Microbiology Research Journal International

30(4): 56-67, 2020; Article no.MRJI.56626
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

The Isolation of Antimicrobial Resistant *Aeromonas* spp. from Aquaculture Samples and their Susceptibility to Medicinal Plant Extracts

C. I. Chikwendu[1*], R. K. Obi[1], K. C. Ibe[1] and J. C. Orji[1]

[1]Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors CIC and JCO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CIC and KCI managed the analyses of the study. Authors CIC and RKO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i430213
Editor(s):
(1) Laleh Naraghi, Iranian Research Institute of Plant Protection, Iran.
Reviewers:
(1) Brian Kipng'etich, University of Eldoret, Kenya.
(2) Igiebor Francis Aibuedefe, Wellspring University, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56626

Original Research Article

Received 02 March 2020
Accepted 07 May 2020
Published 02 June 2020

ABSTRACT

Aeromonas spp, ubiquitous in both terrestrial and aquatic environments are becoming renowned as enteric pathogens of serious public health concern as they have a number of virulence and resistant determinants that are linked to both human and aquatic diseases due to consistent and incorrect use of antibiotics in aquaculture. The effect of crude aqueous and ethanol extracts of some medicinal plants on antimicrobial resistant *Aeromonas* spp. isolated from aquaculture water and fish samples was studied. Two hundred and forty (240) *Aeromonas* isolates, made up of 168 *Aeromonas hydrophila* and 72 *Aeromonas salmonicida,* were recovered from aquaculture water and fish gill samples collected from different commercial fish ponds using selective media. The isolates were assessed for their antibiotic susceptibility against ten (10) conventional antibiotics using the Kirby-Bauer technique. Extracts from three medicinal plants, *Vernonia amygdalina*, *Ocimum gratissimum* and *Garcinia kola* were also analyzed for their antimicrobial effects on the isolates that were resistant to the conventional antibiotics. *Aeromonas hydrophila* isolates expressed the highest resistant rates of 100%, 78.6% and 70.8% to aztreonam, cefotaxime and

**Corresponding author: E-mail: chinwe.chikwendu@futo.edu.ng, chinwechikwendu@gmail.com;*

neomycin respectively, and the *A. salmonicida* isolates also had a similar trend of high resistant rates of 100%, 87.5% and 77.8% to aztreonam, neomycin and cefotaxime respectively. Antimicrobial resistant analyses with the plant extracts showed 100% inhibition of the isolates at 100 mg/ml for both aqueous and ethanol extracts. Phytochemical screening identified the presence of certain phytochemicals like alkaloids, saponins, tannins, flavonoids, steroids and glycosides which could have accounted for the antimicrobial effects of the plant extracts under study. It can be inferred then, that extracts from *Vernonia amygdalina*, *Ocimum gratissimum* and *Garcinia kola* can inhibit resistant aquaculture *Aeromonas* isolates and so can present an alternative source of antimicrobials in the effort to combat the increasing incidence of antimicrobial resistance in aquaculture.

Keywords: Aeromonas hydrophila; Aeromonas salmonicida; furunculosis; Garcinia kola; Ocimum gratissimum; Vernonia amygdalina.

1. INTRODUCTION

Aeromonas is a known ubiquitous genus, having been isolated from many different sources like aquatic environments [1], even meat products [2], chicken [3] and chicken waste samples [4]. They are important pathogens of both cold and warm-blooded animals [5]. They are known to be Gram negative, oxidase positive, facultative anaerobes and catalase positive. Due to their ubiquitous nature, humans are easily colonized and become infected with pathogenic species of *Aeromonas*. According to Odeyemi and Ahmad [6], infections are however determined by strain type and virulence factors. Some of these virulence factors include: amylase, haemolysin, aerolysin, lipase, protease and DNase [7,8].

Apart from bacteremia, respiratory tract infections, gastroenteritis, urinary tract infections and diarrhea [3,9,10] which *Aeromonas* spp. have been associated with, they have also been linked to both water and food-borne infections in different parts of the world. This is especially so in the developing countries because of poor personal hygiene and lack of quality water [6].

Aeromonas salmonicida is common in fish farms worldwide [11], where it causes furunculosis in fish [12]. Antibiotics are used widely for the treatment of furunculosis and this has led to an increasing number of antibiotic resistant isolates. Diverse antibiotic resistant genes conferring resistance to important antibiotics, borne on plasmids have been found on *A. salmonicida,* making it an important reservoir of drug resistance genes that should be more extensively monitored [11].

Due to the misuse of antibiotics, antimicrobial resistance has increasingly become a world-wide problem. This has in effect created a large pool of bacteria that have developed resistance to many antibiotics and has led to failure of most efforts to combat bacterial infections. There is also the added risk of possibility of transfers of antimicrobial resistance markers particularly plasmids between unrelated species. A number of mobile genetic elements, including plasmids and transposons have been found in association with both clinical and environmental *Aeromonas* isolates [13,14,15,16]. *Aeromonas* species have also been shown to possess integrons [15,16, 17], which are capable of antibiotic resistance gene acquisition and/or loss [18]. These antibiotic resistant species include some species of *Aeromonas* in particular clinical and environmental isolates. There has indeed been a large incidence of antimicrobial resistance in *Aeromonas* isolates recovered from heavily polluted waters [19,20].

