Microbiological Studies on *Aeromonas* and *Pseudomonas* Species Isolated From Contaminated Fish Foods

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Abstract: Three bacterial isolates related to the Genus *Aeromonas* and eleven isolates related to the Genus *Pseudomonas* isolated from contaminated fish foods were studied in this work. The study included the identification of these isolates and their susceptibility to the crude extracts of five weeds and four new synthesized thiazolidinone derivatives. The isolates were identified as *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas stutzeri*. The antibacterial activities investigations of the extracts and the thiazolidinone derivatives were performed on solidified growth plates and in liquid media. On solid plates, the highest inhibitory effects (inhibition zones) were recorded to the crude extract of *Brassica tournefortii* inhibiting the growth of all tested species with inhibition zones 8-18mm diameters. The lowest effects were recorded to the extract of *Convolvulus arvensis* inhibiting only the growth of *Pseudomonas putida*. Among the four thiazolidinone derivatives tested, the highest effects were recorded to the derivatives (1d) and (2d) and the lowest effects were recorded to the derivative (4a) inhibiting the growth of *Aeromonas hydrophila* only. In liquid media, the growth of different *Aeromonas* and *Pseudomonas* species-in terms of optical densities at 660nm-was affected by most of the crude extracts and thiazolidinone derivatives.
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Key words *Aeromonas, Pseudomonas*, fish food, plant extract, thiazolidinone.

1. Introduction

Some fishermen use contaminated fish foods in bottom traps and barriers in Nile river branches to attract fish. Microbiological investigations showed that such fish foods are highly contaminated by bacteria and fungi. Bacterial contaminants were found to be related to the bacterial Genera *Aeromonas*, *Pseudomonas*, *Bacillus* and actinomycetes. *Aeromonas species* and *Pseudomonas species* were the most abundant (**Alne-na-ei and Shaaban, 2003**).

Many species of *Aeromonas* and *Pseudomonas* are known as fish pathogens causing severe diseases to different fishes. Skin diseases of fish in polluted water are very important mortal diseases caused by microorganisms, the most important of them are *Aeromonas* species and Pseudomonas species (**Ventura and Grizzle, 1988; Alne-na-ei and Shaaban, 2003**). Species of *Aeromonas* and *Pseudomonas* infecting fish isolated from fish by **Twiddy (1995)** were found to be highly resistant to antibiotics, and many of them were found to be listed as human pathogens.

Plants and their extracts have been used many centuries ago as remedies for curing diseases caused by microorganisms. **Lucas and Lewis (1944)** extracted plant substances active against Gram +ve bacteria, Gram –ve bacteria and fungi. The application of these biologically active extracts provides new, safe source of chemical control against many pathogenic microorganisms and diseases caused by them (**Balandrin *et al.*, 1985**).

With many of constrains on using herbs in curing diseases, several species of wild or cultivated plants all over the world have been examined. Among different types of extracts of eleven wild weeds collected from the cultivated lands in Menoufiya Governorate, Egypt, extracts of *Urtica urens* and *Anagallis arvensis* showed antimicrobial (inhibitory) activities against Gram +ve bacteria, Gram –ve bacteria and yeasts (**El-Abyad *et al.*, 1990**).

Shaaban (1988) investigated the antimicrobial potency of the extracts of some weeds extracted by different methods and different solvents. The study showed inhibitory effects caused by extracts of *Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens* against Gram +ve bacteria, Gram –ve bacteria and yeasts. Extracts and purified substances extracted from many other plants are known in folk medicine uses due to their antibacterial activities against Gram +ve bacteria and Gram –ve bacteria (**Shaaban *et al.*, 2011; 2012**).

Thiazolidinones are thiazole derivatives (**El-Sayed *et al.*, 2008; 2009**). Thiazole derivatives in general, are considered as one of the most important classes of heterocyclic compounds, characterized by high biological activities of great applications in

medicine, being antibacterial (**Tsuji and Ishikawa, 1944**) and antifungal (**Lopez-Garcia *et al*., 2003**).

