EFFICIENCY AND CHARACTERIZATION OF AN ANTIBACTERIAL SUBSTANCE FROM (*ORIGANUM VULGARE*) AGAINST *AEROMONAS HYDROPHILA* IN SOME FRESHWATER FISHES.

DIAB, A. S., M. E. ABO EL- ATTA AND DOAA A. EL- ARABY

Fish Health and Management Department Central Laboratory For Aquaculture Research (CLAR), Abbassa, Sharkia, Egypt.

(Manuscript received 1st October 2006)

Abstract

This study was applied to isolate and identify *Aeromonas hydrophila* which is causative agent of Motile Aeromonas septicemia (MAS) in freshwater fishes (*Oreochromis niloticus and Clarias gariepinus)* and to study the efficacy and characterization of an antibacterial agent from *(Origanum Vulgare)* against *Aeromonas hydrophila* in vitro which is causative agent of (MAS).

This study was applied on 100 *O. niloticus* weighted 80 ± 5 g and 100 *C. gariepinus* weighted 150 ± 5 g *O. niloticus* were suffering from lose of appetite, lethargic, swim near to the water surface, hemorrhage at the fins bases ulcer behind the head roughness of scales (bristle out), slight distended abdomen and redness of anal opening. Internally the fish had distended gall bladder, enlarged kidney and accumulation of serious fluid in abdominal cavity. While *C. gariepinus* showed hemorrhage, erosion and ulcer on different area of the body specially the lateral and ventral parts of the body. Internally, there were congested liver, kidney and spleen with hemorrhage is peritoneal cavity and the intestine was free from any food particles.

The bacteriological examination of samples taken from clinically affected fishes revealed the isolation of *Aeromonas hydrophila* . The incidence of infection in *O. niloticus* reach 58% but in *C. gariepinus* was 45% with a total percentage in both fishes 51.5%. The experimental infection with the isolated *Aeromonas hydrophila* intraperitoneally resulted in 90% mortality in *O. niloticus* and 80% mortality in *C. gariepinus.* The antibiogram sensitivity test showed that ciprofloxacin in a dose 5mg is highly effective in control of *Aeromonas hydrophila.* The separation of the active ingredient and its purification was performed using both thin layer chromatography (TLC) and column chromatography techniques. The physico-chemical characteristics of the purified antibacterial agent viz. color, melting point, solubility, elemental analysis, spectroscopic characteristics and assay of total phenolics have been investigated.

The active extract of *Origanum vulgare* a suggested empirical formula of C11 H19 O8N was evaluated as antibacterial agent against *A. hydrophila* organism.

INTRODUCTION

Diseases pose a serious problem for the development of aquaculture especially for bacterial diseases that cause massive fish mortalities. Motile Aeromonas Septicemia (MAS) cause 13 – 22% mortalities in catfish (Duarte *et al., 1993*) and cause 80%

mortalities in Tilapia (Plumb 1999) *and Shoemaker et al.*, 2000). MAS. affects fresh-water, occasionally brackish water and marine Azad *et al.*, (2001). *Aeromonas hydrophila* is common water borne bacterium, which may be present in the tissues of apparently normal fish (Bullock and Sniesko, *1996*). *Aeromonas hydrophila* is considered as an opportunistic pathogen for human causing soft tissue wound and diarrhea (Altwegg and Geiss, 1989). The risk associated with use of chemical and antibacterial agent may lead to increase antibiotic resistant bacteria, increase human infection and increase of residue, which may cause toxic and allergic reaction (Dixon, 1994). Herbs can be used in treatment of bacterial diseases in aquatic animals. They are natural products, which are usually safe for consumers. Many kinds of herbs may be used, including "*Origanum vulgare*". The antimicrobial substances extracted from herbs are newly used for the treatment of bacterial diseases of fish. Consequently, interest has been focused on alternatives to chemicals particularly for best and disease control. The use of natural products from medicinal plant extracts for disease control are considered as one of the strategies available but much experimental work is being carried out to assess their commercial applicability (Kosar *et al.*, 2005).

Origanum plants belonging to different species and ecotypes (biotypes) are widely used in agriculture and the pharmaceutical and cosmetic industries as a culinary herb, flavoring substances of food products, alcoholic beverages and perfumery for their spicy fragrance. It has been also used as a traditional remedy to treat various ailments such as a spasmodic, antimicrobial, expectorant, carminative and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems (Aligiannis *et al.*, 2001). In previous studies, it has been demonstrated that the content of essential oil and extracts of medicinal plants like Origanum species containing antibacterial activities on many bacteria, namely (Escherichia coli, Enterobacter spp., Bacillus spp., Salmonella spp., Staphylococcus aureus, Klebsiella pneumoniae, Listeria monocytogenes and Campylobacter jejuni (Sahin *et al.*, 2004). Antioxidant and other biological activities may be changed based on the deference's in cultivation, origin, vegetative stage and growing seasons of the plants (Deans *et al.*, 1992, Milos *et al.*, 2000). The chemical compositions of *O. vulgare* subspecies vulgare are caryophyllene, spathulenol, germacrene-D and a terpineol (Sahin *et al.*, 2004). From aforementioned data, this work was planned to study the effect of the pure extract of Origanum vulgare as antimicrobial agent on the causative agent of Motile Aeromonas Septicaemia in freshwater fishes.

MATERIALS AND METHODS

Naturally infected fish: A total number of 100 *O. niloticus* weighted 80 + 5 g and 100 *C. gariepinus*, weighted 150 + 5 g were collected from ponds of Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt and subjected to full clinical and postmortem examinations according to methods described by (Plumb and Bowser, 1983).

