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Antibacterial Potentials of Selected Synthetic Antibiotics and Clove Basil, *Ocimum gratissimum* against some Bacteria of the Skin and Guts of *Clarias garienpinus* Juveniles

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Abstract

The antibacterial activity of some selected antibiotics and *Ocimum gratissimum* aqueous leaf extract against some bacteria isolated from *Clarias garienpinus* juveniles obtained from selected farms at Ijebu-Ode was investigated. *Escherichia coli, Staphylococcus saprophyticus, Streptococcus* spp., *Aeromonas hydrophilia, Pseudomonas aeruginosa, Bacillus mycoides, Pseudomonas fluoresces, Proteus vulgaris, Klebsiella oxytoca* and *Micrococcus* spp. were isolated from the skin and gut of 50 *C. gariepinus* samples (6.30g/fish), using standard microbiological methods. The total bacteria count (X 10^6CFU/mL) of skin (2.46±1.31) and gut (2.16±2.29) were obtained from the samples. Result of sensitivities of identified bacteria varied significantly ($p<0.05$) to the synthetic antibiotics and aqueous extract of *O. gratissimum*. All the synthetic antibiotics tested showed greater inhibition zones (IZ) around the identified pathogen than the aqueous extract of the test plant. Streptomycine had the greatest IZ (mm) on all the bacteria (*S. saprophyticus,* 17.50±0.71; *E. coli,* 22.50±0.71; *P. fluorescence,* 20.50±0.71; *P. mirabilis,* 19.00±1.41; *B. subtilis,* 25.00±1.41; *Streptococcus* spp., 32.00±0.00; *A. hydrophilia,* 18.50 ±0.71; *P. aeruginosa,* 22.50±0.71; *K. oxytoca,* 17.50±0.71 and *Micrococcus* spp., 17.00±0.00). However, the aqueous extract had antibacterial potentials (CLSI Standard = IZ ≥ 14mm) in the control of *P. mirabilis* (IZ, 14.50±0.71mm) and *Micrococcus* spp (IZ, 17.50±0.71mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous extract on *P. mirabilis* were 50% both, while on *Microccocus* spp., it was 50% (MIC) and 75% (MBC). The antibacterial efficacies of *O. gratisssimum* extract against *P. mirabilis* and *Micrococcus* spp are a pointer to its potential use in the formulation of new and more potent antibacterial drugs of natural origin against *C. gariepinus* juveniles pathogens.

Keywords: Synthetic Antibiotics, *Ocimum gratissimum*, Bacteria, *Clarias gariepinus.*

Introduction

Fish contain about 60% protein, and it is a very good source of protein which is cheaper compared to other livestock products. As the world population grows, there is need for more production, hence this results in the establishment of aquaculture at a minimum cost for the production of enough fish to meet up with the demand.

Clarias garienpinus is one of the most common cultured fish in Nigeria and indeed Africa, and third in the world. Nigeria is the highest producer of this Clariid catfish in the world (Williams *et al*., 2007).

Parasites and diseases, which are caused by the

presence of pathogenic microbial flora, reduce fish production by affecting the normal physiology of fish which if left uncurtailed, can result in mass mortalities of fish, or in some cases, infection of man and other vertebrates that consume them (Shawn, 1997). The gut flora consists of the microorganisms that normally live in the digestive tract of animals (O'Hara and Shanahan, 2006). Bacteria, including the non pathogenic and pathogenic, are usually present in small numbers in most fish and in normal situation seldom cause any problem as the fish possess adequate immune system capable of fending off infections (Shawn, 1997).

The microorganisms present in the gut and the skin perform a host of functions such as fermenting unused energy substrates, preventing growth of harmful species (Sears, 2005), regulating the development of the gut and producing vitamins for the host. However, in certain conditions, some species are thought to be capable of causing disease by causing infection to the host (Beaugerie and Petit, 2004).

