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AN *IN VITRO* SCREENING OF ESSENTIAL OILS AGAINST BACTERIA ISOLATED FROM FRESH WATER FISH PONDS

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ABSTRACT

Twenty eight bacterial isolates belongs to *Aeromona hydrophila*, *Pseudomonas aeruginosa*, *Vibrio Cholarea*, *Entrococcus* sp., *Streptococcus sp.* and *Staphylococcus* sp. were isolate and identified on the basis of primary and secondary biochemical tests. The bacterial isolates were tested for antimicrobial susceptibility test against clove, lemon and citronella essential oils at different concentrations. It was observed that the clove oil was found most effective against all the bacteria isolates as compared to other EOs. Thus use of essential oils or other plants originating compounds instead of antibiotics can help in diseases control and safe for environment due to its organic nature, furthermore it will reduce bio-magnification and antibacterial resistant strains.

KEYWORDS: Fresh water, bacteria, fish diseases, essential oils, control.

1. INTRODUCTION

Aquaculture industry plays important contribution to aquatic source foods for national food requirement. India is the second largest fish producing nation after China in the world (DADF, 2016). Among animal food resources, fish provides high quality protein, essential nutrients such as vitamins, polyunsaturated fatty acids (PUFA) and minerals (Wanka *et al.*, 2018). Fish protein approximately 16% of the world's population animal protein consumed (Akila & Kumaran, 2018). This sector has shown tremendous growth from traditional practices to commercial methods of culture and enhanced the fish production from a mere 7.5 lakh ton in 1950-51 to 107.95 lakh ton during 2015-2016, while earnings has reached of Rupees 33,441 crore in 2014-15 through export of fish or aquaculture products to various countries of the world (DADF, 2016; FAO, 2016).

There is global need for food and nutritional security especially among underdeveloped and developing countries (Tripathi, 2012). So, in order to meet with this increasing demand the rearing systems have been changed from extensive to super-intensive farming and it eventually resulted in sudden disease outbreaks in aquaculture (Chinabut, 2001; FAO, 2017). At present, aquaculture is suffering from high economic loss due to infectious diseases which results in lesions and high mortality rates by micro-organisms (Kim *et al.*, 2014). About 15% of fish production loss annually due to diseases in aquaculture (Faruk *et al.*, 2004). Bacteria are the leading diseases causative agents of fishes all over the world (Zorrilla, 2003). Some of the pathogenic bacteria which are responsible for diseases in captivity and causes for columnaris diseases, kidney disease, dropsy, vibriosis, tuberculosis, motile aeromonad septicemia, enteric red-mouth, bacterial gill disease, mouth fungus and tail & fin rot (Banu, 1996; Austin & Austin, 1999).

Medicinal plants are rich source of wide variety of secondary metabolites viz. tannins, terpenoids, alkaloids and flavonoids, which possess enormous antimicrobial and immune-simulative properties (Verma *et al.*, 2013). Approximately 25 to 50 % of current pharmaceuticals are derived from plants. Most of them were found effective against many pathogenic bacteria, fungi, viruses and even found active against drug-resistant microorganisms (Nascimento *et al.*, 2000). Besides this, few antimicrobials such as essential oils (Yang *et al.*, 2010), plant extracts (Mohana *et al.*, 2008; Ravikant *et al.*, 2015) and pure compounds have shown broad-spectrum antimicrobial activity against pathogens (Acharya *et al.*, 2009).

2. MATERIALS AND METHODS

2.1 Bacterial isolation

The water samples were collected from fresh water fish ponds and sample dilutions were prepared following standard protocols and spread over the nutrient agar (NA) plates which were incubated in B.O.D at 37±2^{o}C for 24 hr. Pure colonies of different bacteria were obtained on NA plates by further sub culturing single

isolated colonies by streaking method. The different bacterial isolates were identified on the basis of standard morphological and biochemical tests for their specific identification (Kreig & Holt, 1984; Quinn *et al.*, 1994).

2.2 Efficacy of essential oils

Antimicrobial activity of three essential oils (EOs) Clove, Citronella and Lemon were checked against bacterial isolates by agar well diffusion method. The dilutions of essential oils prepared in final volume of 2ml were 100%, 50%, 25% (v/v) and control (DMSO). The inoculums were prepared by growing the various bacterial isolates on agar plates which were further divided into four sections and marked (100%, 50%, 25% and control).