Bacteriological examination:-

Samples were taken under aseptic condition from the affected lesions (skin ulcer, tail and fins, gills, liver, kidnies, spleen and ascitic fluid), then inoculated into tryptic soy broth (Difco) and incubated at 29°C for 24 hrs, then streaked on Aeromonas base agar with supplement (Ampicillin) (Biolife). Pure colonies were inoculated on nutrient agar slant for further identification by standard microbiological procedures according to Schaperclaus *et al.* (1992).

Experimental infection:

A total number of 30 apparently healthy *O. niloticus* and 30 *C. gariepinus* were collected from (Central Laboratory for Aquaculture Research). Fishes were acclimatized to laboratory condition for 2 weeks in glass aquaria. The *O. niloticus* divided into 3 groups, each group contains 10 fish a shown in Table (3). The isolated *Aeromonas hydrophila* was injected I/P with a dose of 0.2 ml of 24 hr broth culture ($5x10^5$ cfu) according to the methods described by Schaperctaus *et al.* (1992).

Antibiogram sensitivity:-

Antibiogram sensitivity was performed using different chemotherapeutic agents; the test was done according to method described with Quinn *et al.*,(1994).

Plant materials:-

Shoot system (leaves and stems) of *Origanum vulgare* (Lamiaceae) were collected from South Sienna. The shoot system (leaves and stems) of *Origanum vulgare* was dried at room temperature, powdered and kept in plastic bags until extraction.

Screening for antibacterial activity:

The antibacterial activity was determined according to Kavanagh (1972).

The extract of *O. vulgare* was tested against standard Gram positive and Gram negative organisms as follow:

1-Gram Positive: *Staphylococcus aureus*, NCTC 7447; *Bacillus subtilis*, NCTC 1040, *Bacillus pumilus*, NCTC 8214; *Acinetobacter baumanni* and *Sarcina maxima*, ATCC 33910.

2- Gram Negative: *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB, 9111.

Also, the extract was tested as specific fish pathogen *A. hydrophila*, which is locally from naturally infected freshwater fishes.

Extraction of plant materials:-

The coarsely powered shoot parts of *Origanum vulgare* (200 gms) were extracted with distilled water, 95 % ethanol and then partitioned using ethyl acetate and chloroform for 6 hours in a Soxhlet, after which the extract was filtered using Whatman filter paper No. 1 after cooling. The excess solvent of crude and partition of aqueous and organic extract was removed under vacuum using rotary evaporator. Each extract was kept in refrigerator for further biological investigation.

Precipitation: -

The precipitation process of the antibacterial agent was carried out using petroleum ether. The compound precipitate was centrifuged at 5000 rpm for 15 min. The antibacterial agent powder was tested for its antibacterial activity by using cup assay method.

Separation:-

Separation of the antibacterial agent into its individual components has been tried by thin layer chromatography using a solvent system composed of chloroform and methanol (24: 1, v/v).

Purification: -

The purification of the antibacterial agent was carried out by using Silica Gel Column Chromatography. A column of 2.5 X 50 cm was used for this purpose. Chloroform and Methanol 10:1 (v/v), was used as an eluting solvent. The column was left for over night until the silica gel (BDH – 60- 120 mesh) was completely settled. One-ml crude extract to be fractionated was added on the silica column surface and the extract was adsorbed on top of silica gel. Fifty fractions were collected (each of 5 ml). Antibacterial activities were performed for each separate fraction.

Assay for total phenolics:-

Total phenolic constituent of the methanol extract of *O. vulgare* ssp. *vulgare* was determined by employing the methods given in the literature Slinkard and Singleton (1997) involving Folin – Ciocalteu reagent and gallic acid as standard.

Physico-chemical properties of antibacterial agent:-

1- Elemental analysis: The elemental analysis C, H, O, N, and S was carried out by the microanalytical center of Cairo University, Egypt.

2- Spectroscopic analysis: The IR, UV, Mass spectrum and NMR-Spectrum were determined at the micro analytical center of Cairo University, Egypt.

Biological activity: The minimum inhibitory concentration (MIC) has been determined by cup method assay.

RESULTS AND DISCUSSION

The results of clinical examination of the moribund *O. niloticus* showed loss of appetite, loss of balance, swimming near to the water surface, scales losses behind the head and roughed scales. These results agree with those mentioned by *Shoemaker et al* (2000); Cipriano, (2001); El-Ashram, (2002); Abou El-Atta, (2003) and Abou El-Atta and Wafeek, (2005). Also, hemorrhage at the fin bases and most parts of the body, slight exophthalmia (Photo.1) and fryed and sloughed tail and fins rot (Photo. 2) were recorded. Internally, the organs were enlarged liver, congested spleen and kidney, distended gallbladder with bile due to constriction of the bile ducts as a result of hepatomegaly. These results accepted with those mentioned by Shoemaker *et al.*, (2000); Cipriano, (2001); El-Ashram, (2002) and Abou El-Atta, (2003). In addition, the intestine was free from any food particles, these lesions may be due to bacterial toxins which secreted by bacteria (Photo. 3). The clinical examination of *C. gariepinus* showed, loss of barbles, hemorrhage, erosion and ulcer especially lateral and ventral surface of the body, congested and hemorrhaged fins and the ulcer margin very clear a round the ulcerated area (Photo. 4&5). These results nearly agree with recorded with that recorded with (Plumb, 1999) Internally, *C. gariepinus* showed congested liver, kidney, spleen, and intestine hemorrhaged and free from food distended gall bladder and hyperemic and congested musculature (Photo. 6)