Ocimum gratissimum (Plate 1) occurs from sea-level up to 1500m altitude in coastal scrub, along lake shores, in savanna vegetation, in sub-montane forest, and disturbed land. *Ocimum gratissimum* also known as "alfavaca" is an aromatic medicinal plant belonging to the family Lamiaceae. The plant has pleasant smell which gives it the common name scent leaf plant. *Ocimum gratissimum* is rich in alkaloid, tannins, phytates, flavonoids, oligosaccharides and has tolerable cyanogenic content (Ijeh, *et. al*., 2004). It is an important herbal medicine found in the tropical and warm regions such as India and sub-Sahara Africa especially in Kenya and Nigeria (Aguiyi *et al*., 2000). In Nigeria, *O. gratissimum is* called "Efinrin" in Yoruba; "Nchoanwu" or "Ahuji" in Igbo; "Aramogbo" in Edo and "Daidoya" in Hausa (Effraim *et al*., 2003). It is naturally used in the treatment of different diseases and infections such as upper respiratory tract infections, diarrhoea, conjunctivitis, skin disease and pneumonia (Ilori *et al*., 1996). This plant contains ocimum oil which is active against both Gram positive bacteria (*Staphylococcus aureus, Listeria monocytogenes*) and Gram negative bacteria (*Escherichia coli, Shigella* spp., *Salmonella* spp. *and Proteus* spp.). The extracts of *Ocimum gratissimum* and *Acalypha* spp. have been reported to have antimicrobial properties (Owolade and Osikanlu, 1999).

Antifungal activities of ocimum oil on *Trichophyton rubrum, T. mentagrophytes, Cryptococcus neoformans, Penicillium* spp. and *Candida albicans* have also been reported (Akinyemi *et al*., 2004; Lopez *et al*., 2005). *O. gratissimum* has proved to be useful in the medication for people living with Human Immuno Deficiency Virus (HIV) and Acquired Immuno Deficiency Syndrome (AIDS) virus (Elujoba, 2000).

This study was designed in order to provide information on the effect of aqueous extract of *Ocimum gratissimum* on the bacteria extracted from the skin and gut of *Clarias garienpinus* fingerlings.

Plate 1: *Ocimum gratissimum* leaves
(Field survey, 2016)

Materials and Methods

Sampling Locations and Sample Collection: Samples of fish were collected from some selected fish farms in Ijebu-Ode, Ogun State, Southwest Nigeria. The city is located 6º49N 3º55E. A number of 50 *C. garienpinus* juveniles (6.30 g/fish) were collected randomly from five selected farms in sterilized container and then taken to the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta Ogun State, for further analysis.

Preparation of Fish Samples and Aqueous Plant Extract: Each of the fish sample was dissected using scalpel to remove the fish gut. The skin of each sample was also peeled off and was placed inside glasswares which has been sterilized in an oven at 160 ºC for 90 minutes.

Fifty grammes of shade-dried powdered leaves of *Ocimum gratissimum* was soaked in (50ml) of water for three days. At the end of the extraction, the extracts were filtered using No.1 Whatman's filter paper. The filtrate was concentrated in vacuum at 30 ºC. After complete evaporation, the extract was weighed and preserved aseptically at 4ºC. A concentration of 25mg/l of the extract was aseptically prepared using distilled water and then subjected to antibacterial activity assay.

Bacteriological Analysis: Bacterial estimation, culture and isolation from gut and skin of fish samples were done according to the method of Miles and Mistra as described by Hedges (2002).

Total Bacteria Counts in Fish Specimen: Each of 1g of the gut and skin sample was aseptically and thoroughly mixed or homogenized with 9 ml sterile distilled water. One ml of these diluents water was then serially diluted in sterile tubes containing 9 ml of sterile water to make $1/10^2$, $1/10^3$, $1/10^4$, $1/10^5$, $1/10^6$, and $1/10^7$ respectively (WHO, 1989). One mililitre from $1/10^6$ dilution was mixed with warm prepared Nutrient agar, gently mixed and allowed to solidify. The plates were incubated at 37°C for 24 hours. Colonies of bacteria found were counted and estimated accordingly.