The 5µl of each essential oils was poured into agar wells with corresponding marking and DMSO in control. The agar plates were incubated at 37±2°C for 24 hr. After incubation, the zone of inhibition was recorded with help of HiAntibiotic ZoneScale™.

2.3 Statistical analysis: Three factor analysis test were applied to obtained data.

3. RESULTS

3.1 Characterization of bacteria : Total twenty eight bacteria (Zoo 1, Zoo 2 (a), Zoo 2(b), Zoo 2 (c), Zoo 3 (a), Zoo 3 (b), Zoo 4, Zoo 5, B.B, F.D, FA (2), B.K 1, B.K 2, F.1, F.2, C.K.1, C.K.2, C, U.K (a)1, U.K (a)2, U.K (b), W.B.P(a), W.B.P(b), P 2 P(a), P 2 P(b), C-1, S-1 and S-2) were isolated and characterized from the collected water samples. Out of twenty eight bacterial isolates 18 isolates were found Gram negative and 10 isolates were Gram positive (Table 1).

Table 1: Primary colony morphological characteristics of bacterial isolates from pond water samples.

Sr. no	Bacterial isolates name	Shape	Margin	Elevation	Colour	Gram stain	Catalase test	Oxidase Test
1.	Zoo 1	Circular	Lobate	Raised	Off White	-Ve (Rod)	+	-
2.	Zoo 2 (a)	Irregular	Undulate	Flat	Creamy	+Ve (Cooci)	+	-
3.	Zoo 2(b)	Circular	Entire	Flat	Creamy	+Ve (Cocci)	+	-
4.	Zoo 2 (c)	Irregular	Undulate	Umbonate	Creamy	-Ve (Rod)	+	+
5.	Zoo 3 (a)	Irregular	Entire	Flat	White	-Ve (Rod)	+	+
6.	Zoo 3 (b)	Irregular	Entire	Flat	White	+Ve (Cocci)	-	-
7.	Zoo 4	Circular	Entire	Flat	Yellowish White	- Ve (Rod)	+	+
8.	Zoo 5	Circular	Entire	Flat	Yellowish White	+Ve (Cocci/Strept)	-	-
9.	B.B	Circular	Entire	Flat	Yellow(White)	-Ve (Rod)	+	-
10.	F.D	Circular	Entire	Convex	Grey	-Ve (Rod)	+	-
11.	FA (2)	Irregular	Fiamentous	Raised	Creamy	-Ve (Rod)	+	+
12.	B.K 1	Circular	Entire	Flat	Creamy	+Ve (Cocci)	+	-
13.	B.K 2	Circular	Entire	Convex	Yellow	-Ve (Rod)	+	-
14.	F.1	Irregular	Undulate	Umbonate	Creamy	-Ve (Rod)	+	+
15.	F.2	Circular	Entire	Flat	Light Grey	+Ve (Cocci/Strept)	-	-
16.	C.K.1	Circular	Entire	Flat	White	-Ve (Rod)	+	+
17.	C.K.2	Circular	Entire	Raised/ Flat	Yellow	+Ve (Cocci)	+	-
18.	C	Circular	Entire	Flat	Off White	-Ve (Rod)	+	+
19.	U.K (a)1	Irregular	Undulate	Umbonate	Off White	-Ve (Rod)	+	+
20.	U.K (a)2	Irregular	Undulate	Raised	Creamy	-Ve(Rod)	+	-
21.	U.K (b)	Irregular	Entire	Flat	White	-Ve (Rod)	+	+
22.	W.B.P(a)	Irregular	Entire	Flat	White	-Ve (Rod)	+	+
23.	W.B.P(b)	Circular	Entire	Flat	White	-Ve (Rod)	+	+
24.	P 2 P(a)	Irregular	Filamentous	Flat	Creamy	-Ve (Rod)	+	+
25.	P 2 P(b)	Irregular	Filamentous	Raised	Creamy	-Ve (Rod)	+	+
26.	C-1	Irregular	Undulate	Raised	White	+Ve (Cocci)	+	-
27.	S-1	Circular	Entire	Umbonate	Creamy	+Ve (Cocci)	+	-
28.	S-2	Irregular	Entire	Flat	White	+Ve (Cocci/Strept)	-	-
+ = Positive; - = Negative								