Antimicrobial resistance is currently said to be one of the major threats to global health owing to factors such as climatic change, globalization as seen in increased travel and food importation and demographic changes have made the situation even worse [21,22]. There is therefore, a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanisms of action for new and re-emerging diseases and also for combating antimicrobial resistant pathogens. According to Ali et al. [23], plant materials are currently the sources of fifty percent (50%) of western drugs, hence medicinal plants can be used as alternative therapy if not first line therapy for the treatment of pathogenic infections. Medicinal plants have also been long exploited for their potential therapeutic purposes since time immemorial [24] and have long been reported to be very useful in healing various diseases. One of the main advantages of these medicinal plants is that they are 100% natural [25].

Different types of herbs have both antibacterial and antifungal activities which can be harnessed to control various diseases. However, very little effort has been made towards the treatment of fish diseases with herbs [26]. This study was therefore carried out to study the efficacy of aqueous and ethanol extracts of *Vernonia amygdalina*, *Ocimum gratissimum* and *Garcinia kola* against antimicrobial resistant *Aeromonas* spp. isolated from aquaculture water and fish samples.

2. MATERIALS AND METHODS

Selective media for *Aeromonas* isolation was used for primary isolation, while furunculosis agar for selective isolation/confirmation of *Aeromonas salmonicida* was used in the secondary isolation. Furunculosis agar, an artificial medium has the following composition: Casein enzyme hydrolysate (10 g/L), Yeast extract (5 g/l), tyrosine (1 g/l), Sodium chloride (2.5 g/l), Agar Agar (15 g/l). On *Aeromonas* Ryan agar, suspect colonies of *A. hydrophila* are green in colour with dark centers, while *Aeromonas salmonicida* appears brown in colour.

2.1 Sampling Areas

Aquaculture water samples were collected from twenty different fish ponds located in Owerri Municipal Council and Mbaitoli Local Government Areas in Imo State, Nigeria. Fish gill samples were also collected from the fishes in the sampled fish ponds.

Plant samples were purchased from the Relief Market in Owerri, Imo State, and identification confirmed at the Crop Science Technology Department of Federal University of Technology, Owerri, Imo Sate, Nigeria.

2.2 Sample Types

The aquaculture samples used in the study included surface and sediment water samples and swab samples from the fish gills of fishes in the pond. Plant samples tested were *Vernonia amygdalina* (bitter leaf), *Ocimum gratissimum* (scent leaf) and *Garcinia kola (*bitter kola*).*

2.3 Sample Collection

Surface and sediment water samples were collected from the fish ponds with sterile plastic containers. After collecting the surface water, the pond was stirred and sediment water collected from the discharge units of the ponds. Swab samples were also collected from fish in the pond, by swabbing the fish gills with sterile swab sticks. Samples were taken to the laboratory in ice chambers and analyzed within 12 hours of sample collection.

Two hundred and fifty (250 g) of *Vernonia amygdalina*, *Ocimum gratissimum*, and *Garcinia kola* each, were purchased as leafy vegetables for *Vernonia amygdalina* and *Ocimum gratissimum* and seeds for *Garcinia kola* from the local market.

Ten antibiotics were analyzed for their efficacy against the *Aeromonas* isolates. These were imipenem (10µg); ciprofloxacin (5µg); ceftazidime (30µg); cefotaxime (30µg); nalidixic acid (10µg), neomycin (30µg); enrofloxacine (5µg); aztreonam (30µg); sulphamethoxazole (23.75µg) and tetracycline (30µg).

2.4 Sample Preparation

Water samples: Two (2) drops of each water sample was enriched in 2 ml sterile alkaline peptone water and incubated at room temperature (28±2°C) for 24hrs. After incubation, a loop-full from each enrichment broth was inoculated onto prepared selective media plates of *Aeromonas* Ryan Agar by the spread plate technique and incubated at room temperature (28±2°C**)** for 48 hrs.

Fish gill swab samples: Swab samples were also enriched for 24 hrs at room temperature using sterile alkaline peptone water. A loop-full from each enrichment tube was also inoculated on the surface of *Aeromonas* Ryan Agar, and incubated at room temperature for 48 hrs.

After 48 hrs incubation, suspect colonies of *Aeromonas salmonicida* with characteristic brown colonies were sub-cultured on furunculosis agar plates for confirmation while suspected *A. hydrophila* isolates were cultured on fresh *Aeromonas* Ryan agar for purification and pure cultures.

Antibiotic susceptibility tests: The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility tests. A loop-full of each of the standardized isolates was inoculated on Mueller Hinton agar plates and test antibiotics were placed on the agar surfaces. These plates were subsequently incubated for 24 hrs at 35°C.

Thereafter, the zones of inhibition around the discs were measured and interpreted according to the recommendation of the National Committee for Clinical Laboratory Standards (CLSI) [27]. The multiple antibiotic resistance (MAR) index was determined for each isolate using the formula 'a/b', where 'a' is the number of antibiotics to which the isolate is resistant to while 'b' is the number of antibiotics tested for each isolate [28].