Bacteriological examination:-

According to morphological and biochemical characters shown in Table (1), the isolated bacteria was proved to be *Aeromonas hydrophila.* It was –ve, short rods 0.3x1.0-3.54, rapid growth rate colonies with in 24 hr at 29°C help its ubiquity these results were almost similar to those described by Enany *et al.,* (1985); Roberts, (1989); Roberts, (1989); Plumb, (1999); El-Ashram, (2002) and Abou El-Atta, (2003). The prevalence and distribution of *Aeromonas hydrophila* in different organs and tissues of *O. niloticus* and *C. gariepinus* shown in Table (2) where the higher percentage of distributions in skin ulcer and tail and fins with 44.66% and 38.83% respectively while lowest percentage were recorded from spleen and ascitic fluid 17.47%. The higher prevalence of *A. hydrophila* could be attributed to its presence as a part of intestinal flora of healthy fresh water fish and marine water fish (Neoman, 1982). The highest recovery rate of *A. hydrophila* from skin ulcer, fins and tail this may be attributed to the primary entrance of systemic infection these results agree with Enany *et al.*, (1985); Azad *et al.*, (2001); El-Ashram, (2002) and Abou El-Atta, (2003).

Experimental infection:-

Injected fishes showed nearly similar clinical signs and postmortem lesions to natural infected fishes. The mortality rate among artificial infected fishes showed in Table (3). The intraperitoneal (I/P) route of injection appear to be more pathogenic than the intramuscular (I/M) route.

Table 1. Important morphological and biochemical characters of Aeromonas.

Test	Reaction
Motiliy	+
Gram staining	-
Gelatin l iquefaction	+
Oxidase	+
O/F	F
Growth on 5% NaCl	-
Indol	+
V.P	+
Methyl red	+
H2S production	-
Catalase	+
Nitrate reduction	+
Citrate utilization	+
Arginin hydrolysis	+
Fermentation of	
Glucose	+
Sucrose	+
Lactose	-
Maltose	+
Galactose	+
Fructose	+
Trehalose	+

+ (positive) - (negative)

Table 2. Distribution of *Aeromonas hydrophila* in different tissues and organs of clinically diseased *Tilapia nilotica* and Catfish *(Clarias gariepinus).*

Fish species	Organ →	Skin ulcer	Tail &Fin	Gill	Liver	Kidney	Spleen	Ascitic fluid
Tilapia nilotica	No	12	11	7	9	8	5	6
	%	20.68	18.96	12.06	15.51	13.79	8.62	10.34
Catfish	No	11	9	6	7	5	4	3
	%	24.44	20.00	13.33	15.55	11.11	8.88	6.66
Total		23	20	13	16	13	9	9
Mean %		44.66	38.83	25.24	31.06	25.24	17.47	17.47

Table 3. Mortality rate among *Tilapia nilotica* and Catfish *(Clarias gariepinus)* inoculated with *Aeromonas hydrophila.*

Fish species	Group	Route of injection	Type and dose of injected material	No of injected fish	No of Dead fish	Mortality %
Tilapia nilotica	I	I.P.	o.2ml of $5X10^5$ cfu of *Aeromonas hydrophila*	10	9	90
	II	I.M.	o.2ml of $5X10^5$ cfu of *Aeromonas hydrophila*	10	8	80
	III	I.P.+I.M.	o.2 ml of sterile broth	5+5	0	0
Catfish	I	I.P.	o.2ml of $5X10^5$ cfu of *Aeromonas hydrophila*	10	8	80
	II	I.M.	o.2ml of $5X10^5$ cfu of *Aeromonas hydrophila*	10	7	70
	III	I.P.+I.M.	o.2 ml of sterile broth	5+5	0	0

Antibiogram sensitivity test:-

The antibiogram sensitivity test revealed that *Aeromonas hydrophila* is sensitive to ciprofloxacin (CIP_5) at a concentration of (5 mg) as shown in Photo. (7). These results were mentioned also by Abou El-Atta and Wafeek, (2005).

Control of pathogenic bacterial growth using Origanum vulgare:

The antibacterial substance produced by Origanum vulgare exhibited various degrees on inhibitions of A. hydrophila growth (Table 4) and Photo (8).

Extraction, Precipitation and Purification of antibacterial activities:-

The coarsely powered shoot parts of *Origanum vulgare* (200 g) were extracted with 95 % ethanol for 6 hours in a Soxhlet, then the extract was filtered using Whatman filter paper No. 1 after cooling. The excess of crude extract was evaporated under vacuum using rotary evaporator. The residual syrup was dissolved in least amount of DMSO and filtered. The filtrates were tested for their antibacterial activity (Table5). The coarsely powered shoot parts of *Origanum vulgare* (200 g) were extracted with 95 % ethanol for 6 hours in a Soxhlet, then the extract was filtered using Whatman filter paper No. 1. Similar results were recorded by Anna *et al.*, (2004). Only one fraction was obtained with petroleum ether (b.p. 40-60°C) by centrifugation at 5000 rpm for 15 minute. Crude deep green powder was tested for their antibacterial activities by using cup diffusion method. Separation of the antibacterial agent into individual components has been carried out by thin layer chromatography (TLC).

The obtained results revealed two bands at R_f 0.46 and 0.87. One band at R_f 0.46 exhibited obvious inhibitory effects against the growth bacterial strains.