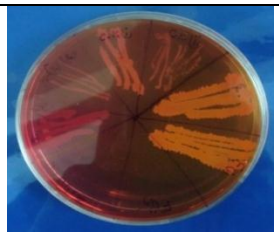
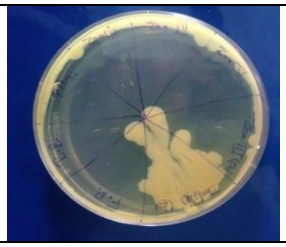
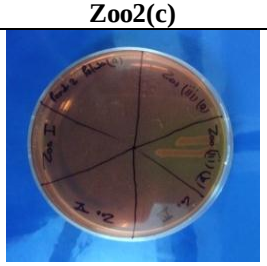
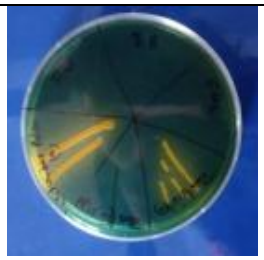
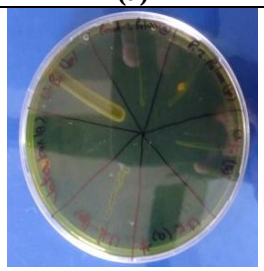
On the basis of primary and specific secondary biochemical tests the bacteria identified as *Staphylococcus* species (Isolate I-VI), *P. aeruginosa* (Isolate I-II), *V. cholerea* (Isolate I-II), *Entero.* species, *A. hydrophila* (Isolate I- IX), *Strept.* species (Isolate I-III) and five isolates were not confirmed and identified at genus level (Table 2 and Plates 1-9).

Table 2: Secondary biochemical tests (on selective media) for bacteria isolated from fresh water fish ponds Biochemical tests (Selective Agar Media).

Sr. No.	Bacterial isolates	MacConkey agar	*Pseudomonas* Agar Base	SS Agar	TCBS Agar	Vogel-johnson Agar Base	M-*enterococcus* Agar Base	Blood Agar Base	Urea Broth Base Agar	*Aeromonas* Isolation Medium Base
1.	Zoo 1	+						+		
2.	Zoo2(a)							+	+	
3.	Zoo2(b)							+	+	
4.	Zoo2(c)	+	+							
5.	Zoo3(a)	+			+			+	+	
6.	Zoo3(b)						+	+	+	
7.	Zoo4	+				+		+		+
8.	Zoo5					+		+		
9.	B.B	+						+		
10.	F.D	+						+	+	
11.	F.A(2)	+						+		+
12.	B.K 1							+	+	
13.	B.K 2	+						+		
14.	F.1	+	+			+		+	+	
15.	F.2							+	+	
16.	C.K.1	+		+		+		+	+	+
17.	C.K.2					+		+	+	
18.	C	+		+		+		+	+	+
19.	U.K(a)1	+		+				+		+
20.	U.K(a)2	+						+		
21.	U.K(b)	+		+	+	+		+	+	+
22.	W.B.P(a)	+			+	+		+	+	+
23.	W.B.P(b)	+						+	+	+
24.	P 2 P(a)	+		+		+		+	+	+
25.	P 2 P(b)	+				+		+	+	+
26.	C-1									
27.	S-1									
28.	S-2								+	
+ = Growth on media										

3.2 Efficacy of essential oils against bacterial isolates

The bacterial isolates were checked for their susceptibility against three essential oils i.e Clove oil, Citronella oil and Lemon oil. The results of inhibitory effect of essential oils revealed in Table- 3 and found that effect of essential oils different for all bacterial isolates. The zone of inhibitions was increased as the concentration of essential oils increases. The maximum zone of inhibition (12mm) was seen in Zoo2(c) and U.K (a)1 by the clove oil with 100% concentration (5.25mg/5µl). Zoo 4 and U.K (a)1 also having maximum 7mm zone of inhibition by the citronella oil at 100% concentration (4.48mg /5µl). Lemon oil was found most effective against Zoo2(b), Zoo 5 and C.k(2) with zone of inhibition (6mm) at 100% concentration (4.25mg/5 µl). F.D and W.B.P(a) were resistant to the lemon oil with 25% concentration (1.06mg/5µl) (Table3 and Plates 10-15).

