HH_2O D - Hot H_2O dried; MET F - Methanol Fresh; MET D - Methanol Dried; HEX F - Hexane Fresh; HEX D - Hexane Dried.

showed similar trend of susceptibility to both cold H_2O and hexane extract of the fresh leaf; 7–14 mm and 7–12 mm, respectively (Table 3). MIC results, indicated that cold H_2O extract of fresh leaf appeared more potent with 12.5, 25, 50, 50 and 100 (mg/ml) against *E. coli, B. cereus, A. hydrophila, Y. enterocolitica* and *S. typhimurium*, respectively. This was followed by hexane extract with 25, 50, 50, 100 and 100 (mg/ml) against *B. cereus, E. coli, S. typhimurium, A. hydrophila,* and *Y. enterocolitica* respectively. Methanolic extract of the fresh dried leaf had 100 mg/ml on *E. coli* only. Hot H_2O extract of the fresh leaf appeared least potent with 150 mg/ml on *E. coli* (Figure 1). However, the cold H_2O fresh extract gave MBC of 3.13, 12.5, 25, 25 and 25 mg/ml for *E. coli, B. cereus, A. hydrophila*, *S. typhimurium* and *Y. enterocolitica*, respectively. While the hexane fresh leaf extract gave MBC of 12.5, 25, 25, 50 and 50 mg/ml for *E. coli*, *B. cereus, Y. enterocolitica A. hydrophila* and *S. typhimurium*, respectively (Table 4).

The phytochemical screening showed that the different

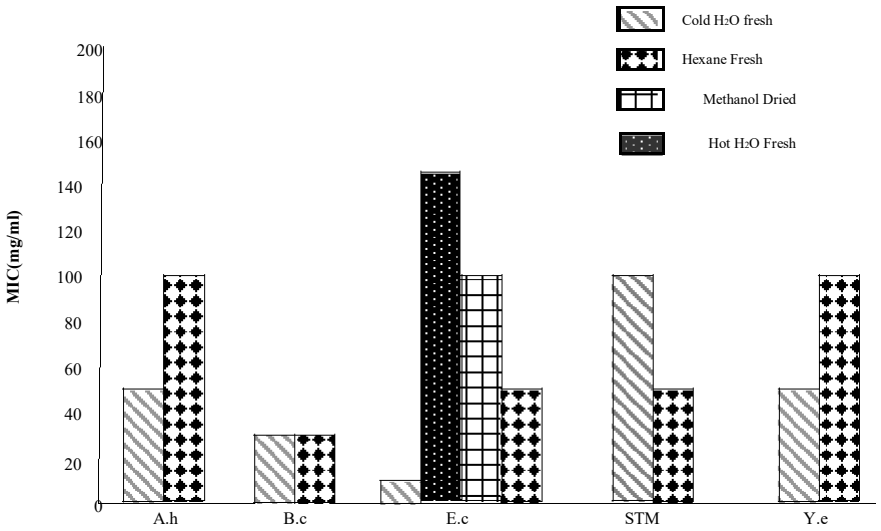

Figure 1. Minimum inhibitory concentrations of leaf extracts of *Ocimum gratissimum.*

Table 4. Results of minimum bactericidal concentrations (MBC) of leaf extracts of *Ocimum gratissimum.*

Isolates	Concentrations of extracts (mg/ml)												
	200	150	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	EXTRACTS	MBC
A.h	-	-	-	-	-	+	+	+	+	++	++	CH_2O F	25
A.h	-	-	-	-	+	+	+	+	++	++	++	Hex F	50
B.c	-	-	-	-	-	+	+	+	++	++	++	CH_2O F	12.5
B.c	-	-	-	-	+	+	+	+	++	++	++	Hex F	25
E.c	-	-	-	-	-	-	-	-	+	+	++	CH_2O F	3.13
E.c	-	-	-	-	-	-	+	+	+	++	++	Hex F	12.5
E.c	-	-	-	+	+	+	++	++	++	++	++	HH_2O F	100
E.c	-	-	-	-	+	+	+	++	++	++	++	MET F	50
STM	-	-	-	-	-	+	+	++	++	++	++	CH_2O F	25
STM	-	-	-	-	+	+	+	++	++	++	++	Hex F	50
Y.e	-	-	-	-	-	+	+	+	++	++	++	CH_2O F	25
Y.e	-	-	-	-	-	+	+	+	++	++	++	Hex F	25