Preparation of plant samples: Test plant samples were thoroughly cleaned and kept to dry under shade for two weeks. These dried plants were then crushed into powder. Two hundred (200 g) of each ground sample was then used to prepare crude aqueous and ethanol extracts The crude aqueous extracts (CAE) were prepared by soaking each sample in 500mls of distilled hot water for 3 days while the ethanol extraction was done by soaking the same amount of dried plant samples in ethanol for 4 days while shaking constantly. The CAE was filtered with Whatmann No1 filter paper after centrifuging at 1500X for 20 mins and stored in dark bottles in the refrigerator at 4°C as CAE for later use.

The ethanol extracts were concentrated by placing in a water bath at 37°C to allow the solvents to evaporate.

2.5 Determination of Phytochemical Constituents of the Plant Extracts

The extracts were subjected to various standard phytochemical analyses to identify the chemical constituents present like tannins, alkaloids, flavonoids, saponins, steroids, plobatamins, cynogenic and cardiac glycosides as described by Amadi et al. [29].

2.6 Determination of Minimum Inhibitory and Bactericidal Concentration of Extracts

The minimum inhibitory concentration (MIC) was carried out using the broth dilution method as described by Dalitha [30] and the micro-dilution susceptibility tests as described by Das et al. [31]. One milliliter (1ml) of peptone water broth was dispensed into test tubes and sterilized by autoclaving at 121°C, 15psi for 15 mins. The various plant extracts were serially diluted from their stock solutions to obtain varying concentrations, of 100, 50 and 25 mg/ml. Thereafter, 0.1 ml of each test isolate was inoculated into the various test tubes containing varying concentrations and then, incubated in triplicate at 28±2°C for 48 hrs. After incubation, the presence or absence of growth on each tube was detected by the presence or otherwise of turbidity of the medium. The MIC was taken as the least concentration in the tube that didn't show visible bacterial growth.

The MBC was also determined by the micro-dilution susceptibility tests described by Das et al. [31]. Aliquots of 10µl of the cell suspensions from the MIC plates were used to inoculate fresh *Aeromonas* growth media and then incubated for 48 hrs at 28±2°C. The MBC was taken as the lowest concentration that hindered the growth of the isolates in a fresh growth medium.

3. RESULTS AND DISCUSSION

A total of two hundred and forty (240) *Aeromonas* spp. were isolated from aquaculture water and fish gill swab samples. One hundred and sixty-eight (168) of these isolates were *Aeromonas hydrophila* while seventy two (72) were *Aeromonas salmonicida*. *A. hydrophilia* was therefore more dominant at 70% than *A. salmonicida* at 30% (Table 1).

Out of the 168 isolates of *Aeromonas hydrophila* assessed for antimicrobial resistance, high resistant rates were observed for aztreonam, cefotaxime and neomycin with resistant rates of 100%, 78.6% and 70.8% respectively. While moderate rates of 63.1%, 58.9%, 56.6%, 53.6% and 50% were observed for nalidixic acid, enrofloxacine, sulphamethoxazole, ciprofloxacine and tetracycline respectively, the lowest resistant rates of 19.6% and 10.7% were observed for ceftazidime and imipenem respectively (Table 2). For *Aeromonas salmonicida* high resistant rates of 100%, 87.5% and 77.8% were equally observed for aztreonam, neomycin and cefotaxime respectively. Resistant rates were moderate for tetracycline, ciprofloxacin nalidixic acid sulphamethozaxole and enrofloxacine at 33.3% - 63.9%, while resistant rate was low for imipenem (13.9%) and ceftazidine (12.5%) (Table 3). Comparative rates of resistance between the isolates showed that *A. hydrophila* expressed higher resistant rates than *A. salmonicida* for the same antibiotics. Resistant rates for neomycin and enrofloxacine were however higher in *A. salmonicida* (87.5% and 63.9% respectively) than in *A. hydrophila* (70.8% and 58.9%) as shown on Table 4.

Table 1. Distribution (%) of *Aeromonas species* from the different aquaculture sample types

Sampling source (aquaculture)	Test organism or isolate		Total
	***A. hydrophila* (n=168)**	***A. salmonicida* (n=72)**	(n=240)
Surface water	58(34.5)	22(30.6)	80(33.3)
Sediment water	62(36.9)	18(25.0)	80(33.3)
Swab from fish gill	48(28.6)	32(44.4)	80(33.3)
Total	168(100)	72(100)	240(100)

Table 2. Frequency (%) of resistant isolates of *Aeromonas hydrophila* from aquaculture samples

Antibiotics	Sample types / resistant rates (%)			
	Surface water (n=58)	**Sediment water (n=62)**	**Fish gill swab (n=48)**	**Total resistance (n=168)**
Aztreonam	58 (100)	62 (100)	48(100)	168(100)
Cefotaxime	57(98.3)	33(53.2)	42(87.5)	132(78.7)
Neomycin	55(94.8)	38 (61.3)	26 (54.2)	119 (70.8)
Nalidixic acid	40 (69.0)	47 (75.8)	19 (39.6)	106 (63.1)
Enrofloxacine	42 (72.4)	25 (40.3)	32 (66.7)	99 (58.9)
Sulphamethoxazole	47 (81.0)	34 (54.8)	14 (29.2)	95 (56.7)
Ciprofloxacine	37 (63.8)	39 (62.9)	14 (29.2)	90 (53.7)
Tetracycline	36 (62.1)	34 (54.8)	14 (29.2)	84 (50.0)
Ceftazidime	11 (19.0)	12 (19.4)	10 (20.8)	33 (19.6)
Imipenem	9 (15.5)	9 (14.5)	0 (0.00)	18 (10.7)

