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In Vivo antimicrobial activities of *Allium cepa* on cultured adult *Clarias gariepinus* (Burchell, 1822)

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Abstract
The design of the study was to evaluate the antimicrobial activity of Allium cepa in cultured Clarias gariepinus. The proximate compositions and Mineral assay of whole A. cepa bulb and experimental diets were determined using standard methods. Microbial susceptibility assay was carried out in vitro on Staphloccocus aureus and Escherichia coli using Agar well diffusion. Bacterial isolation and identification from the different treatments and control was carried out according to standard methods. High moisture content (89.25 %) was recorded followed by carbohydrate (9.45%) and crude protein (7.21%). Experimental and control diets revealed 50 % and above crude protein content. In vitro susceptibility test of the various onion extracts and antibiotic shows susceptibility of the reference strains to the onion extracts. Bacterial isolation and identification showed the presence of pathogenic gram positive and negative coli and bacilli respectively. Results obtained for total fungi counts show the presence of both systemic and superficial fungi. Lower bacteria and fungi counts were observed 14 days after the withdrawal of experimental diets. Conclusively, onion can be used as antimicrobial agent in the culture of Clarias gariepinus.

Keywords: *Allium cepa; Antimicrobial; Bacteria; Clarias gariepinus; Fungi; Proximate composition*

Introduction

Food and Agricultural Organization of United Nations report gave the statistics of the annual production of onions (*Allium cepa* L.*)* at around 66 million tonnes and that it is second most important horticultural crop worldwide, after tomatoes (Benítez *et al.*, 2011). The use of the plant cuts across culinary and medicinal sphere and has been cultivated for over 5 000 years. In the last decade, the world production of onions increased with 25 % reaching 45 million tons. Increased research carried out on the onion plant can be attributed to its health benefit (Griffiths *et al.*, 2002). The bulb contains various biologically active compounds which include compounds of sulphur, seleno and flavonoids; however, it is not still clear their biochemical mechanism of action (Bonaccorsi *et al.*, 2008). Anthocianins and quercetins which give the onion skin its varying colours (red, yellow and brown respectively) belong to flavonoids (Benítez *et al.*, 2011). Onion compounds possess anticarcinogenic, antidiabetic, antithrombotic, antiasthmatic, antioxidant, antimicrobial and anti-aging properties (Benmalek *et al.*, 2013).The World Health Organization (WHO) (Corzo-Martínez and Villamiel, 2012) report describes hypoglycemic and platelet anti-aggregation effects of onion bulbs *in vivo*.

Recently, over 50% of studies conducted on the incorporation of medicinal plants and natural herbs into fish feed to determine their effect on growth performance, nutrient utilization and immunostimulant activity have shown huge success (Adegbesan *et al.,* 2017; Adegbesan *et al.,* 2018; Awad and Awaad, 2017). The majority of plants that have been used include marjoram (El-Dakar *et al.,* 2004), licorice roots (Shalaby *et al*., 2003), black seeds and roquette seed (Abd –Elmonem *et al*., 2002), basil leaves (El-Dakar *et al*., 2008), caraway seed (El-Dakar *et al.,* 2004), fennel seed (El-Dakar *et al.,* 2004), fenugreek seeds Shalaby, 2004), onion and walnut (Bello *et al*., 2014) etc. The study of Bello *et al.* (2014) revealed that differences in formation and function of blood cells from the African catfish fed diets containing onion and walnut can also be an indication of dietary manipulations.

Experiments have been carried out using natural herbs, medicinal and aromatic plants for therapeutic purpose; however, few studies has been documented on the use of these plants in fish feeding both on the experimental and commercial scales. Also, there is paucity of information on the use of the onion bulb as feed supplements in fish. The study design was therefore aimed at investigating the *in vivo* antimicrobial activity of *A. cepa* on cultured *Clarias gariepinus* (sharp tooth African catfish) sub-adult fed diet containing different concentration of fresh *A. cepa.*