		
MacConkey Agar : Plate- 1: Isolate No- C.K.1, C.K 2, C, F.1,F.2,Zoo2(a),Zoo2(b), Zoo2(c)	***Pseudomonas* Isolation Agar Base: Plate- 2: Isolate No- Zoo2(c)**	**Blood Agar Base No. 2: Plate- 3: Isolate no- Zoo1,Zoo2(a),Zoo2(b),Zoo3(a), Zoo3(b),Zoo4,Zoo5**
		
SS agar (*Salmonella Shigela* agar): Plate- 4: Isolate no- Zoo3(b)	**TCBS Agar : Plate- 5: Isolate no – Zoo3(a),Zoo3(b),W.B.P (a)**	**Vogel-Johnson Agar Base : Plate- 6: Isolate no – F.1,C.K.1,C.K.2,C**
		
M- *Enterococcus* Agar Base : Plate-7: Isolate no – Zoo 3(b)	***Aeromonas* Isolation Medium Base : Plate- 8: Isolate no – U.K(a)1, U.K(b), W.B.P(a), W.B.P(b), P2P(a), P2P(b)**	**Urea Broth Base Agar : Plate-9: Isolate no –F.1,C.K.1,C.K.2,C, B.K 1**

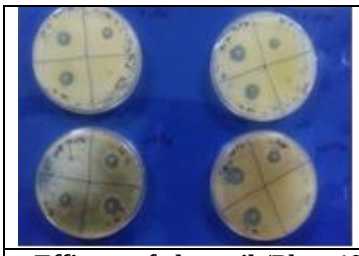
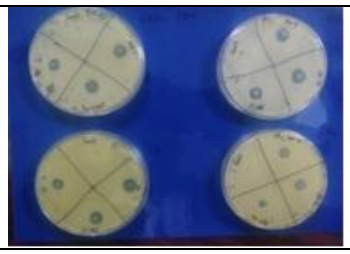
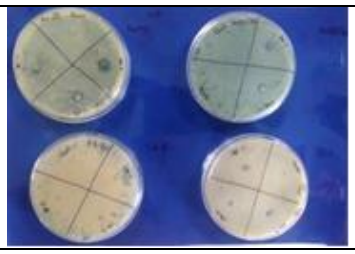
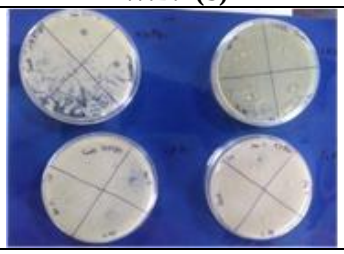
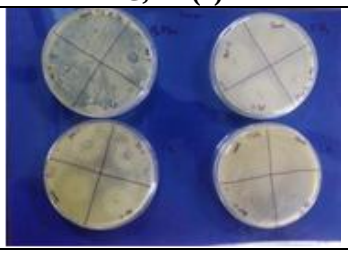
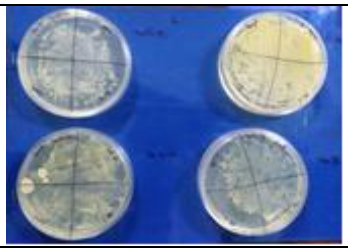
		
Efficacy of clove oil (Plate 10) : IsolatenoP2P(a),P2P(b),W.B.P(a), W.B.P(b)	**Efficacy of clove oil (Plate 11): Isolate no- C.K.(1),F.1, C, FA(2)**	**Efficacy of citronella oil (Plate 12): Isolate no- Zoo4,Zoo3(b),C.K.1,C**
		
Efficacy of citronella oil (: Plate 13) Isolate no- W.B.P(b),U.K (a)1,U.K(b),P2P(a)	**Efficacy of lemon oil (Plate 14): Isolate no- P2P(b),FA(2),C,C.K.1**	**Efficacy of lemon oil (Plate 15): Isolate no- Zoo2(a), Zoo2(b),Zoo2(c), Zoo3(a)**

Table 3: Inhibitory effect of essential oils against the bacterial isolates using well diffusion method.