Thiazolidinone derivatives were reported to show a variety of pharmacological properties (**El-Sayed *et al*., 2008**) being antibacterial and antifungal (**DeLima *et al*., 1992**), cyclooxygenase and lipoxygenase inhibitors (**Unangst *et al*., 1993**), cardiotonic (**Anderiani *et al*., 1993**) and anthelmentic (**Vagdevi *et al*., 2006**).

Some thiazolidinone derivatives (1,3 thiazolidinones) were recorded as potent antimicrobial agents, showing clear inhibitory effects against many Gram +ve bacterial pathogens (**Singh *et al*., 1981**). **Nasr *et al*. (2003)** reported that chemical modifications at different positions of thiazolidine ring increases the antimicrobial activities of the compound. Similar results obtained by **El-Sayed *et al*. (2009).**

Different methods have been used in the investigation of antimicrobial potencies of plant extracts and chemicals. These methods included the investigation of the antimicrobial or inhibitory effects on solidified plates and in liquid cultures (**Shaaban and El-Sharif, 2001; Shaaban *et al*., 2012**)

So, this study was interested in the identification of the *Aeromonas* isolates and *Pseudomonas* isolates isolated from contaminated fish foods and investigation of susceptibility of the identified *Aeromonas species* and *Pseudomonas species* to the crude extracts of some weeds collected from the cultivated lands in Menoufiya Governorate, Egypt and four new synthesized thiazolidinone derivatives on solidified growth plates and in liquid cultures.

2. Material and Methods

Test microorganisms

The test microorganisms included three *Aeromonas* isolates (A_1, A_2, and A_3) and eleven *Pseudomonas* isolates (P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11}) were isolated from contaminated fish foods in our study, **(Al-ne-na-ei and Shaaban, 2003).**

Test Plant Extracts

The crude extracts of five weeds collected from the cultivated lands in Menoufiya Governorate, Egypt. The weeds which were investigated for their antibacterial and antifungal potencies in our study, **(Shaaban, 1988)** are *Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens*.

Test new chemical derivatives

Four new 2,4 disubstituted thiazolidinone derivatives (1d), (2d), (4a) and (4d)- soluble in 12.5% Dimethyl sulfoxide (DMSO) which were synthesized and tested for their inhibitory effects against Gram +ve bacteria, Gram –ve bacteria and actinomycetes in our study, (**El-Sayed *et al*., 2009**).

Identification of the bacterial isolates

The bacterial isolates A_1, A_2, A_3, P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11} were identified according to **Bergey's Manual** of Systematic Bacteriology (1984). The identification criteria included the growth features (colony, shape, color, elevation and margin), growth conditions (aerobiosis, optimum temperatures, ability to grow at different temperatures, special requirements in their growth media, suitable growth media), morphology of cells (shape and dimensions), physiological characteristics (motility, number and position of flagella and Gram reaction), production of enzymes (oxidase, catalase and lecithinase), production of starch, hydrolysis of starch, denitrification and utilization of different carbon and nitrogen sources.

Investigation of the antibacterial activity

Different techniques have been applied including the investigation a- on solidified plates using filter paper discs method. Nutrient agar plates in Petri-dishes were inoculated with test species, allowed to dry for 15-20 minutes. Nine test substances could be tried against one species in one Petri-dish. Filter paper discs (8mm in diameter) were prepared and sterilized in an autoclave (1 atm for 20 minutes). Sterile filter paper discs were soaked in the crude plant extracts and thiazolidinone derivatives for four hours to reach saturation. The saturated filter paper discs were then taken and placed at the top of agar seeded with the test organism. The Petri-dishes containing the test microbe and the filter paper discs were then incubated at the optimum temperature for 24-48 hours. The inhibitory effect of a substance was detected by the inhibition zone appeared surrounding the discs of +ve activity; the diameter of this inhibition zone is a measure of the antibacterial potency (**Egorov, 1985; Izzo *et al*., 1995**). b- In liquid cultures. 45 ml of nutrient broth in 100 ml conical flasks were autoclaved, cooled and then 5 ml of sterilized and filtered crude plant extract or the thiazolidinone derivatives were added to each flask, inoculated by the test *Aeromonas* or *Pseudomonas* species, incubated at the optimum temperature of the species handled, in an incubator with shaker (at 150 rpm). The growth was followed up at standard intervals (4 hours). Changes in turbidity (in terms of the optical densities OD) were taken as the growth measure, measured in a spectrophotometer at wave length of 660nm (**Farag *et al*., 1989; Lortie *et al*., 1992; Shaaban, 2001; Shaaban and El-Sharif, 2001**).