Materials and methods

Experimental fish collection and acclimation

One hundred and eighty adults *Clarias gariepinus*, 12-14 weeks old, of average weight 421g purchased from a reputable commercial fish farm within the study area and transported in aquarium to the experimental site. Fish samples were allowed to acclimatize for seven (7) days and fed three times daily with commercial fish feed. Water quality parameters such as dissolved oxygen, pH and temperature of the culture medium were also maintained at recommended range using water quality test kit HANNA Multiparameter Water Quality Meter (HI98194)

Experimental design

Completely Randomized Design (CRD) was adopted for the study, in which the experimental fish were kept without food for 24 hours prior to the study commencement followed by, random placement of experimental fish samples into 50l plastic containers at a ratio of 10:1 fish per container for seven (7) days acclimatization. Experimental fish were grouped into two categories: A and B, treated and control groups. Fish in group A were fed with onion diet while group B served as negative control without any exposure to the onion bulb. Group A consist of five (5) treatments based on inclusion concentration of the onion bulb slurry.

Collection, preparation and extraction of *Allium cepa* bulb

Fresh *Allium cepa* bulbs weighing 1kg were peeled and blended and percolated in 1.3 l of methanol, acetone and n-hexanee. The mixture was then shaken thoroughly on an electronic flask shaker (SM/DR-10, Singifield Medicals, England) for 15 Hours. Filtration was with Whattman no 1 filter paper. Rotary evaporator was used to separate solvents from the extracts which were then concentrated at $40^{o}C$ and kept for further use.

Proximate composition of *A. cepa* and experimental diets

Whole *A. cepa* bulb and diets were analysed for the Moisture content (MC) and Fat content as described by AOAC (2005) while crude protein (CP) content was by the macro kjeldahl method and Total ash (Kirk *et al*., 1991). The crude fiber (CF) was then determined according to the procedure of

(AOAC, 2005). Nitrogen free extract was then calculated from results on proximate composition

Determination of the mineral profile of *Allium cepa*

Ashing of *A. cepa* bulb sample was done at 550^{O}C and boiled in 10ml of 20% Hydrochloric acid. filtered into a 100ml standard flask and made up to the mark with deionized water. The minerals content was profiled for Sodium (Na) and potassium using the standard flame emission photometer (Shalaby, 2004). Phosphorus (P) was determined colorimetrically using the spectronic 20 (Gallenkamp, UK) (Kirk and Sawyer, 1991)) and Calcium (Ca), Magnesium (Mg) and Iron (Fe) were then determined using Atomic Absorption Spectrophotometer (AAS model SP9).

Fish feed formulation and processing

Six different experimental diets containing 200g, 150g, 100g, 50g and 25g of fresh onion bulb and the control / standard diet without onion bulb inclusion were formulated and compounded containing 50% crude protein.

In vitro* antimicrobial susceptibility activities of *Allium cepa

In vitro antimicrobial susceptibility testing of *Escherichia coli* (ATCC 25922) and *Staphlococcus aureus* (ATCC 33591) organisms to different *A. cepa* extracts was determined using the agar well diffusion method. Briefly, each wells of 6 mm diameter bored into the agar after spreading the bacteria inoculums over the agar surface was filled with different concentration of 1000mg, 750mg and 500mg of the plant extracts and 10µg/ml of streptomycin as standard. Incubation of Petri dishes was at 37^{o}C for 24 hours. Inhibition of bacterial growth was measured in millimetre using meter rule.

In vivo* assessment of the antimicrobial activities of *Allium cepa* fed *Clarias gariepinus

Changes in total bacteria count on fish sample organs after introduction of*A. cepa* extract was carried out according to method of Miles and Mistra described by Hedges (2002) and isolated bacteria were characterized according to Cowan and Steel (1993). Fungal strains obtained on Potato Dextrose Agar (PDA) (Oxoid, UK) supplemented with Streptomycin (100 mg/ml) were identified by distribution and arrangement of chlamydospore, sporangiospore, hypha, globose, conidiophore and spore in Lactophenol-cotton blue stain protocol according to Chessbrough (1998). Fungi strains were evaluated on malt extract agar (MA) as previously described (Hedges, 2002).

Data analysis

Significance of the bacteria load and fungi evaluation in *A. cepa* fed fishes was determined using ANOVA. Separation of treatment means was by Duncan Multiple Range Test at $P<0.05$.