Zone of inhibition (mm)										
Isolate no.	Control (DMSO)	Clove oil			Citronella oil			Lemon oil		
		25% (1.31mg/ 5µl)	50% (2.62mg/ 5µl)	100% (5.25mg/ 5µl)	25% (1.12mg/ 5µl)	50% (2.24mg/ 5µl)	100% (4.48mg/ 5µl)	25% (1.06mg/ 5µl)	50% (2.12mg/ 5µl)	100% (4.25mg/ 5µl)
Zoo 1	0	5	8	10	0	3	2	0	1	4
Zoo2(a)	0	3	5	7	3	4	2	4	4	5
Zoo2(b)	0	2	6	8	3	3	2	3	5	6
Zoo2(c)	0	6	9	12	3	4	6	3	5	5
Zoo3(a)	0	4	7	5	2	3	3	2	3	5
Zoo3(b)	0	4	7	6	2	3	4	1	2	3
Zoo4	0	2	4	5	3	4	7	1	3	4
Zoo5	0	6	8	11	5	5	6	3	5	6
B.B	0	3	4	6	1	2	4	3	5	5
F.D	0	5	7	9	1	2	5	0	3	4
F.A(2)	0	3	5	7	0	0	0	0	0	0
B.K 1	0	3	6	7	2	2	4	3	4	4
B.K 2	0	7	9	11	0	0	0	2	5	5
F.1	0	5	7	7	2	3	4	2	3	5
F.2	0	3	6	8	0	0	0	3	4	5
C.K.1	0	4	6	6	0	3	4	0	0	0
C.K.2	0	3	5	6	4	6	5	4	5	6
C	0	6	7	7	2	1	4	0	0	0
U.K(a)1	0	0	11	12	3	5	7	2	3	5
U.K(a)2	0	4	6	8	2	3	5	1	2	4
U.K(b)	0	4	6	6	0	1	3	0	0	0
W.B.P(a)	0	7	9	6	3	4	3	0	3	4
W.B.P(b)	0	5	10	11	4	2	3	5	3	4
P 2 P(a)	0	5	7	7	0	0	0	0	0	0
P 2 P(b)	0	4	9	6	0	0	0	3	2	5
C-1	0	4	5	5	2	4	4	3	4	5
S-1	0	3	4	6	2	4	6	2	3	3
S-2	0	2	3	6	4	5	5	4	5	4

Factors	C.D.	SE(d)	SE(m)
Factor(A)	0.927	0.472	0.334
Factor(B)	0.303	0.154	0.109
Intraction A X B	1.606	0.817	0.578
Factor(C)	0.303	0.154	0.109
Intraction A X C	N/A	0.817	0.578
Intraction B X C	N/A	0.267	0.189
Intraction A X B X C	N/A	1.415	1.001

The effect of factor A (bacterial isolates), factor B (essential Oils) and factor C (concentrations) had been found significant. The two factor interaction between factor A and factor B also found significant whereas rest of the two factor and three factor interactions were insignificant.