The purification of the antibacterial substance was carried out by using silica gel column chromatography. The active fractions were concentrated. The maximum activity was recorded at fraction No. 9&10 (Table 6). These results were similar that mentioned by Tohamy *et al*, (2006)

The excess of crude extract evaporated under vacuum using rotary evaporator. The extract was concentrated and treated with petroleum ether (b.p. 40-60°C) for precipitation process where only one fraction was obtained in the form of deep green ppt.

Separation of antibacterial substance into individual components has been tried by thin-layer chromatography using a solvent system composed of chloroform and methanol (24:1, v/v) as developing solvent. The band with R_f value of 0.46 and 0.78 there is one band at R_f 0.46 exhibited obvious inhibitory effects against the growth bacteria strains. For the purpose of purification process, the antibiotic were allowed to pass through a column chromatography packed with silica gel and eluting solvent was composed of chloroform and methanol (10:1, v/v), fifty fractions were collected and tested for their activities. The maximum activity was recorded at fraction No. 9&10. Similarly, many workers used a column chromatography packed with silica gel and an eluting solvent composed of various ratios of chloroform and methanol. Similarly; Yoko *et al.*, (2001) and Ueno *et al.*, (2002),

Physico-Chemical properties of the antibacterial substance:

The physical characteristics of the antibacterial substance denored a melting point are 175°C and soluble in, ethanol, water, chloroform, DMSO and methanol but insoluble in, petroleum ether, n-Butanol, hexane and benzene.

A-Elemental analysis:

The elemental analytical data of the antimicrobial substance indicated that: **C**=46.31; **H**=6.31; **N**= 4.85; **O**= 42.53 and **S**= 0.0

This analysis indicate a suggested empirical formula of: C_{11} H_{19} O_8N.

The spectroscopic characteristics of antibacterial substance revealed the presence of the maximum absorption peak in UV. at 188 nm, infra-red absorption spectrum represented by 22 peaks and Mass-spectrum showed that the molecular weight is 294.

B- Spectroscopic characteristics:

The ultraviolet (UV) absorption spectrum of the antibacterial substance recorded a maximum absorption peak at 188 nm (Fig.1). The infrared (IR) spectrum of the antibacterial substance showed characteristic band corresponding to 22 peaks (Fig.2). The Mass spectrum of antibacterial substance showed that the molecular weight at 294 (Fig.3) and NMR-spectrum has been also investigated (Fig. 4).

Total of phenolic compounds:

Based on the absorbance value of the methanol extract solution, reacted with Folin – Ciocalteu reagent an compared with the standard solutions of gallic acid equivalents as described above, the amount of total phenolics was estimated as 235 ug/ml dry extract (24%,w/w). The amount of total phenolics constituent of the methanol extract of *O. vulgare* was estimated as 235 ug/ml dry extract (24%, w/w). Similar results were recorded by Sahin *et al.,* (2004)

The physico-chemical characteristics of the purified antibacterial substance revealed that, the melting point are 175°C and soluble in, ethanol, water, chloroform, DMSO and methanol but insoluble in, petroleum ether, n-Butanol, hexane and benzene. Similar results were recorded by Ueno *et al.*, (2002) , Anna, *et al.*, (2004), Sahin *et al.*, (2004) and Kosar *et al* .,(2005).

C- Biological activities of the purified antibacterial substance:-

Data of the antibacterial spectrum of antibacterial agent indicated that the antibacterial agent is fairly active against Gram-positive and Gram negative bacteria (Table7). The MIC of antibacterial agent under study exhibited various activities against gram positive and gram-negative bacteria. Similar investigations and results were attained by Atta, *et al.* (2003) Sahin, *et al.* (2004), Kosar, *et al*, (2005) and Tohamy *et al.*,(2006).

CONCLUSION

From the results mentioned above the antibacterial substance extracted from *Origanum vulgare* can be used for control of bacterial diseases caused by Gram positive and Gram negative bacteria instead of chemical antibiotics for their safety for human and environment.

Table 4. Mean diameters of inhibition zones (mm) caused by 100µl of the antibacterial activities from *Origanum vulgare* in the agar plate diffusion assay (The diameter of the used cup assay was 10 mm).

Test organism	*Mean diameters of inhibition zones (mm)
Aeromonas hydrophila	32

Table 5. Extraction of antibacterial substance of *Origanum vulgare*.

Extract Type	*Mean diameters of inhibition zones (mm)
	Aeromonas hydrophila
Crude aqueous extract	0.0
Ethyl Acetate	10
Ethyl Alcohol	33
Acetone	28
Chloroform	0.0

* Mean values of 3determinations.

Table 6. Isolation, precipitation and purification steps of antibacterial substance from *Origanum vulgare*

Step	*Mean diameters of inhibition zones (mm)
	Aeromonas hydrophila
1-Isolation	32± 0.15
2-Precipitation	31± 0.20
3-Purification by Column chromatography	28± 0.23

* Mean values of 3determinations.

Table 7. Antibacterial spectrum of the Purified antibacterial substance by applying the cup method assay.