Table 3. Frequency (%) of antibiotic resistance of *Aeromonas salmonicida* from aquaculture samples

Antibiotics	Sample types / resistant rates (%)			
	Surface water (n=22)	**Sediment water (n=18)**	**Fish gill swab (n=32)**	**Total resistance (n=72)**
Aztreonam	22 (100)	18 (100)	32 (100)	72 (100)
Neomycin	20 (90.9)	16 (88.9)	27 (84.4)	63 (87.5)
Cefotaxime	19 (86.4)	13 (72.2)	24 (75.0)	56 (77.8)
Enrofloxacine	20 (90.9)	10 (55.6)	16 (50.0)	46 (63.9)
Sulphamethoxazole	20 (90.9)	9 (50.0)	12 (37.5)	41 (56.9)
Nalidixic acid	16 (72.7)	16 (88.9)	7 (21.9)	39 (54.2)
Ciprofloxacine	11 (50.0)	10 (55.6)	9 (28.1)	30 (41.7)
Tetracycline	10 (45.5)	9 (50.0)	5 (15.6)	24 (33.3)
Imipenem	3 (13.6)	5 (27.8)	2 (6.3)	10 (13.9)
Ceftazidime	4 (18.2)	2 (11.1)	3 (9.4)	9 (12.5)

Table 4. Comparative rates (%) of antimicrobial resistance between *A. hydrophila* and *A. salmonicida* isolated from aquaculture samples

Antimicrobials	***A. hydrophila* (n=168)**	**A. *salmonicida* (n=72)**
Aztreonam	168 (100)	72 (100)
Cefotaxime	132 (78.6)	56 (77.8)
Neomycin	119 (70.8)	63 (87.5)
Nalidixic acid	106 (63.1)	39 (54.2)
Enrofloxacine	99 (58.9)	46 (63.9)
Sulphomathoxazole	95 (56.6)	41 (56.9)
Ciprofloxacine	90 (53.6)	30 (41.7)
Tetracycline	84 (50.0)	24 (33.3)
Ceftazidime	33 (19.6)	9 (12.5)
Imipenem	18 (10.7)	10 (13.9)

The low resistant rates observed in this study for imipenem is in contrast to that of De Silva et al., [32] who recorded high resistant rates for imipenem among his *Aeromonas* isolates from scallops. While De Silva et al., [32] recorded low rates for ciprofloxacine, also in contrast to this study where resistant rates were moderate at 50%, resistant rates for nalidixic acid at 60% were consistent with his results with resistant rates for nalidixic acid being 65% and 53.6% and 58.9% respectively for ciprofloxacine and enrofloxacine. The results however agree with Hossain et al., [5] who also observed high and low resistant rates of 65.15% and 6.98% for imipenem and ciprofloxacine respectively among his isolates. The most effective antibiotics against the isolates in this study were imipenem and ceftazidime, while the highest resistant rates were among the monobactam, aztreonam at 100%. The major resistant mechanism for most aeromonads is the inducible chromosomal β-lactamases and according to Zhiyong et al. [33], the expression of metallo-β-lactamases acts against carbapenems. Extended spectrum β-lactamases have also been identified amongst aeromonads [34], as well as other resistance genes like tetracycline and plasmid mediated quinolone resistant genes [5].

There was a high level of variability among the *Aeromonas* spp. with *A. hydrophila* isolates exhibiting a total of fifty-nine (59) different resistant patterns, the most predominant pattern being IMP ATM CAZ TE CIP SXT ENR CTX NA N exhibited by 26 isolates. Likewise, the *A. salmonicida* isolates expressed a total of 42 patterns with IMP+ATM+ CAZ+TE+CIP+SXT+ ENR+CTX+NA+N being the most predominant pattern but expressed by 8 isolates (Tables 5 and 6). All the isolates also expressed MAR index of between 0.3 and 1. This implies that the isolates have a high risk of infection being that their MAR indices were greater than 0.2 [28]. All the isolates were observed to be multi-resistant isolates.

Plant extracts were tested for their efficacy against the *Aeromonas* isolates. The Twenty one (21) *Aeromonas hydrophila* isolates tested were inhibited only at 100 mg/ml concentration of the extracts, both for aqueous and alcohol extracts. There was no inhibition at 25 mg/ml and only moderate inhibition at 50 mg/ml concentration. A similar trend was observed for *A. salmonicida* isolates where none of the five isolates tested was inhibited at 25 mg/ml concentration. Also at 100 mg/ml, all the isolates were inhibited by aqueous and ethanol extracts of *Garcinia kola Ocimum gratisinum* and *Vernonia amygdalina.* At 50 mg/ml however, there was only moderate inhibition of the isolates with *G. kola* extracts inhibiting the least number of isolates (Tables 7 and 8). Phytochemical analysis of both the crude aqueous and ethanol extracts showed the presence of the following phytochemicals; alkaloids, tannins, saponins, flavonoids and glycosides in the plants samples (Table 9).