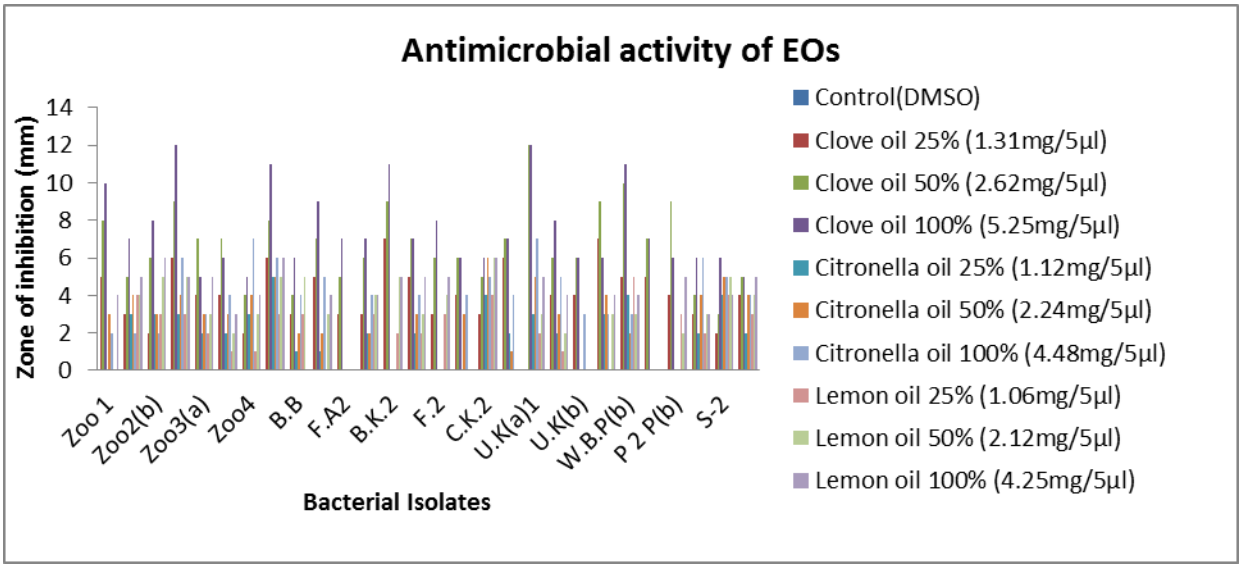


Figure 1: Inhibitory effects of essential oils against bacterial isolates.

4. DISCUSSION

Bacterial species are ubiquitous in nature, these shows tremendous growth whenever better condition occurs. This growth may cause diseases to other organisms inhabiting the same environment. So, in present study the pond water samples were taken for isolation of bacterial isolates which was highly successful sampling method. The 18 Gram negative and 10 Gram positive bacterial isolates were studied during this invetigation. Al-Imarah (2008) also isolated 28 isolates of *A. hydrophila*, 41 isolates of *P. fluorescence*, 27 isolates of *E. coli*, 21 isolates of *K. pneumoniea*, 18 isolates *of Pseudomonas* sp. and 17 isolates of *Proteus vulgaris* from the farmed carps and water samples. Sharma *et al*. (2013) isolated bacterial flora from the surface lesions of EUS affected fish as well as from their muscle and gut revealed the occurrence of *A. hydrophila, Pseudomonas, Streptococcus, Shigella, Cellobiosococcus, Acinetobacter, Micrococcus, Streptococcus grp Q1* in the different farms around Hisar, Haryana, India. They found that *A. hydrophila* was predominantly present in all the fish samples collected from all fish farms and similar results were found in our study also.

Medicinal plants such as Indian ginseng (*Withania somnifera*) was reported to reduce mortalities of greasy grouper (*Epinephelus tauvina*) against infection by *V. harveyi* (Harikrishnan *et al*., 2012). Fish fed with dietary ginger develop stronger non-specific immunity and subsequently reduces the susceptibility to *V. harveyi* infection. A study using garlic (*A. sativum*) revealed that the plant has immune-stimulant (Verma *et al*., 2019), therapeutic and antimicrobial effects that increase the immunity against *V. harveyi* leading to improved growth and survival (Talpur *et al*., 2012). Verma *et al.* (2013) reported the ameliorating effect of neem leaf powder on pathology of *A. hydrophila* infection in Common carp.

Rahman *et al.* (2017) found that all the isolates *E. faecalis* were resistant to many antibiotics but highly susceptible to the crude extracts of two medicinal herbs, *S. aromaticum* and *A. sativum*. Methanol and acetone extracts of *S. aromaticum* and methanol extracts of *A. sativum*, when used as both preventive and therapeutic significantly reduced the mortality of tilapia fish artificially infected with *E. faecalis*. Dahiya *et al.* (2012) incorporated probiotics rich fish feed to eliminate *A. hydrophila* in experimental fishs

Ajay (2018) reported that zone of inhibition increased whenever the concentration of essential oils increases. In accordance with this, similar results were found in our study i.e. size of zone of inhibition was increased with increase in concentration.