Culture and Isolation of Bacteria: Isolates obtained from the nutrient were sub-cultured on Nutrient agar and McConkey agar and was incubated at 37°C for 24 hours to observe the growth. Isolated colonies were further sub-cultured to obtain pure isolate.

Colonial Examination and Microscopic Examination of Isolates: The colonial and morphology characteristics such as size, shape, colour, consistency, elevation and edges were examined according to Cowan and Steel (1993). Microscopic examination was performed by gram staining to examine the cell morphology.

Biochemical Identification

Biochemical Identification of Pure Isolates: Standard biochemical tests were performed to further characterize the bacteria isolates according to Cowan and Steel (1993). These include sugar fermentation tests, oxidase, catalase, urease, indole, citrate utilization and methyl-red tests.

Phytochemical Analysis of *Ocimmum gratissimum:* The phytochemical components of the powdered *O. gratissimum* leaves were analysed according to the methods described by Trease and Evans (1985). Biochemical compounds analysed for include Saponins, Tannins, Phlobatanins, Cardiac glycosides, Anthraquinones, Flavonoids, Terpenoids, Deoxy-sugar and Alkaloids.

In-Vitro* Antimicrobial Susceptibility Test of *Ocimmum gratissimum

Pure bacterial isolates of 0.5McFarland were spread on Mueller-Hinton Agar and allowed to dry at room temperature. Concentration of 25.0 mg/ml of the prepared *O. gratissimum* extract and synthetic drugs were tested against the bacteria isolates by the Kirby Bauer disc diffusion method on Mueller Hinton agar according to Bauer *et al* (1996). Sterile discs which have been impregnated with the *O. gratissimum* extract and antibiotic discs (Streptomycine, 5mg/ml; Cefuroxime, 10mg/ml; Amoxicillin, 10mg/ml; Cotrimoxazole, 5mg/ml) were placed at about 20mm distance from each other on the inoculated agar and incubated at 37°C for 18 to 24 hours. The inhibition zones were measured using graduated metre ruler to determine the diameter of the inhibition zones and interpreted as sensitive and resistant, according to the Clinical and Laboratory Standard Institute guidelines (CLSI, 2007).

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of Plants Extracts

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in test tubes according to macro broth dilution techniques (Akinyemi *et al*., 2005). To 0.5 ml of varying concentrations of the extracts and drugs (mg/ml) in test tubes, 2 ml of nutrient broth was added and then a loopful of the moxifloxacin resistant *E. coli*. The culture tubes were then incubated at 37^0C for 24 hrs. After incubation the tubes were then examined for microbial growth by observing for turbidity. The MIC was read as the least concentration that inhibited the growth of the test organism. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile nutrient agar by streaking. Nutrient agar plates were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37^oC for 24 hrs. After incubation, the concentration that yielded no visible growth was considered as the Minimum Bactericidal Concentration (MBC) (Akinjogunla *et al*., 2009).

Results

Bacterial Enumerated and Identified in the Fish Samples

Specimens of skin and gut analysed for the total bacterial counts (TBC) of isolates showed that the mean TBC (CFU/mL) of skin ranged from 1.0 to 5.2 is 2.46±1.31 and gut is 2.16±2.29 (range, 0.6 to 8.5) (Table 1).