3. Results

As shown in table 1, the three isolates relating to the Genus *Aeromonas* (A_1, A_2 and A_3) were found to have the same morphological, physiological and biochemical characteristics. All of them form white circular convex colonies with entire margin. All of them are Gram –ve, non capsule formers, motile by one, polar flagellum. The cells of all of the three isolates are of straight rod shape with cell lengths of 2.0-2.5µm and cell width of 0.5µm. The optimum temperature for growth was the same for all (28˚C), but all of them were able to grow at 37˚C in nutrient broth, sodium was not required for growth of any of them. The three isolates were able to produce oxidase enzyme, lipase enzyme, gas from glucose and H_2S from cystiene, able to ferment mannitol and sucrose, able to reduce nitrate NO_3 to nitrite NO_2. All of them were able to utilize D-mannitol, histidine, L-arginine and L-arabinose. According to these characteristics and according to Bergey's Manual for Systematic Bacteriology (1984), the *Aeromonas* isolates no. A_1, A_2 and A_3 were identified as *Aeromonas hydrophila*.

Eleven *Pseudomonas* isolates (P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11}) were characterized for their morphology of colonies, physiological activities and biochemical behaviors listed in Bergey's Manual for Systematic Bacteriology. The results classified the eleven isolates to three groups. The first group included the isolates P_1, P_5, P_6 and P_8; the second group included the isolates P_2, P_7, P_9 and P_{10}; the third group included the isolates P_3, P_4 and P_{11} (Table 2).

The isolates P_1, P_5, P_6 and P_8 were found to form white colonies. The cells are rod shaped with cell length of 3µm and cell diameter of 0.8µm, Gram –ve, aerobic, motile by one polar flagellum. The optimum temperature for them was 37˚C but all of them were able to grow at 41˚C. All of the isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of them was able to form levan, produce or hydrolyse starch. None of them accumulated poly-β-hydroxyputyrate PHB. All of the isolates were able to denitrify or reduce nitrate NO_3 to nitrite NO_2. All of them produced fluorescent pigments. All of isolates were able to utilize mannitol, N-butanol, D&L-arabinose. These characteristics were coincident to the characteristics of *Pseudomonas aeruginosa* listed in Bergey's Manual for Systematic Bacteriology, so the isolates P_1, P_5, P_6 and P_8 were identified as *Pseudomonas aeruginosa*.

Isolates P_2, P_7, P_9 and P_{10} formed white colored colonies. The cells were rod shaped with 2.5µm cell length and cell diameter of 0.8µm, Gram –ve, aerobic, motile with a polar taft of flagella. The optimum temperature was found to be 30˚C, but all of them were able to grow at 4˚C, unable to grow at 41˚C. All of the isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of the isolates formed levan, neither produced or hydrolyzed starch and none of them accumulated poly-β-hydroxybutyrate PHB in their cells. None of the isolates were denitrifying bacteria. All of them produced fluorescent pigments but none of them produced diffusible pigments in the growth medium. All the isolates were able to utilize sorbitol, mannitol, N-butanol, D&L-alanine, lactate acetate, pyruvate, succinate and L-arabinose. These characteristics were coincident to the characteristics of *Pseudomonas putida* listed in Bergey's Manual for Systematic Bacteriology, accordingly, the isolates P_2, P_7, P_9 and P_{10} were all identified as *Pseudomonas putida*.