5. CONCLUSION

Based on primary and secondary biochemical tests the bacteria identified as *Staphylococcus* species (Isolate I-VI), *Pseudomonas aeruginosa* (Isolate I-II), *Vibrio cholerea* (Isolate I-II), *Enterococcus* species, *Aeromonas hydrophila* (Isolate I- IX), *Streptococcus* species (Isolate I-III) and five isolates were not confirmed and identified at genus level. The inhibitory effect of essential oils i.e. zone of inhibition were increased as when the concentration of essential oils increases. Clove oil was found to most effective as compared citronella and lemon oil against on all bacterial isolates. This study provides future encouragement to use plant's originating compounds against microflora of aquatic environment that may be harmful for aquaculture. These can also be used in place of industrial antibiotics which increases bio-magnification in food chain and also because of its eco-friendly nature as these are organic compounds.

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Conflict of interest: The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

REFERENCES

1. Acharya, P., Barua, N.C. & Sharma, A. Anti microbial activity of a pseudoguaianolids isolated from *Parthenium hysterophorus Linn. Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, 2009; 10(2): 281-282.
2. Ajay Evaluation of antibacterial activity of essential oils against bacterial flora of the field rodents in Hisar. M.Sc. thesis, CCS HAU, Hisar, 2018; 45-51.
3. Akila, N. & Kumaran, R. Isolation and identification of prevalent bacterial pathogens from an exotic fish *Tilapia zillii* and *Oreochromis. International journal of green pharmacy*, 2018; 12(03): 497.
4. Al-Imarah, E.A. Distribution of some aerobic bacteria in an infected *Cyprinus carpio L*. fish farm in Basrah and its resistance to antibiotics. *Journal of Kerbala University*, 2008; 6(4): 209-215.
5. Austin, B. & Austin, D.A. Bacterial fish pathogens: Diseases in farmed and wild fish. (3rd Ed.), Springer-Praxis Publication, 1999; 457.
6. Banu, G.R. Studies on the bacteria *Aeromonas spp.* in farmed fish and water in Mymensingh region. MSc. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh, 1996.
7. Chinabut, S. Health management for sustainable aquaculture. In Responsible Aquaculture Development in South-east Asia. Proceedings of the Seminar-Workshop on Aquaculture Development in South-east Asia organized by the SEAFDEC Aquaculture Department, 2001; 12-14.
8. DADF Guidelines - Central Sector Scheme on Blue Revolution: Integrated Development and Management of Fisheries. Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India, India, 2016.
9. FAO Opportunities and challenges. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy, 2014.
10. FAO National Aquaculture Sector Overview, Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations for a world without hunger, India, 2016.
11. FAO Statistics and Information Branch, Fisheries and Aquaculture Department/ FAO. Fishery and Aquaculture Statistics. Global Production by Production Source 1950–2015 (FishstatJ). FAO Fisheries and Aquaculture Department (online), Rome, 2017.
12. Faruk, M.A.R., Sarker, M.M.R., Alam, M.J. & Kabir, M.B. Economic loss from fish diseases on rural freshwater aquaculture of Bangladesh. *Pakistan Journal of Biological Sciences*, 2004; 7(12): 2086-2091.
13. Harikrishnan, R., Balasundaram, C., Jawahar, S. & Heo, M. Immunomodulatory effect of *Withania somnifera* supplementation diet in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) against *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, 2012; 32: 94-100.
14. Kim, M.S., Cho, J.Y. & Choi, H.S. Identification of *Vibrio harveyi*, *Vibrio ichthyoenteri*, and *Photobacterium damselae* isolated from olive flounder *Paralichthys olivaceus* in Korea by multiplex PCR developed using the rpoB gene. *Fisheries Science*, 2014; 80: 333–39.
15. Kreig, N.R. & Holt, J.G. Identification of bacteria In: *Bergey's Manual of Systematic Bacteriology*. Williams & Wilkins Baltimore, London, 1984; 1: 24-26.
16. Mohana, D.C., Satish, S. & Raveesha, KA. Antibacterial evaluation of some plant extracts against some human pathogenic bacteria. *Biological Research*, 2008; 2(3-4): 49-55.
17. Mohana, D.C., Satish, S. & Raveesha, KA. Antibacterial evaluation of some plant extracts against some human pathogenic bacteria. *Biological Research*, 2008; 2(3-4): 49-55.
18. Nascimento, G.G.F., Locatelli, J., Paulo, C., Freitas-Giuliana, L. & Silva, S. Antibacterial activity of plant extracts and Phytochemical on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, 2000; 31: 247-256.
19. Quinn, P.J., Carter, M.E., Markey, B.K. & Carter, G.R. Zoonosis and control of infectious diseases. *Clinical Veterinary Microbiology*, Wolfe Publishing, Spain, 1994; 460-486.
20. Rahman, M., Rahman, M.M., Deb, S.C., Alam, M.S., Alam, M.J., & Islam, M.T. Molecular identification of multiple antibiotic resistant fish pathogenic *Enterococcus faecalis* and their control by medicinal herbs. *Scientific reports*, 2017; 7(1): 1-11.
21. Ravikant, Dahiya,T., Gahlawat S.K. and Sihag, R. C. Restorative effect of garlic (*Allium sativum* Linn.) treatment on haematological parameter changes in *Cyprinus carpio* (L.) experimentally infected with *Aeromonas hydrophila*, in proceeding of National seminar on "Innovative Researches in Life Science" organized by Department of Zoology, MDU Rohtak, held on February, 2015; 21: 95-100. ISBN: 978-81-920945-5-7.
22. Sharma, P., Sihag, R.C. & Bhardwaj, A. Isolation and identification of pathogenic bacteria and fungi isolated from skin ulcers of *Cirrhinus mrigala*. *Indian Journal of Animal Research*, 2013; 47(4): 283-291.
23. Talpur, A.D. & Ikhwanuddin, M. Dietary effects of garlic (*Allium sativum*) on haematoimmunological parameters, survival, growth, and disease resistance