Table 1: Total Bacteria Count (CFU/mL) of Isolates from fish and Water Samples

Sample	Mean ($X10^6$)	Std. Deviation
Skin	2.46	1.31
Gut	2.16	2.29

CFU = Colony forming unit

Table 2: Bacteria species Identified from Fish Samples

SN	LABEL	A	B	C	D	E	F	G	H	I	J
1	1-SKIN	+	+	+	-	-	+	-	+	-	-
2	2-GUT	+	+	+	+	+	+	-	-	+	-
3	3-GUT	+	+	+	+	-	-	+	+	-	-
4	5-GUT	+	-	-	+	+	-	+	-	+	+
5	5-SKIN	+	+	-	+	-	+	-	+	-	-
6	7-GUT	+	-	-	+	+	-	+	-	+	-
7	6-GUT	-	+	-	-	+	-	+	-	+	-
8	6-SKIN	+	-	+	-	+	-	+	-	+	-
9	7-SKIN	+	+	-	+	-	+	-	+	-	-
10	4-GUT	+	+	+	-	+	-	+	-	+	-
11	9-GUT	+	-	+	-	+	-	-	+	-	-
12	10-SKIN	+	+	+	+	+	-	+	-	+	-
13	8-SKIN	+	+	+	-	+	+	-	+	+	-
14	4-SKIN	+	+	+	-	+	+	+	+	-	-
15	2-SKIN	+	+	+	+	-	+	-	+	+	+
16	1-GUT	+	+	+	+	-	+	+	+	+	-
17	10GUT	+	+	+	+	+	+	+	+	+	+
18	9-SKIN	+	+	+	+	-	-	+	-	+	-
19	8-GUT	+	+	-	+	-	+	+	-	+	-
20	3-SKIN	+	+	+	+	+	+	+	+	+	+
21	8-SKIN	+	+	+	+	+	+	+	+	+	-

Keys: *A= Escherichia coli, B= Staphylococcus saprophyticus, C= Streptococcus species, D= Aeromonas hydrophilia, E= Pseudomonas aeruginosa, F= Bacillus mycoides, G= Pseudomonas fluoresces, H= Proteus vulgaris, I = Klebsiella oxytoca, J= Micrococcus species,* + = PRESENT, = ABSENT

Analysis of bacteria present in the experimental samples showed that a number of ten different bacterial species were identified (Table 2). The result showed that *Escherichia coli, Staphylococcus saprophyticus, Streptococcus species, Aeromonas hydrophilia, Pseudomonas aeruginosa, Bacillus mycoides, Pseudomonas fluoresces, Proteus vulgaris, Klebsiella oxytoca,* and *Micrococcus species* were present in both skin and gut specimen of sampled fish.

Phytochemical Constituents of *Ocimum gratissimum* Leaf Extract

The phytochemical characteristic of *O. gratissimum* leaf is summarized in Table 3 below. The result revealed the presence of medicinally active constituent in the plant studied. Saponins, Cardiac glycoside, Alkaloids, Tannins, Phlobatanins, Anthraquinones, Flavonoids and Terpenoids were present while Deoxy-sugar was not detected.

Table 3: Qualitative Phytochemical Constituents of *Ocimum gratissimum Leaf Extract*

Phytochemical Constituents	*Ocimum gratissimum*
Saponnins	+++
Tannins	+
Phlobatanins	+
Cardiac glycoside	++
Anthraquinones	+
Flavonoids	+
Terpenoids	+
Deoxy-sugar	-
Alkaloids	++

Keys: - = Not detected; + = Detected/present

Sensitivities of Identified Bacteria to Synthetic Antibiotics and Aqueous Extract of *Ocimum gratissimum*

Result of sensitivities of identified bacteria varied significantly ($p<0.05$) to the synthetic antibiotics and aqueous extract of *Ocimum gratissimum* (AQEx) (Table 4). Inhibition zones ranged from 3.00±0.00mm (AQEx) to 32.00±0.00mm (Streptomycin). Most of the organisms except *E. coli* and *A. hydrphilia* were highly susceptible to most of the synthetic drugs as they have inhibition zone ≥ 14mm (CLSI standard) around them. *Proteus mirabilis* and *Micrococcus* species were highly susceptible to the aqueous extract of the test plant (inhibition zone > 14 mm), while others were resistant to it (inhibition zone less than 14 mm).

Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of Synthetic Antibiotics and Aqueous Extract of *Ocimum gratissimum* on Identified Bacteria

Results of MIC and MBC of synthetic antibiotics and aqueous extract of *Ocimum gratissimum* were presented in Tables 5 and 6 respectively. The results showed that the activities of all antibiotics tested were more effective than the aqueous extract. Streptomycine was most active on all the bacteria than others as its MICs and MBCs were least. MICs (%) of synthetic drugs ranged from 2 to 64 (Table 5), while their MBCs (%) ranged from. 1 to 32 (Table 6). The highest MICs and MBCs ranged from 50 to >100 (Tables 5 and 6) and were produced by the aqueous extract of *Ocimum gratissimum.*

Table 4: Sensitivities of Identified Bacteria to Synthetic Antibiotics and Aqueous Extract of *Ocimum gratissimum*

	Inhibition Diameter, mm (Mean±SD)									
Antibiotics	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
COT	30.50±0.71^{a}	4.50±0.71^{d}	18.50±0.71^{bc}	9.50±0.71^{e}	27.50±0.71^{a}	26.50±0.71^{b}	12.50 ±0.71^{c}	15.50±0.71^{b}	21.50±0.71^{a}	19.50±0.71^{a}
AMX	13.50±0.71^{d}	18.50±0.71^{b}	19.50±0.71^{ab}	22.50±0.71^{b}	24.50±0.71^{bc}	26.50±0.71^{b}	22.00 ±0.00^{a}	15.50±0.71^{b}	18.50±0.71^{b}	14.50±0.71^{c}
STP	17.50±0.71^{e}	22.50±0.71^{a}	20.50±0.71^{a}	19.00±1.41^{c}	25.00±1.41^{b}	32.00±0.00^{a}	18.50 ±0.71^{b}	22.50±0.71^{a}	17.50±0.71^{b}	17.00±0.00^{b}
CEF	22.50±0.71^{b}	12.50±0.71^{c}	17.50±0.71^{c}	29.50±0.71^{a}	23.00±0.00^{c}	27.50±0.71^{b}	10.50 ±0.71^{c}	3.50±0.71^{d}	8.50±0.71^{c}	10.50±0.71^{d}
AQEx	5.50±0.71^{e}	5.50±0.71^{d}	3.00±0.00^{d}	14.50±0.71^{d}	9.00±1.41^{d}	7.50±0.71^{5}	9.00 ±0.00^{d}	5.50±0.71^{c}	4.00±1.41^{d}	17.50±0.71^{b}

Means of different superscripts are significantly different ($p<0.05$) along the column
A = *Staphylococcus saprophyticus,* B = *Echerichia coli,* C = *Pseudomonas fluorescence* D = *Proteus mirabilis,* E = *Bacillus subtilis,* F = *Streptococcus* species, G = *Aeromonas hydrophilia,* H= *Pseudomonas aeruginosa,* I = *Klebsiella oxytoca,* J = *Micrococcus* species, COT = Cotrimoxazole, AMP = Amoxicilin, STP = Streptomycine, CEF = Cefuroxime, AQEx = Aqueous Extract of *Ocimum gratissimum*

Table 5: Minimum Inhibitory Concentrations (MICs) of Synthetic Antibiotics and Aqueous Extract of *Ocimum gratissimum on* Identified Bacteria

	MIC (%)									
Antibiotics	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
COT	4	16	32	64	8	8	64	64	8	8
AMX	32	32	64	8	4	2	2	8	8	32
STP	32	16	16	16	16	4	2	4	16	32
CEF	4	16	64	16	2	4	16	32	64	64
AQEx	>100	>100	>100	75	>100	>100	>100	>100	>100	50

A = *Staphylococcus saprophyticus,* B = *Echerichia coli,* C = *Pseudomonas fluorescence,* D = *Proteus mirabilis,* E = *Bacillus subtilis,* F = *Streptococcus* species, G = *Aeromonas hydrophilia,* H= *Pseudomonas aeruginosa,* I = *Klebsiella oxytoca,* J = *Micrococcus* species, COT = Cotrimoxazole, AMP = Amoxicilin, STP = Streptomycine, CEF = Cefuroxime, AQEx = Aqueous Extract of *Ocimum gratissimum.*

Table 6: Minimum Bactericidal Concentrations (MBCs) of Synthetic Antibiotics and Aqueous Extract of *Ocimum gratissimum* on Identified Bacteria