Results and Discussion

Proximate and mineral profile of onion bulb

High moisture content (89.25 %) was recorded followed by carbohydrate (NFE) (9.45%) and crude protein content (7.21%) while low fat and ash were observed. Significant level of iron, copper, and sodium were estimated (Figure 1).

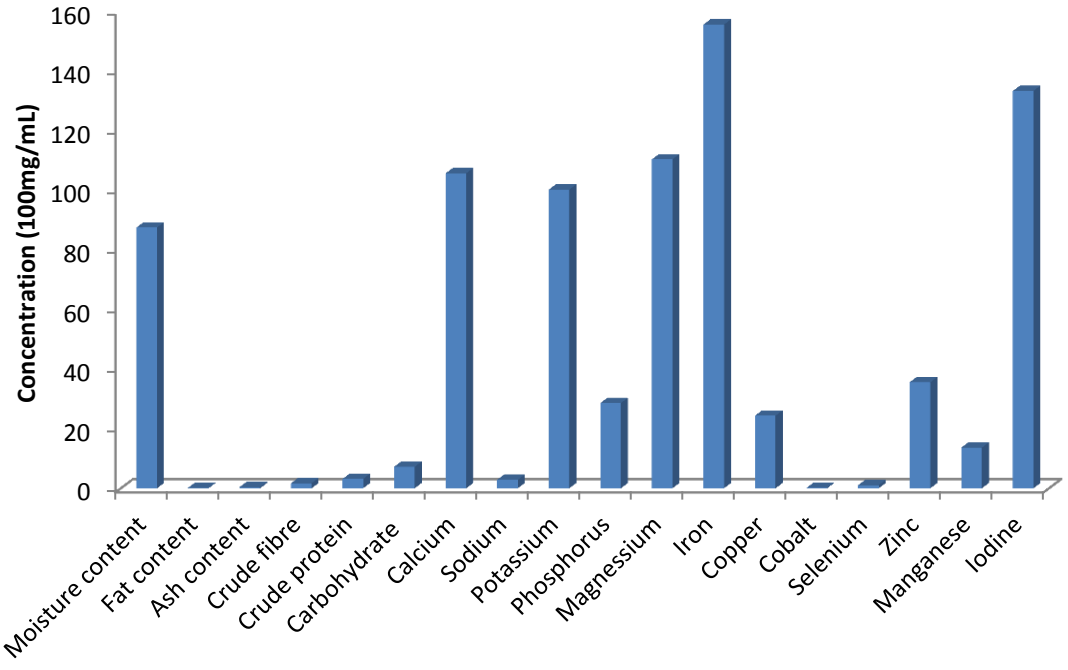

Figure 1: Proximate composition and mineral profile of the onion bulb.
Key: y- axis showing the concentrations of the measured parameters

The proximate composition of the onion bulb and the different diets showed high moisture content in onion bulb followed by, carbohydrate (NFE) and crude protein with less content of fat and crude protein. Finding corroborates the result documented by Ugwoke and Ezugwe (2010) on the proximate composition of the onion bulb. High amounts of carbohydrates (NFE) in the bulb could be of significant effect to health as carbohydrates are used in various biochemical reactions. Bhattacharjee *et al.* (2013) also reported high carbohydrate levels than protein in two varieties of the onion bulb from different origin. The high level of the carbohydrate (crude fibre and NFE) in the onion bulb had been document to be a determinant of the biological activity of the bulb as it provides a substrates aromatic amino acids and phenolic compounds production via the Shikimic acid pathway.

Proximate and gross composition (%) of experimental diets

Proximate and gross composition of experimental and control feeds are as shown in Table 1. The crude protein content of the various experimental feeds were 50 % and above while the carbohydrate content was in the range of 16.5 and 18.9. Percentage composition of the diets is shown in Table 1.