against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture*, 2012; 364–365: 6–12.

24. Tejpal Dahiya, Ravi Kant Verma, Gajender Singh. Effect of probiotics on growth performance of Indian magur (*Clarius batrachus* L.). *Annals of Agri. Bio Res.*, 2012; 17(2): 121-127.
25. Tripathi, S.D. Need for diversification of species and systems as resource-based, region-specific freshwater aquaculture. In: Swain, S.K., Swain, P., Pillai, B.R., Raghunath, M.R., Jayasankar, P. (eds.). Lead papers on strategies for Aquaculture Development. ICAR-Central Institute of Freshwater Aquaculture. Bhubaneswar, India, 2012.
26. Verma, R.K., Kumari, M. and Singh,G. Ameliorating effect of Neem (*Azadirachta indica*) leaf powder on pathology of *Aeromonas hydrophila* infection in Common carp(*Cyprinus carpio* L). *Annals of Biology,* 2013; *29*(3): 418-424.
27. Verma,R.K., Gahlawat, S.K. and Sihag, R.C. Hematological and immune biological changes in common carps on induced pathogenicity of *Aeromonas hydrophila* infection following herbal *Alium sativam* (*L.*). In: International seminar on "Sustainable environment & agriculture under global climate change" on February 21, 2019, organized by Department of Environmental Science, Maharshi Dayanand University, Rohtak-124001 in collaboration with Society for Sustainable Agriculture and Resource Management(SSARM) Hisar, India, 2019; 136.
28. Wanka, K.M., Damerau, T., Costas, B., Krueger, A., Schulz, C. & Wuertz, S. Isolation and characterization of native probiotics for fish farming. *BMC microbiology*, 2018; 18(1): 119.
29. Yang, E.J., Kim, S.S., Moon, J.Y., Oh, T.H., Baik, J.S., Lee, N.H. & Hyun C.G. Inhibitory effects of Fortunella japonica var. margarita and Citrus sunki essential oils on nitric oxide production and skin pathogens. *Acta Microbiologica et Immunologica Hungarica*, 2010; 57(1): 15-27.
30. Zorrilla, I. Bacteria recovered from diseased cultured gilthead sea bream (*Sparus aurata L.)* In:Southwestern Spain. *Aquaculture*, 2003; 218: 11-20.