	MBC (%)									
Antibiotics	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
COT	1	8	4	4	4	4	32	16	2	2
AMX	16	16	32	4	2	2	1	4	4	16
STP	16	8	8	8	8	1	1	1	4	16
CEF	2	8	16	8	1	1	4	16	16	32
AQEx	>100	>100	>100	50	>100	>100	>100	>100	>100	75

A = *Staphylococcus saprophyticus,* B = *Echerichia coli,* C = *Pseudomonas fluorescence,* D = *Proteus mirabilis,* E = *Bacillus subtilis,* F = *Streptococcus* species, G = *Aeromonas hydrophilia,* H= *Pseudomonas aeruginosa,* I = *Klebsiella oxytoca,* J = *Micrococcus* species, COT = Cotrimoxazole, AMP = Amoxicilin, STP = Streptomycine, CEF = Cefuroxime, AQEx = Aqueous Extract of *Ocimum gratissimum*

Discussion

Analysis of bacteria present in the experimental samples showed that a number of ten different bacterial species were identified. The result showed that *Escherichia coli, Staphylococcus saprophyticus, Streptococcus species, Aeromonas hydrophilia, Pseudomonas aeruginosa, Bacillus mycoides, Pseudomonas fluoresces, Proteus vulgaris, Klebsiella oxytoca,* and *Micrococcus species* were present in both skin and gut specimen of sampled fish.

The phytochemical characteristic of *O. gratissimum* revealed the presence of medicinally active constituent in the plant studied. It was observed that Saponins, Cardiac glycoside, Alkaloids, Tannins, Phlobatanins, Anthraquinones, Flavonoids and Terpenoids were present in the test plant while Deoxy-sugar was not detected. It has been reported that the leaves and stems of some plants are rich in alkaloids, flavonoids, tannins and saponins (Obi and Onuoha, 2000). Moreover, the qualitative screening results confirmed the possible use of the test plant as a source of antimicrobial agent against pathogenic diseases of fish (Ogundare, 2011).

Result of sensitivities of identified bacteria varied significantly ($p<0.05$) to the synthetic antibiotics and aqueous extract of *Ocimum gratissimum*. Streptomycine had the highest activity as it inhibited growth of all the organisms. The least inhibition was produced by aqueous extract of the test plant. All the drugs tested showed greater antibacterial potentials in the control of most of the identified pathogen than the aqueous extract of the test plant. This was in line with the study of Okwu (2003), where greater antibacterial efficacy of synthetic antibiotics than the plants tested were observed. Results of MIC and MBC of antibiotics and aqueous extract of *Ocimum gratissimum* also showed that all the synthetic activities used were more effective than the aqueous extract. This was supported by Sharif *et al*, (2015) who observed similar trend in their study. However, the aqueous extract had an antibacterial potential in the control of *Proteus mirabilis* and *Micrococcus* spp as it had inhibition zones greater than 14 mm on these organisms when compared to the standard (CLSI, 2007). In other words, *Proteus mirabilis* and *Micrococcus* spp were susceptible to the test plant. The antibacterial potential of the test plant could be as a result of the bio-active constituents present in it.

Conclusion and Recommendation

The result obtained from the phytochemical screening of the leaf extracts of *Ocimum gratissimum* revealed the presence of Saponins, Cardiac glycoside, Alkaloids, Tannins, Phlobatanins, Anthraquinones, Flavonoids and Terpenoids, while Deoxy-sugar was not detected. However, further study on quantitative phytochemical analysis of these constituents can be carried out in order to ascertain their concentrations in the test plant. Aqueous extract of *O. gratissimum* exhibited some levels of biological activity against fish pathogens especially *Proteus mirabilis* and *Micrococcus* spp. The sensitivity of these fish microbes to the plant extract is a pointer to its potential antibacterial use in fish hatcheries. Further studies can be done using higher concentration or dosage of the extract to test for bacterial activity because the higher dosage will give room for wider activity.

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