Aeromonas spp. have primarily been found to be sensitive to many medicinal plants like *Olea europa, Myrtus communis, Thymus vulgaris, Rosmariniu sofficinalis* and *Achillea falcats* [35], black pepper, *Glycine max,* nutmeg [36], guava and neem extracts [37]. *Eugenia caryophyllus, Spondias pinnata, Teriminalia chebula, Annona coniosus* and *Citrus senensis* have also inhibited the growth of *Aeromonas* sp. according to Rahman and Hossain [38] and Lawal et al. [39]. Crude extracts of *Vernonia amygdalina* have also been found to be effective against some bacterial pathogens [40].

Emphasis has been given to medicinal plants and efforts are being geared towards identifying compounds which are biologically active from extracts of known medicinal plants [41]. According to Anyanwu and Okoye [42], they have become potential sources of new antimicrobial molecules, thereby creating a renewed interest in antimicrobial of plant origin.

Table 5. Antibiotic resistant patterns of *Aeromonas hydrophila*

S/N	Pattern	No of Isolates	MAR
1	IMP ATM CAZ TE CIP SXT ENR CTX NA N	26	1
2	IMP ATM CAZ TE SXT ENR CTX NA N	11	0.9
3	IMP ATM CAZ TE CIP SXT CTX NA N	7	0.9
4	ATM CAZ TE CIP SXT ENR CTX NA N	7	0.9
5	IMP ATM TE SXT ENR CTX NA N	6	0.8
6	IMP ATM TE CIP SXT ENR CTX NA N	5	0.9
7	IMP ATM CAZ TE CIP SXT ENR CTX N	5	0.9
8	IMP ATM CAZ TE CIP SXT ENR NA N	5	0.9

S/N	Pattern	No of Isolates	MAR
9	IMP ATM CAZ TE CIP ENR CTX NA N	4	0.9
10	IMP ATM CAZ TE CIP ENR SXT CTX	4	0.8
11	IMP ATM CAZ TE SXT CTX NA N	4	0.8
12	ATM CAZ TE SXT ENR CTX NA N	4	0.8
13	IMP ATM CAZ TE SXT ENR NA N	4	0.8
14	ATM CAZ TE SXT CTX N	4	0.6
15	IMP ATM CAZ TE CIP CTX NA N	3	0.8
16	ATM TE N	3	0.3
17	ATM CAZ TE CTX NA N	3	0.6
18	IMP ATM TE ENR CTX CAZ NA N	2	0.8
19	IMP ATM CIP TE ENR CTX NA N	2	0.8
20	ATM TE CIP SXT ENR CTX NA N	2	0.8
21	ATM CAZ TE SXT CTX NA N	2	0.7
22	IMP ATM CAZ TE SXT ENR CTX N	2	0.8
23	IMP ATM TE CIP SXT ENR NA N	2	0.8
24	ATM CAZ TE CIP SXT CTX NA N	2	0.8
25	ATM CAZ TE CIP ENR CTX NA N	2	0.8
26	IMP ATM CAZ TE CIP SXT CTX NA	2	0.8
27	IMP ATM CAZ SXT ENR CTX NA N	2	0.8
28	IMP ATM TE SXT ENR N	2	0.6
29	IMP ATM CAZ TE SXT NA N	2	0.7
30	ATM TE SXT ENR NA N	1	0.6
31	IMP ATM CAZ TE CTX NA N	1	0.7
32	IMP ATM CAZ TE CTX N	1	0.6
33	IMP ATM TE CAZ SXT ENR CTX NA	1	0.8
34	ATM CAZ TE SXT CTX NA	1	0.6
35	IMP ATM TE SXT CTX NA N	1	0.7
36	ATM CAZ CIP SXT ENR CTX NA	1	0.7
37	ATM CIP ENR CTX	1	0.4
38	IMP ATM CAZ TE SXT CTX N	1	0.7
39	ATM CAZ TE ENR NA N	1	0.6
40	IMP ATM CAZ CIP SXT CTX NA	1	0.7
41	ATM CAZ TE CIP ENR NA N	1	0.7
42	ATM CAZ TE CIP SXT NA N	1	0.7
43	IMP ATM CAZ TE CIP ENR NA	1	0.7
44	ATM CAZ TE CIP ENR NA	1	0.6
45	IMP ATM CAZ TE CIP SXT NA N	1	0.8
46	ATM CAZ TE CIP NA N	1	0.6
47	ATM CAZ TE CIP SXT ENR NA N	1	0.8
48	IMP ATM CAZ TE SXT CTX	1	0.6
49	ATM CAZ TE CIP SXT NA N	1	0.7
50	ATM CAZ TE CIP SXT ENR CTX N	1	0.8
51	IMP ATM TE SXT CTX NA N	1	0.7
52	IMP ATM CAZ TE SXT ENR CTX N	1	0.6
53	ATM CAZ TE SXT CTX NA N	1	0.7
54	IMP ATM CAZ TE CTX NA	1	0.6
55	IMP ATM CAZ TE CIP SXT CTX NA N	1	0.9
56	IMP ATM TE CIP ENR N	1	0.6
57	IMP ATM TE N	1	0.4
58	IMP ATM ENR NA N	1	0.5
59	IMP ATM TE CIP ENR NA N	1	0.7