Organism	Code	MIC (µg/ml) concentration
1-Gram Positive Bacteria:		
Micrococcus lutea,	ATCC 9341	11.7
Staphylococcus aureus,	NCTC 7447	11.7
Staphylococcus haemolyticus	NCTC 29968	15.6
Bacillus subtilis	NCTC 1040	20.8
Bacillus pumilus	NCTC 8214	20.8
Sarcina maxima	ATCC 33910	7.8
2-Gram Negative Bacteria		
Escherichia coli	NCTC 10416	23.4
Klebsiella pneumonia	NCIMB 9111	31.25
Salmonella typhi,	ATCC 10145	62.5
3-Bacteria isolated from Fish		
Aeromonas hydrophila		60.5

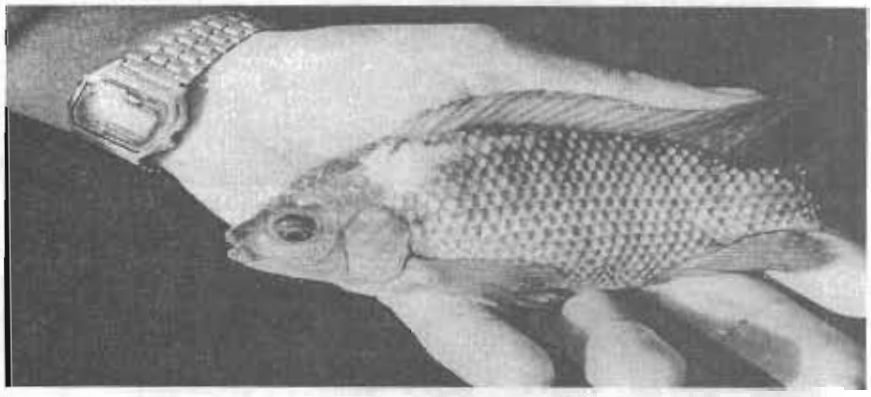

Photo 1. *O. niloticus* clinically showed scale losses behind the head, roughed scales, haemorrhage at the base of the fin and slight exophthalmia.

Photo 2. *O. niloticus* clinically showed fryed & sloughed tail and fin rot

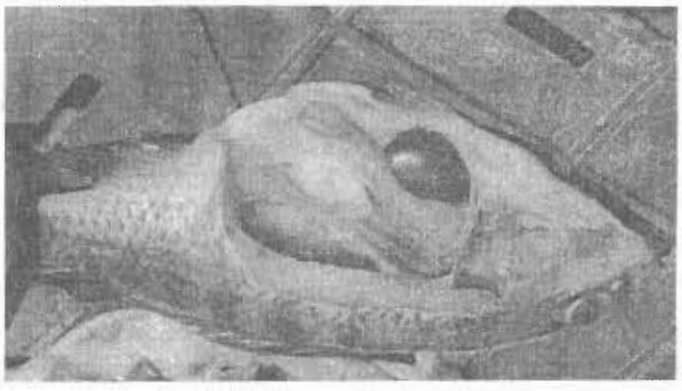

Photo 3. *O. niloticus* internally showed enlarged liver, gall bladder, congested kidney and spleen.

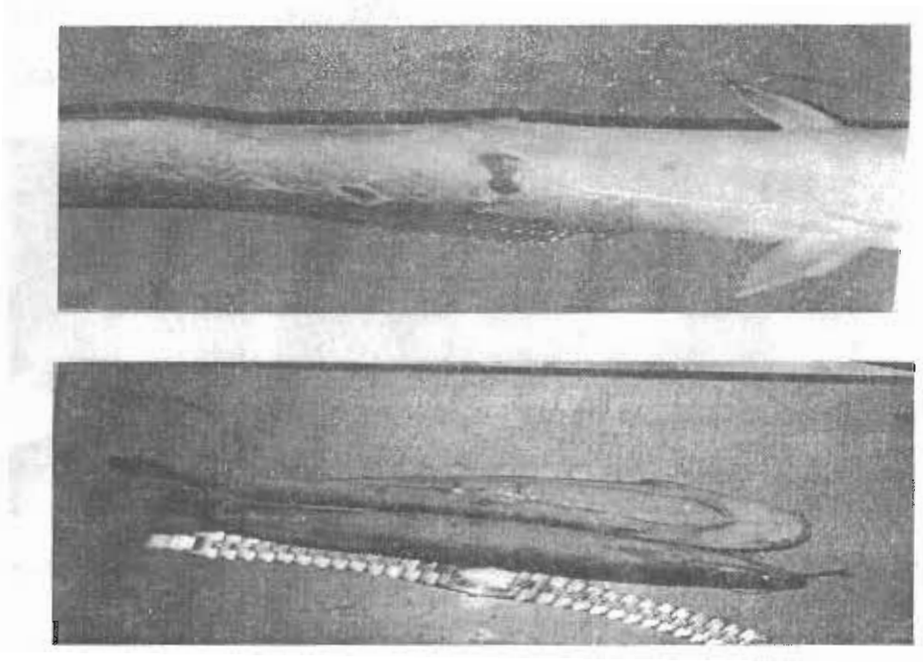

Photo (4 & 5) *C. gariepinus* clinically showed loss of barbles, erosion and ulcer especially at the lateral and ventral surface of the body

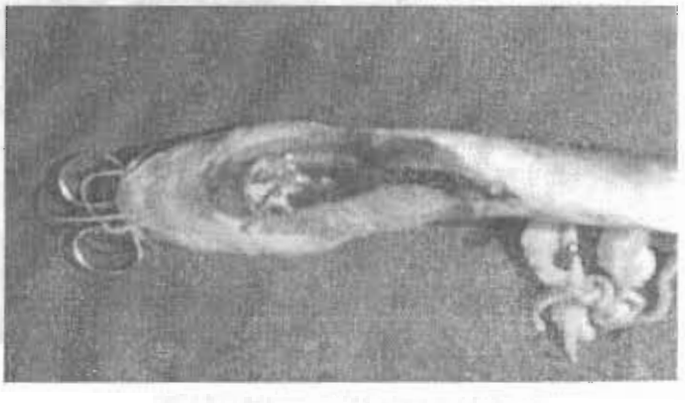

Photo 6. *C. gariepinus* internally showed congested liver, spleen, kidney the intestine haemorrhaged and free from food.