Table 1: Proximate and gross composition (g/100 g) of the experimental feeds

Parameters	Diets					
	1	2	3	4	5	6
Moisture content	8.3±0.06	5.8±0.03	7.5±0.04	6.0±0.088	7.4±0.021	8.2±0.023
Ether extract	5.3±0.03	6.2±0.02	5.6±0.02	6.0±0.06	5.8±0.02	5.5±0.02
Ash	11.5±0.03	13.7±.05	11.3±.06	13.1±.05	12.3±0.01	11.8±0.04
Crude fiber	3.6±0.07	4.7±0.02	3.9±0.07	4.4±0.06	4.1±0.03	3.9±0.1
Crude Protein	55.3±0.06	50.7±0.02	54.8±0.11	52.0±0.10	53.6±0.02	54.4±0.27
Carbohydrate	16.8±0.67	18.9±0.11	16.6±0.31	18.5±0.19	16.5±0.22	16.5±0.07
Fish meal	23	23	23	23	23	23
Soybean meal	45	45	45	45	45	45
Maize	30	30	30	30	30	30
Fish premix	0.5	0.5	0.5	0.5	0.5	0.5
DCP	1	1	1	1	1	1
Toxin binder	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1	0.1
Onion	20	15	10	5	2.5	0

Key:1 = 200g/kg onion inclusion diet, 2 = 150g/kg onion inclusion diet, 3 = 100g/kg onion inclusion diet, 4 = 50g/kg onion inclusion diet, 5 = 25g/kg onion inclusion diet, 6 = control diet with 0g/kg of A. cepa inclusion. SBM= Soya Bean Meal, DCP= Dicalcium Phospahte.

Microbiology

***In vitro* antimicrobial susceptibility screening**

In vitro susceptibility test of the various onion extracts and antibiotics reveals that the onion bulb has antibiotic activity (Table 2). Highest inhibition by the extracts was exhibited in the methanolic extracts for both organisms (12mm and 14mm) while acetone extract exhibited lowest inhibition (8.3mm) for *E. coli*. The extracts of onion bulb showed higher activity against gram positive test organism than the gram negative organisms.

Table 2: Sensitivity of the organisms to the extracts and control

Organisms	FOBM	FOBA	FOBH	Streptomycin
E. coli (mm)	12.0	8.3	10.0	24.0
S. aureus (mm)	14.0	13.7	9.3	32.0

Key: FOBM fresh onion bulb methanolic extracts, FOBA fresh onion bulb acetone extracts, FOBH fresh onion bulb n-hexane extracts. Zone of inhibition is given in millimeter.

The susceptibility of test bacteria to the different extracts of *A. cepa* could be attributed to the presence of these carbohydrates as FOS in the onion bulb and their various biochemical activities conferring on the plant its antioxidant and antibacterial activities (Benítez *et al.*, 2011). Also, studies have shown that the onion bulb has immune stimulating ability therefore, boosting the immune system to combat invading bacteria (Amrevuawho *et al*., 2016). However, gram negative and positive bacteria *Escherichia coli* and *Staphlococcus aureus* were more sensitive to fresh onion bulb methanolic extracts. This finding is in

agreement with the documentation of (Purseglove, 2005), that extract from onion has antibacterial properties. Hence, the antibacterial activity of the onion extracts compared favourably with commercially available antibiotic (streptomycin) used in this research. The study also observed a higher inhibition for gram positive bacterial than the gram negative bacteria. Similar result was reported in the study of Sharma *et al*. (2018). They reported mild resistance of gram negative bacteria used in their study to the onion bulb extract.

***In vivo Allium cepa* antimicrobial assessment**

Bacteria and fungi strains were identified in both control and experimental fish showing systemic and superficial infectivity. Fungi and bacteria loads were high in both kidney and liver of the onion exposed treatments at 14th day of administration but reduced load was observed after withdrawal of treatment (Table 3 and 4). Also, microbial load was recorded to be higher in the liver and least in the kidney in all the treatments and control groups. However, no significant difference ($P< 0.05$) was observed in the bacterial load between treatments and control except for experimental fish exposed to 150g/Kg onion inclusion diet which was extremely higher than the control in the kidney on the 14 day of experiment. Also, fungi count showed no significant variation between the treatments and control on 14 day of experiment but there was significant variation on the 14 day after treatments were withdrawn.