Key: ATM (Aztreonam), CXT (Cefotaxime), N (Neomycin), NA (Nalidixic Acid), ENR (Enrofloxacine), SXT (Sulphamethoxazole), CIP (Ciprofloxacine), TE (Tetracycline), CAZ (Ceftazidime), IMP (Imipenem), MAR; multiple antibiotic resistance index

Table 6. Antibiotic resistant patterns of *Aeromonas salmonicida*

S/N	Pattern	No of Isolates	MAR
1	IMP ATM CAZ TE CIP SXT ENR CTX NA N	8	1
2	IMP ATM TE CIP SXT ENR CTX NA N	6	0.9
3	IMP ATM CAZ TE CIP SXT CTX NA N	5	0.9
4	IMP ATM CAZ TE CIP SXT ENR CTX N	4	0.9
5	ATM CAZ TE CIP SXT ENR CTX NA N	2	0.9
6	IMP ATM TE SXT ENR CTX NA N	2	0.8
7	IMP ATM CAZ TE CIP SXT ENR NA N	2	0.9
8	IMP ATM CAZ TE SXT ENR CTX NA N	2	0.9
9	IMP ATM CAZ TE CIP ENR SXT CTX	2	0.8
10	IMP ATM CAZ TE SXT CTX NA N	2	0.8
11	ATM CAZ TE SXT ENR CTX NA N	2	0.8
12	IMP ATM CAZ TE CTX ENR NA N	2	0.8
13	IMP ATM TE N	2	0.4
14	IMP ATM ENR NA N	2	0.5
15	IMP ATM TE CIP ENR NA N	2	0.7
16	IMP ATM TE CIP SXT ENR NA N	1	0.8
17	ATM TE CIP SXT NA N	1	0.6
18	IMP ATM CAZ TE CTX NA N	1	0.7
19	IMP ATM TE CIP ENR CTX NA N	1	0.8
20	IMP ATM CAZ TE CIP SXT CTX N	1	0.8
21	IMP ATM TE CIP CTX NA N	1	0.7
22	IMP ATM TE CIP SXT ENR N	1	0.7
23	IMP ATM TE SXT CTX NA N	1	0.7
24	ATM CAZ TE CIP ENR CTX NA N	1	0.8
25	IMP ATM CAZ SXT CTX NA N	1	0.7
26	ATM CAZ CIP CTX N	1	0.5
27	ATM CAZ TE CIP SXT NA N	1	0.7
28	ATM CAZ CIP SXT ENR NA N	1	0.7
29	ATM CAZ TE CIP SXT ENR CTX NA	1	0.8
30	ATM CAZ TE SXT ENR	1	0.5
31	ATM TE N	1	0.3
32	ATM CAZ TE CTX NA N	1	0.6
33	IMP ATM TE SXT ENR N	1	0.6
34	IMP ATM CAZ TE SXT ENR NA N	1	0.8
35	IMP ATM TE CIP ENR CTX N	1	0.7
36	ATM CAZ TE SXT CTX N	1	0.6
37	IMP ATM CAZ TE SXT NA N	1	0.7
38	ATM CAZ TE CIP SXT ENR CTX N	1	0.8
39	IMP ATM TE CIP SXT ENR CTX	1	0.7
40	ATM ENR CTX N	1	0.4
41	IMP ATM TE SXT NA N	1	0.6
42	ATM TE SXT NA	1	0.4

Key: ATM (Aztreonam), CXT (Cefotaxime), N (Neomycin), NA (Nalidixic Acid), ENR (Enrofloxacine), SXT (Sulphamethoxazole), CIP (Ciprofloxacine), TE (Tetracycline), CAZ (Ceftazidime), IMP (Imipenem), MAR; multiple antibiotic resistance index

It can be inferred then, that extracts from *Vernonia amygdalina*, *Ocimum gratissimum* and *Garcinia kola* can inhibit resistant aquaculture *Aeromonas* isolates and so can present an alternative source of antimicrobials in the effort to combat the increasing incidence of antimicrobial resistance in aquaculture.

Table 7. Inhibitory activities of the crude plant extracts against *A. hydrophila*

		Aqueous extracts			Ethanol extracts		
Conc. (mg/ml)	**No of isolates**	***O. gratissimum***	***V. amygdalina***	***G. kola***	***O. gratissimum***	***V. amygdalina***	***G. kola***
100	21	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
50	21	5 (23.8)	6 (28.6)	9 (42.9)	5 (23.8)	6 (28.6)	9 (42.9)
50	21	21 (100)	21 (100)	21 (100)	20 (95.2)	20 (95.2)	21 (100)

Table 8. Inhibitory activities of the crude plant extracts against *A. salmonicida*

		Aqueous extracts			Ethanol extracts		
Conc. (mg/ml)	**No. of isolates**	***O. gratissimum***	***V. amygdalina***	***G. kola***	***O. gratissimum***	***V. amygdalina***	***G. kola***
100	5	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
50	5	2(40)	1 (20)	2(40)	1 (20)	1 (20)	3 (60)
25	5	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)