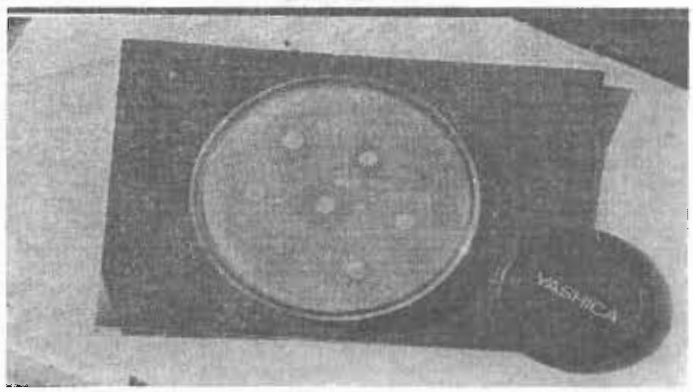

Photo 7. Antibiogram sensitivity test on MÜller,s Hinton agar media showed zone of inhibition around the antibiotic discs.

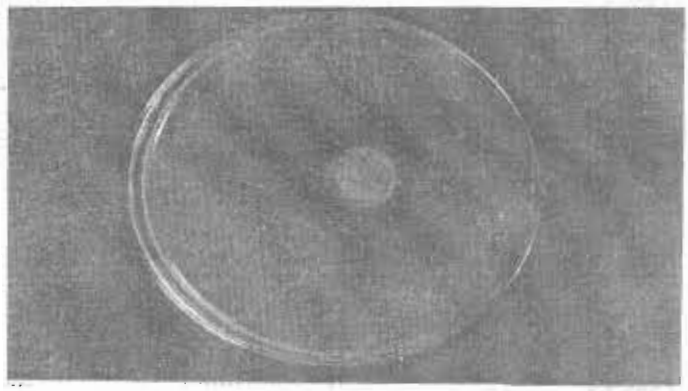

Photo 8. The antibacterial substance produced from *Origanum vulgare* exhibited various degree of inhibition on A. hydrophila growth.

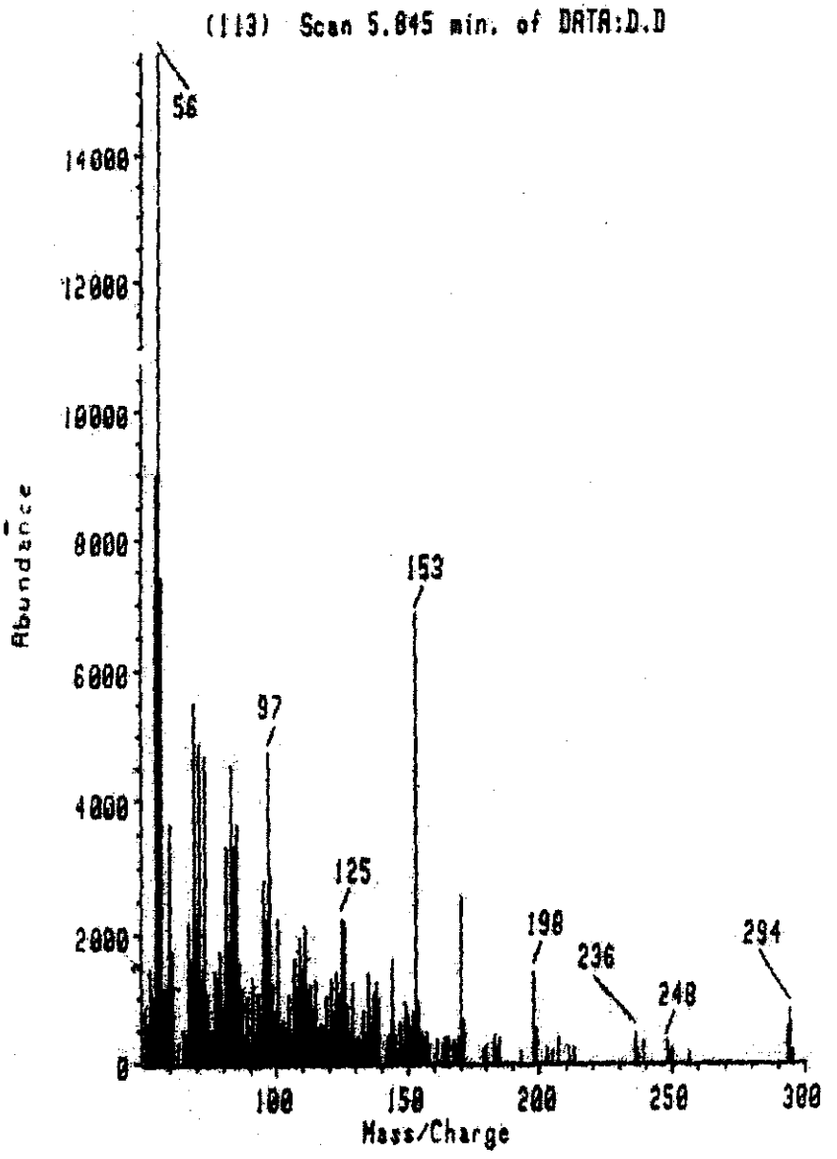

Fig. 1. The mass spectrum of the purified antimicrobial substance produced by *Origanum vulgare*.