Table 3: Bacterial load in kidney and liver of *Clarias gariepinus*

Treatments	Organisms	Microbial load (cfu/ml)	Organisms	Microbial load (cfu/ml)
14 Day of exposure			14 day after withdrawal of treatment	
Kidney				
200g/kg	1,2,3,5,6,7,8,9,10,12,13	1.3 ± 0.06^{cd}	1,3,4,5,6,7,9,10,11,13	1.03 ± 0.03^{a}
150g/Kg	2,3,5,6,7,8,9,10,11,12,13	1.9 ± 0.12^{a}	1,2,4,5,6,7,9,10,11,12,13	0.93 ± 0.03^{a}
100g/kg	1,2,3,4,5,7,8,9,10,12	1.23 ± 0.03^{cd}	1,2,4,5,6,7,9,11,13	1.20 ± 0.00^{a}
50g/kg	1,2,3,4,5,6,8,9,11,13	1.3 ± 0.17^{cd}	1,2,4,5,6,7,8,9,10,11,13	1.20 ± 0.17^{a}
25g/kg	2,3,4,6,7,9,10,11,12,13	1.5 ±0.06^{bc}	1,2,4,7,8,10,11,12,13	1.60 ± 0.00^{b}
0g/kg	2,3,4,5,6,8,9,11,12,13	1.30 ± 0.17^{cd}	2,3,4,5,6,8,9,11,12,13	1.80 ± 0.06^{b}
Liver				
200g/kg	1,2,3,4,5,6,8,9,10,11,12	1.30 ± 0.06^{g}	1,2,4,5,6,7,9,10,11,12	1.27 ± 0.20^{cd}
150g/Kg	1,2,3,4,5,7,8,9,10,11	2.00 ± 0.00^{ef}	1,3,5,6,9,11,12,13	1.33 ± 0.03^{bc}
100g/kg	1,2,5,7,8,9,10,11,13	2.20 ± 0.12^{abcd}	1,2,3,4,5,7,9,10,11,12	1.70 ± 0.06^{ab}
50g/kg	1,2,4,5,7,8,9,10,11,12,13	2.33 ± 0.12^{cde}	1,2,4,5,7,8,10,12,13	1.20 ± 0.06^{cd}
25g/kg	1,2,3,5,7,8,10,11,12,13	2.40 ± 0.15^{bcd}	1,2,5,6,7,8,9,10,11,12	1.30 ± 0.00^{bcd}
0g/kg	1,2,4,5,6,7,8,9,11,12,13	2.53 ± 0.09^{ab}	1,2,4,5,6,7,8,9,11,12,13	1.50 ± 0.06^{abc}
Water	1,2,3,4,5,6,7,8,9,10,11,13		1.85 ± 0.09^{ab}	

Treatment means bearing same superscript in the columns showed no significant variation. All values were expressed in means ± SE.

KEY: += Present, -= Absent, 1 = *Bacillus subtilis*,2 = *Enterobacter cloaca*,3 = *Proteus mirabilis*,4 = *Streptococcus specie*,5 = *Staphylococcus saprophyticus*,6 = *Escherichia coli*,7 = *Pseudomonas fluoresce*,8 = *Pseudomonas aeruginosa*,9 = *Bacillus mycoides*,10 = *Micrococcus specie*,11 = *Klebsiella oxytoca*,12 = *Citrobacter freudii*,13 = *Bacillus megaterium*.

In vivo assessment of the antimicrobial activities of *A. cepa* in experimental fish showed the likely effect of the onion bulb to actively resist influx of micro-organism even after withdrawal of the diets as shown in the drop in microbial load in experimental fish between end of treatment and 14 days after withdrawal of the treatment. Not much study has been conducted on the effect of the onion bulb on microbial load of cultured fish. However, this findings confirms the findings of the author in her Master's Thesis on the 'Efficacy of selected plant extracts and synthetic antibiotics on cultured *C. gariepinus* (Burchell 1822)' that after withdrawal of onion treatments, fish muscles showed presence of residue which was still probably in action hence, the decreased number of microbial organism 14 days after the withdrawal of the diets observed in this study.