Table 9. Phytochemical constituents of the crude aqueous and ethanol plants extracts

	Plant extracts					
	Ocimum grattisimum		*Vernonia amygdalina*		*Garcinia kola*	
Phytochemicals	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**
Alkaloids	+	-	-	-	+	-
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+
Steroids	-	-	-	-	+	-
Glycosides	+	+	-	+	+	+

Key: - (absent); + (present)

4. CONCLUSION

At the end of the study, high rates of resistance were identified amongst the *Aeromonas* spp isolates from aquaculture. Also, the crude aqueous and ethanolic extracts of the three plants tested were found to be effective against the isolates and so could be good candidates for alternative sources of antimicrobials in aquaculture in view of increasing resistance amongst *Aeromonas* spp in that environment to commercial antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Janda JM, Abbot, SI. The genus *Aeromonas*: Taxonomy, pathogenicity and infection. Clin. Microbiol. Rev. 2010;23:35-73
2. Dallal MS, Yazdi MS, Avadisians. Study of prevalence and antibiotic resistance in *Aeromonas* sp. isolated from minced meat and chicken samples in Iran. Afr. J. Microbiol. Res. 2012;6:460-464.
3. Papadakis V, Poniros N, Kalsibardi K, Cherissiadou AE, Anastasppoulos, J, Polychronopoulou, S. Fulminant *Aeromonas hydrophila* infection during acute lymphoblastic leukemia treatment. J. Microbiol. Immunol. Infect. 2012;45:154-157.
4. Igbinosa IH. Antibiogram profiling and pathogenic status of *Aeromonas* sp. recovered from chicken. Saudi J. Biol. Sci. 2014;21:481-485.
5. Hossain S, Dahanayake PS, Del Silva BCJ, Wickramanayake MVKS, Wimalasen SH MP, Heo GJ. Multi drug resistant *Aeromonas* sp. isolated from Zebra fish (*Daniorerio*): Antibiogram, antimicrobial resistance genes and class I integrin gene cassettes. Lett. Appl. Microbiol. 2019;68: 370-377.
6. Odeyemi OA, Ahmad A, Antibiogram and resistogram profiling of *Aeromonas* sp. isolated from Malaysian coastal sea water. Pollution Res. 2014;33:487-492.
7. Aberoum A, Jovyandeh HA. Review on occurrence and characterization of the *Aeromonas* sp. from marine fishes. World J. Fish Mar. Sed. 2010;2:519-523.
8. Sharma A, Deo AD, Riteshkumar ST, Chanu TI, Das A. Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in Labeo rohita (Hamilton) fingerlings. Fish Shellfish Immunol. 2010; 29:508-512.
9. Hochedez P, Hope-Rapp E, Olive C, Nicolas M, Beaucaire G, Cabblé A. Bacteremia caused by *Aeromonas hydrophila* complex in the Caribbean Islands of Martinique and Guadeloupe. Am. J. Trop. Med. Hyg. 2010;83:1123-1127
10. Igbinosa H, Igumbor EU, Aghdasi F, Tom M, Okoh AI. Emerging Aeromonas sp. infections and their significance in public health. Scient. World J. 2012;2012: 625023-625035.
11. Trudel MV, Vincent AT, Attére SA, Lebbé M, Derome N, Culley AI,Charette SJ. Diversity of antibiotic-resistance gene in Canadian isolates of *Aeromonas salmonicida* Subsp. *Salmonicida*: dominance of pSn254b and discovery of pAsa8. Sci. Rep. 2016;6(35617):1-10.
12. Dallaire-Dufresne S, Tanaka, KH, Trudel MV, Lafaille A, Charette SJ. Virulence, genomic features and plasticity of *Aeromonas salmonicida* Subsp. *Salmonicida*, the causative agent of fish furunculosis. Vet. Microbiol. 2014;169:1-7.