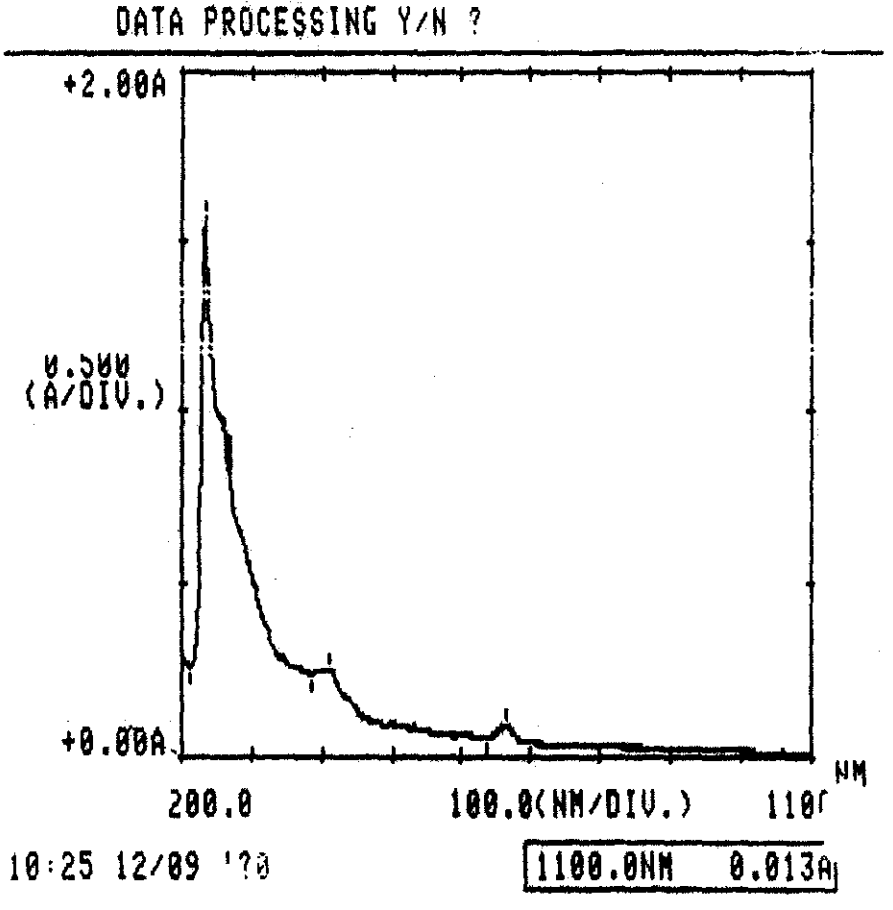

Fig. 2. The UV spectrum of the antimicrobial substance produced by *Origanum vulgare*.

13. Duarte, S. A., M. P. Masser and Plumb, J. A. 1983. "Seasonal occurrence of diseases in cage reared channel catfish 1987-1991. Journal of Aquatic Animal Health 5, 223-229.
14. El-Ashram, A. M. 2002. On *Aeromonas hydrophila* infection among cultured tilapias: A biological, histopathological and management study. . J. Aquat. Biol. &Fish. Vol. 6, No. 3:181-202.
15. Enany, M., Nareeman Abd El-Galil and A. M. Ammar. 1985. Aclinico-pathological study of aeromoniasis in experimentally inoculated Armout catfish. First Int. conf. App. Sci., 4: 1753-1767.
16. Kavanagh, F. 1972. Analytical Microbiology. Vol. 2, Acad. Press, New York.
17. Kosar, M., H. Dorman and R. Hiltunen 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chemistry 91, (525-533).
18. Milos, M., J. Mastelic and I. Jerkovic. 2000. Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare L.*ssp.*hirtum* .Food Chemistry. 71: 79 – 83.
19. Newman, S. G. 1982. *Aeromonas hydrophila*: A review with emphasis on its role in fish diseases. In Antigens of fish pathogens. Development and production for vaccine and serodiagonositics, ed. D. P. Anderson, M. Dorson and Phdubouret, pp: 87-117 Lyon: foundation Marcel Merieux.
20. Plumb J. A and P. R. Bowser. 1983. "Microbial Fish disease Laboratory Manual. Alabama Agriculture experimental Station Auburn University.
21. Plumb J. A. 1999. Health maintenance and principal diseases of cultured fishes. Iowa Press. U. S. A.
22. Pridham, T. G. and R. G. Gottlieb. 1966. A section of media for maintenance and taxonomic study of Streptomycetes. Antibiotics Ann. pp. 947-953.
23. Quinn P. J., M. E. Carter, B. K. Markey and G. R. Carter 1999. Clinical Veterinary Microbiology Published by Wolf Publishing, Book Europe Limited.
24. Roberts, R. J. 1989. Fish pathology. SaillíereTindal, London.
25. Sahin, F., M. Guluce, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, G. Agar and H. Ozer. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food Control 15: (549-557).
26. Schaperclaus, W., H. Kulow and K. Schreckenback. 1992. Fish Disease Vol.I A.A. Balkema / Rotterde.

27. Shoemaker, C. A., P. H. Klesius and J. J. Evans. 2000. Disease of tilapia with emphasis on economically important pathogens 5th Int. symposium on tilapia aquaculture in 21th century, Brazil, 2:565- 572.