Table 4: Total fungi count in kidney and liver of *C. gariepinus*

Treatments	Organisms	Microbial load (cfu/ml)	Organisms	Microbial load (cfu/ml)
14 Day of exposure			14 day after withdrawal of treatment	
Kidney				
200g/kg	B,D	0.13 ± 0.03 ^{c}	-	0.00 ± 0.00^{a}
150g/Kg	A,B	0.37 ± 0.03 ^{a}	B,E	0.10 ± 0.06 ^{a}
100g/kg	A,B,C	0.20 ± 0.00 ^{b}	B	0.03 ± 0.03 ^{a}
50g/kg	B,D,F	0.40 ± 0.00 ^{a}	A	0.03 ± 0.03 ^{a}
25g/kg	A,B	0.40 ± 0.00 ^{a}	-	0.00 ± 0.00 ^{a}
0g/kg	B,G	0.20 ± 0.00 ^{b}	B,G	0.3 ± 0.00 ^{b}
Liver				
200g/kg	A,B,C,F	0.63 ± 0.03^{b}	A,B,C,D,G	0.23 ± 0.03^{bc}
150g/Kg	A,B,C,E	0.83 ± 0.15^{b}	B	0.10 ± 0.06 ^{c}
100g/kg	A,C,G	0.83 ± 0.15^{b}	A,B	0.20 ± 0.06^{bc}
50g/kg	A,B,C,E	0.63 ± 0.15^{b}	A,C	0.10 ± 0.06 ^{c}
25g/kg	A,C,D,E	1.17 ± 0.09^{a}	A,D	0.13 ± 0.03 ^{c}
0g/kg	A,B,E,F,G	0.83 ± 0.12^{b}	A,B,E,F,G	0.83 ± 0.00 ^{a}
Water	A,B,C,D		1.10 ± 0.06 ^{a}	

Treatment means bearing same superscript in the columns showed no significant variation (P> 0.05). All values were expressed in means ± SE

KEY: + = present, - = negative, A = *Fusarium oxysporum*, B = *Penicillium notatum*, C = *Fusarium solani*, D = *Aspergilus niger*, E = *Aspergilus flavus* , F = *Geotrichum species*, G= *Fusarium species**

Conclusion and Recommendations

The antibacterial activity recorded in this study for the onion bulb against the tested pathogenic bacteria makes it a baseline medicinal plant for the isolation and source of active compounds for the manufacture and processing of new antimicrobial drugs for use in the health sector both for man and animals. The onion bulb exhibited antimicrobial activity 2 weeks after withdrawal of medicated diet. The onion bulb has high levels of carbohydrate (crude fibre and nitrogen free extract). Based on the findings of this study, there is need for further research work to be carried out on the antimicrobial activity *in vivo* of this plant in the development of new drugs and on the effect of onion residue in animal models.

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References

Abd –Elmonem, A., Shalaby, S. and El-Dakar, A. (2002). Response of red tilapia to different levels of some medicinal plants by-products: black seed and roquette seed meals. The 1st Scientific Conference of Egyptian Aquaculture Society, 13-15 December pp. 247-260.

Adegbesan, S. I., Obasa, S. O. and Abdulraheem, I. (2017). Metabolic activities and health indices of African catfish (*Clarias gariepinus*) fed varying levels of *Zingiber officinale* root. *Journal of Applied Biology and Biotechnology*, 5(4): 21-28.

Adegbesan, S. I., Obasa, S. O. and Abdulraheem, I. (2018). Growth performance, haematology and histopathology of African catfish (*Clarias gariepinus*) fed varying levels of *Aloe barbadensis* leaves. *Journal of Fisheries*, 6(1): 553–562.

Amrevuawho, M.O., Akinyemi, A.A., Oyewusi, A.J., Bankole, O.M. and Ezeri, G.N.O. (2016). Effects of onion (*Allium Cepa)*and chloramphenicol on haematological parameters, histopathology and survival of catfish *Clarias gariepinus* (Burchell, 1822)sub-adult infected with *Pseudomonas aeruginosa*. *Journal of Veterinary Science*, 11:1-12.

Association of Official Analytical Chemist (2005). *Official Methods of Analysis of Association of Official Analytical Chemists (AOAC)*. 18th Edition, Washington, DC.

Awad, E. and Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish Shellfish Immuno.* 67(2017): 40-54.

Benítez, V., Mollá, E. and Martín-Cabrejasa, M.A. (2011) "Study of bioactive compound content in different onion sections," *Plant Foods for Human Nutrition*, 66(1):48–57.