13. Son R, Rusul G, Sahilah AM, Zainuri A, Raha AR, Salmah I. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, telapia (*Telapia mossambica*). Letters Appl. Microbiol. 1997;24:479–482.
14. Rhodes G, Huys G, Swings J, Mcgann P, Hiney M, Smith P, Pickup RW. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant TetA. Appl. Environ. Microbiol. 2000;66:3883–3890.
15. Schmidt AS, Bruun MS, Dalsgaard I, Larsen JL, Characterization of class 1 integrons associated with R-plasmids in clinical *Aeromonas salmonicida* isolates from various geographical areas. J. Antimicrob. Chemother. 2001a;47:735–743.
16. Schmidt AS, Bruun MS Dalsgaard I, Larsen JL. Incidence, distribution and spread of tetracycline resistance determinants and integron associated antibiotic resistance genes among motile aeromonads from a fish farming environment. Appl. Environ. Microbiol. 2001b;67:5675–5682.
17. Sørum H, L'Abee-Lund TM, Solberg A, Wold A. Integron-containing IncU R plasmids pRAS1 and pAr-32 from the fish pathogen *Aeromonas salmonicida*. Antimicrob. Agents Chemother. 2003;47: 1285–1290.
18. Fluit AC, Schmitz FJ. Class 1 integrons: Gene cassettes, and epidemiology. Europ. J. Clin. Microbiol. Infect. Dis. 1999;18:761–770.
19. Huddleston JR, Zak JC, Jeter RM. Antimicrobial susceptibilities of *Aeromonas* sp. isolated from environmental sources. Appl. Environ. Microbiol. 2006;72:7036-7042
20. Aravera-Román M, Inglis TJJ, Henderson, B, Riley, TV, Chang, BJ. Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. Antimicrob. Agents Chemother. 2012;56: 1110-1112.
21. Cheng G, Dai M, Ahmed S, Hao H, Wang X, Yuan Z. Antimicrobial drugs in fighting against antimicrobial resistance. Front. Microbiol. 2016;7:470-480.
22. Miranda CD, Tello A, Keen PL. Mechanism of antimicrobial resistance in fin fish aquaculture environments. Front Microbiol. 2013;4:233-238.
23. Ali SM, Ravikumar S, Saravanan V, Anuradha V, Valliammal N. Antibacterial activity of medicinal herbs plants against urinary tract infection pathogens. Asian J. Exp. Biol. Sc. 2013;4(3):388-392.
24. Yusuf L, Oladunmoye MK, Akinyosoye FA, Hassan G, Momoh AO. Comparative antibacterial studies of mistletoes growing on two different host plants in Akure North, Nigeria. Intl. J. Med. Med. Sci. 2013;3(5): 009-011.
25. Orhue PO, Momoh AR, Igumbor EO, Esumeh FI. Antibacterial effect of Azadirachta indica (CN: Neem or Dongo Yaro) parts on some urinary tract bacterial isolates. Asian J. Plant Sci. Res. 2014; 4(2):64-67.
26. Raham, MM, Hossain, MN. Antibiotic and herbal sensitivity of some *Aeromonas* sp. isolates collected from diseased carp fishes. Progress Agric. 2010;21(1 and 2): 117-129.
27. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility testing, twentieth Informational Supplement, CLSI Document M100-S20, Wayne, PA: Clinical laboratory Standards Institute. M100-S20. 2011;31(1):42-48.
28. Krumperman PH. Multiple antibiotic resistance indexing of *E. coli* to identify high risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 1983;46: 165-170.
29. Amadi BA, Agomuo EN, Ibegbulam CO. Phytochemical tests: Research Methods in Biochemistry. Supreme Publi. Owerri. 2004;89-95.
30. Dalitha MK. Manual on the Antimicrobial Susceptibility testing of the Indian Association of Medical Microbiologists. 2008;10-21.
31. Das A, Jaman K, Singh AV. Antimicrobial and antioxidant activities of *Callistemon linearis* DC leaf extract. Pharmacology. 2008;3:875-881.
32. De Silva BCJ, Hossain S, Dahanayaki PS, Heo GJ. *Aeromonas* sp. from marketed Yesso Scallop (*Patino pectenyessoensis*): molecular characterization, phylogenetic analysis, virulence properties and antimicrobial susceptibility. J. Appl. Microbiol. 2018;126:288-299.
33. Zhiyong X, Xiaoju L, Yaniju G. *Aeromonas hydrophila* infection: Clinical aspects and

therapeutic options. Rev. Med. Microbiol. 2002;13:151-162.

34. Chikwendu CI, Egbadon EO, Amadi ES, Ibe SN, Okpokwasili GC. Beta-lactamase Genes in Multi-resistant *Aeromonas* spp. Isolated from River and Aquaculture Water Sources in Nigeria. Nig. J. Microbiol. 2017; 31(2):3970-3978.
35. Al Laham SA, Al Fadel FM. Antibacterial activity of various plant extracts against antibiotic resistant *Aeromonas hydrophila*. Jundishapur J. Microbiol. 2014;7(7): e11370.
36. Dorman HTD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 2000; 88(2):308-316.
37. Malfuzul HMD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S. Antibacterial activity of guava (*Psidium guayava*) and neem (*Azadira chtaindica* A. Juss) extracts against food borne pathogens and spoilage bacteria. Food Borne Pathog. Dis. 2007;4:481-488.
38. Rahman MM, Hossain MN. Antibiotic and herbal sensitivity of some *Aeromonas* sp. isolates collected from diseased carp fishes. Progress. Agric. 2010;21(1 & 2); 117-129.
39. Lawal D, Yunusa I, Bala I. A study of the phytochemical properties and synergistic antibacterial activity of *Annona comosis* (LINN) MERR PEL and *Citrus senensis* peel extracts on *Aeromonas hydrophila* and *Salmonella* sp. Bayero J. Pure Appl. Sci. 2013;6(1):40-45.
40. Habtom S, Gebrehiwot S. *In-Vitro* antimicrobial activities of crude extracts of *Vernonia amygdalina* and *Croton macrostachyus* against some bacterial and fungal test pathogens. J. Phytopharmacol. 2019;8(2):57-62.
41. Steep R. The role of weeds as sources of pharmaceuticals. J. Ethnopharmacol. 2004;92:163-166.
42. Anyanwu MU, Okoye RC. Antimicrobial activities of Nigerian Medicinal plants. J. Intercultural Ethnopharmacology. 2017; 6(2):240-259.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56626