28. Slinkard, K. and V. L. Singleton. 1997. Total phenol analyses automation and comparison with manual methods. American Journal of Enology and Viticulture, 28: 49 – 55.

29. Tohamy E. Y., H. M. Atta , M. Hazah , M. E. Abou El Atta and D. A. El Arabe. 2006. Purification and characterization of an antibacterial agent from (*Origanum vulgare*) against of some pathogenic bacteria isolated from fishes. Egypt. J. Biotechnol. Vol. 22, February, 2006.p. 348-366.

30. Ueno, R., N. Urano, M. Suzuki and S. Kimura. 2002. Isolation, characterization, and fermentative pattern of a novel thermotolerant *Prototheca zopfii* var. *hydrocarbonea* strain producing ethanol and CO_2 from glucose at 40°C. Arch. Microbiol. 177: 244-250.

31. Yoko, H., U. Masashi, O. Gen, O. Rie, U. Ron, H. Koki and O. Hiroyuki. 2001. Isolation and Biological properties of a new cell cycle inhibitors curvularol, Isolation from *curvulaia Sp RK97-F166.* J. of antibiotics. 54 (1):10-16.

كفاءة وخصائص العامل المضاد للبكتريا المستخلص من نبات الزعتر ضد ميكروب الإيروموناس هيدروفيلا في بعض أسماك المياه العذبة.

أحمد سعيد دياب – محمد السيد أبو العطا – دعاء أحمد العربى محمد

المعمل المركزى لبحوث الثروة السمكية بالعباسة – مركز البحوث الزراعية– القاهرة

تم إجراء الدراسة لعزل وتصنيف ميكروب الايروموناس هيدروفيلا المسبب لمرض التسمم الدموي المتحرك في أسماك المياه العذبة وخاصة أسماك البلطي وأسماك القراميط وكذلك دراسة كفاءة وخصائص العامل المضاد للبكتيريا المستخلص من نبات الزعتر ضد الميكروب المعزول معمليا.

حيث تتم هذه الدراسة على ١٠٠ سمكة من أسماك البلطي ٨٠ ± ٥جم كانت تعاني من أعراض التسمم الدموي وكانت هذه الأعراض الإكلينيكية فقدان الشهية بطء في الحركة والعوم قريبا من سطح الماء ووجود أنزفة وقرح على معظم أجزاء جسم السمكة وامتلاء الجيوب القشرية بالماء مما يجعل ملمس القشور خشن عند تحريك اليد في عكس اتجاه القشور وتآكل الذيل والزعانف وتساقط القشور خلف منطقة الرأس وتضخم حجم البطن وبهتان لون الخياشيم وتضخم حجم الكبد وشحوب لونه وتضخم حجم الحويصلة المرارية وتضخم في حجم الكلي سائل أوديمي في التخويف ألبطني.

وكذلك تمت الدراسة على ١٠٠ سمكة من أسماك القراميط كانت تزن ١٥٠ ± ٥جم وكانت تعاني تآكل الزعانف وخاصة الزعنفة الذيلية وتآكل وتعفن الشوارب ووجود أنزفة عند قاعدة الزعانف؛ داخليا كانت هذه الأسماك تعاني من احتقان الكبد والكلي والطحال ونزيف في التجويف البطني والأمعاء خالية من الغذاء.

بعمل الفحص البكتربولوجي للأسماك المصابة تم عزل وتصنيف ميكروب الإيروموناس هيدروفيلا على حسب الخواص المورفولوجية والبيوكيميائية.

وكانت نسبة الإصابة في أسماك البلطي ٥٨% و ٤٥% في أسماك القراميط بإجمالي ٥١,٥% إصابة في النوعين (البلطي والقراميط) وكانت أعلى نسبة لعزل الميكروب من التقرحات الجلدية والزعانف والذيل ٤٤,٦٦% و ٣٨,٨٣% على التوالي.

وتم عمل العدوى الصناعية التجريبية بواسطة ميكروب الإيروموناس المعزول حيث أثبتت النتائج أن الحقن في التجويف البطني (I / P) أشد ضراوة من الحقن العضلي(I /M) وحيث أدى إلى نفوق ٩٠% في البلطي و ٨٠% في القراميط بالمقارنة بالحقن العضلي الذي أدى إلى نفوق ٨٠% البلطي و ٧٠% في القراميط.

وبإجراء اختبار الحساسية للميكروب المعزول وجد أنه حساس للسيبروفلوكساسين تركيز ٥ملجم حيث أعطى منطقة منع نمو حول قرص المضاد الحيوي قدرت بحوالي ٣٢ملميتر. وتم التحكم في نمو تلك العزلات البكتيرية عن طريق استخلاص نبات الزعتر بواسطة الكحول الايثيلى بنسبة ٩٥%. كما تم

فصل المواد الفعالة وتنقيتها باستخدام الكروماتوجراف بنوعية الرقائق الورقية وعمود الكروماتوجرافى. وقد تم أيضا دراسة الخواص الفيزيوكيميائية للمركب الناتج مثل اللون، الذوبانية، درجة الانصهار، وكذا تحليل العناصر والخواص الطيفية والكمية (UV , IR , NMR, Mass Spectra). حيث أشارت تلك التحاليل إلى أن التركيب الجزئي للمركب هو $(C_{11}H_{19}O_8N)$. كما تم قياس نسبة الفينولات الكلي للمركب وتحديد أقل تركيز مثبط لنمو البكتريا (MIC).