Bello, O.S., Olaifa, F.E. and Emikpe, B.O. (2014). Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed walnut (*Tetracarpidium conophorum* Mull Arg.) leaf and onion (*Allium cepa* Linn) bulb supplemented diets. *American Journal of Experimental Agriculture*, 4(12):1593-1603.

Bhattacharjee, S., Sultana, A. Sazzad, M.H., Islam, M.A., Ahtashom, M.M., and Asaduzzaman. (2013). Analysis of the Proximate Composition and Energy Values of Two Varieties of Onion (Allium Cepa L.) Bulbs of Different Origin: A Comparative Study. *International Journal of Nutrition and Food Sciences*, 2,(5):246-253. doi:10.11648/j.ijnfs.20130205.16

Bonaccorsi, P.B., Caristi, C.A., Gargiulli, C.G. and Leuzzi, U.U. (2008). Flavonol glucosides in *Allium* species: A comparative study by means of HPLC-DAD-ESI-MS-MS, *Food Chemistry*, 107(8): 166-167.

Benmalek, Y., Yahia, O.A., Belkebir, A. and Fardeau, M.L., (2013). Anti-microbial and antioxidant activities of *Illicium verum*, *Crataegus oxyacanthassp monogyna* and *Allium `cepa* red and white varieties. *Bioengineered* 4: 244-248.

Cowan, S.T. and Steel, K.J. (1993). Enterobacteriacea. In G.I Barrow, R. K. A. Felthan, (Eds). *Manual for the Identification of Bacteria* (3rd edition), Cambridge University press, United Kingdom, 213-218pp.

Chessbrough, M. (1998). *Medical Laboratory Manual for Tropical Countries* Vol.II Microbiology ELBS Publication 284-289 pp.

El-Dakar, A. Y., Hassanien, G.D.I., Gad, S.S.

and Sakr, S.E. (2004) Use of medical and aromatic plants in fish diets: I. Effect of dried marjoram leaves on performance of hybrid tilapia *Oreochromis niloticus* × *Oreochromis auraus*, fingerlings. *J. Egypt. Acad. Soc. Environ. Dev.* 5:67–83.

El-Dakar, A.Y., Hassanien, G.D., Gad, S.S. and Sakr, S.E. (2008). Use of dried basil leaves as a feeding attractant for hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fingerlings. *Mediteranian Aquaculture Journal*, 1(1): 35-44

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. and Smith, B. (2002) "Onions—a global benefit to health," *Phytotherapy Research*, 16(7): 603–615.

Hedges, A.J. (2002), Estimating the precision of serial dilution and viable bacteria count. *International Journal of Food Microbiology*, 76(3): 207-214.

Kirk, R.S. and Sawyer, R. (1991) Pearson's Composition and Analysis of Foods. Longman Group, UK Limited, London.

Shalaby, S.M.M. (2004). Response of Nile tilapia, *Oreochromis niloticus*, fingerlings to diets supplemented with different levels of fenugreek seeds (Hulba). *Journal of Agriculture Mansoura University*, 29:2231–2242.

Shalaby, S.M.M., Abd Elmonem, A.I. and El-Dakar, A.Y. (2003) Enhancement of growth performance, feed and nutrient utilization of nile tilapia (*Oreochromis niloticus*), using of licorice roots (Erksous) as a feed attractive. *J. Egypt. Acad. Soc. Environ. Develop.* 4:119–142.

Sharma, K., Mahato, N. and Lee, Y. R. (2018). Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions. Journal of Food and Drug Analysis, 26(2): 518-528. https://doi.org/10.1016/j.jfda.2017.06.009.

Marta Corzo-Martínez and Mar Villamiel (2012). An overview on bioactivity of onion. In: Onion Consumption and Health. Editors: C. B. Aguirre et al. 49 p. Nova Science Publishers, Inc.

Purseglove, J.W. (2005). Tropical crops [Monocotyledon]. Longman Scientific and Technical, England. Pp 27-38.

Ugwoke, C.E.C. and Ezugwe, C.O. (2010). Phytochemical screening and proximate composition and onion bulb (*Allium cepa* L). *Journal of Pharmaceutical and Allied Sciences*, 7:2.