No growth
+ Growth; ++Heavy growth; CH_2O F - Cold H_2O Fresh; CH_2O D - Cold H_2O dried; HH_2O F - Hot H_2O fresh; HH_2O D - Hot H_2O dried; MET F - Methanol Fresh; MET D - Methanol Dried; HEX F - Hexane Fresh; HEX D - Hexane Dried.

extracts of *O. gratissimum* contain alkaloids, tannin, resins, saponin, glycosides, flavonoids, cardiac glycoside, steroidal ring, steroidal terpenes and carbohydrates at different concentrations. However, some phytochemicals were absent in some extracts (Table 5).

DISCUSSION

The results obtained in this study revealed the antimicrobial efficacy of cold water extract of the fresh leaf *O. gratissimum* on all the test isolates. Its highest inhibitory activity, suggests that the active component of this plant may be a highly polar compound. This is similar to the findings of Ijeh et al. (2005), but in contrast to the report of Obi and Onuoha (2000), who reported alcohol to be the best solvent for the extraction of most plant active principles of medical importance. From this study alcohol could not have been the best plant solvent, since the entire test isolates were completely resistant to methanol fresh extracts, except for *E. coli* that was particularly susceptible to methanol dried extracts with zones of inhibitory ranged from 6-14 mm. The susceptibility of *E. coli* to extracts of fresh and dried leaf, confirms the antimicrobial activity reported by (Sofowora, 1982) using both fresh and dried leaf of the plant, but the none inhibitory activity of the dried leaf extract, suggest that active principle of the plant may be heat labile, and likely lost during drying. It is not unusual to observed antimicrobial activity of plant extract to have been contributed by

Table 5. Phytochemical analysis of leaf extracts of *Ocimum gratissimum.*

EXTRACTS COMPOUNDS	CH_2OF	HH_2OF	METF	HEXF	CH_2D	HH $_2$OD	METD	HEXD
	PRESENT							
Alkaloids	++	+	+	++	+	-	+	+
Tannins	++	+	+	++	+	-	-	+
Resins	++	+	+	++	+	-	+	+
Saponin	++	+	+	+	+	-	+	+
Glycosides	++	-	+	++	-	-	-	-
Flavonoids	++	+	+	++	+	+	+	+
Anthraquinone	+	-	-	+	-	-	-	-
Cardiac Glycoside	+	-	-	+	-	-	-	-
Steroidal ring	+	-	+	+	+	-	-	+
Steroidal Terpens	++	+	+	++	+	+	+	+
Carbohydrates	++	++	++	++	++	++	++	++

+ Trace
++ Present in appreciable quantity Absent
CH_2OF = Cold Water (Fresh leaf)
- HH_2OF = Hot Water (Fresh leaf)
- METF = Methanol (Fresh leaf)
- HEXF = Hexane (Fresh leaf)
- CH_2D= Cold Water Dried leaf)
. HH_2OD = Hot Water Dried leaf)
. METD = Methanol (Dried leaf)
. HEXD = Hexane (Dried leaf)

solvents of extraction, but in this study solvents used to reconstitute extracts were observed not to possess any antibacterial effect.

The minimum inhibitory concentrations observed for cold water and hexane extracts of the fresh leaf ranged from 12.5–100 mg/ml while minimum bactericidal concentration (MBC) gave a range of 3.13–50 mg/ml. The variation in results imply that the MBC results obtained after plating on various dilutions of extracts is more reliable compared to MIC results obtained usually using turbidity as an index.

The observed antimicrobial effects of *O. gratissimum* leaf on the bacterial isolates used, though *in vitro* appear interesting and promising. This implies that the plant extracts may indeed be effective in management of gastroenteritis, supporting its ethnomedicinal use; thus the plant may be presented as potential source of novel antimicrobial drugs.

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