The isolates P_3, P_4 and P_{11} formed white colonies. The cells of the isolates were found to be rod shaped with cell length of 2.3µm and cell diameter of 0.7µm, Gram –ve, aerobic, motile by one polar flagellum. The optimum temperature for growth was 36˚C, able to grow at 41˚C, but unable to grow at 4˚C. The isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of the isolates was able to form levan, produce starch or accumulate poly-β-hydroxybutyrate PHB inside their cells. The isolates were able to denitrify nitrogen compounds and hydrolyze starch. Neither fluorescent pigments nor diffusible pigments produced. They were able to utilize mannitol, N-butanol, D&L-alanin, lactate, acetate, pyruvate and succinate, but none of them was able to utilize sorbitol, sucrose, or L-arabinose. These characteristics were coincident with that listed in Bergey's Manual for Systematic Bacteriology for *Pseudomonas stutzeri*. Thus P_3, P_4 and P_{11} were identified as *Pseudomonas stutzeri*.

The antimicrobial potencies of the plant crude extract of five weeds were handled in this study against the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri*. The inhibitory effects in terms of inhibition zone diameter as shown in table 3 were different from one extract to the other, and the responsibility of bacteria tested differed from one species to the other. The crude extract of *Brassica tournefortii* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* with inhibition zones of 18mm, 8mm, 8mm and 10mm diameter respectively. The highest effect of this extract was against *Aeromonas hydrophila*, followed by *Pseudomonas stutzeri*. The crude extract of *Anagallis arvensis* exhibited its inhibitory effects against three only of the test organisms, *Aeromonas hydrophila* with inhibition zone diameter of 22mm, *Pseudomonas aeruginosa* with 9mm diameter

inhibition zone and *Pseudomonas atutzerii* with inhibition zone diameter of 6mm. the growth of *Pseudomonas putida* showed no susceptibility to the crude extract of *Anagallis arvensis* (no inhibition zone appeared). The crude extract of *Convolvulus arvensis* inhibited the growth of *Pseudomonas putida* only showing an inhibition zone of 6mm diameter. No inhibition zones appeared on the plates of *Aeromonas hyrophila*, *Pseudomonas aeruginosa* or *Pseudomonas stutzeri*. The extract of *Rumex dentatus* inhibited the growth of *Aeromonas hydrophila* exhibiting inhibition zones of 15mm and 10mm diameters respectively. *Pseudomonas putida* and *Pseudomonas stuzerii* were found to be resistant to this extract (no inhibition zones appeared). The extract of *urtica urens* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri* exhibiting inhibition zones of 17mm, 11mm and 8mm diameter, respectively. *Pseudomonas aeruginosa* was resistant to the extract of *Utrica urens* (no inhibition zones appeared).

In regard to the inhibitory effects of the four thiazolidinone derivatives (1d), (2d), (4a) and (4d) against *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri*, the results are shown in table 4. The thiazolidinone derivative (1d) inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomonas putida* showing inhibition zones on the growth plates of 21mm, 11mm and 7mm diameters, respectively, while *Pseudomonas stutzeri* was resistant to this derivative. The derivative (2d) showed inhibitory effects against the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomoans stutzeri* exhibiting inhibition zones of 24mm, 8mm and 7mm diameters, respectively. *Pseudomonas putida* was resistant to the derivative (2d). The thiazolidinone derivative (4a) affected only the growth of *Aeromonas hydrophila* showing an inhibition zone of 8mm diameter. No inhibition zones appeared on the growth plates of *Pseudomonas aeruginosa*, *Pseudomonas putida* or *Pseudomonas stutzeri*. The derivative (4d) inhibited the growth of *Aeromonas hydrophila*, *pseudomonas stutzeri*. The derivative 4d inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri* causing inhibition zones of 6mm, 8mm and 7mm diameters, respectively. No inhibitory effect was exhibited by the derivative (4d) against the growth of *Pseudomonas aeruginosa* i.e. no inhibition zone appeared. The solvent (12.5% DMSO) was used as a control. No inhibition zones appeared around the filter paper discs saturated by (DMSO) on any of the bacterial species studied.

In liquid cultures, following up the bacterial growth- in terms of optical densities at wavelengths 660nm- in presence of crude extracts and thiazolidinone derivatives. The results obtained showed different effects of different crude extracts and different thiazolidinone derivatives upon the growth of different bacterial isolates. A matter depending on the agent used and the bacterial species studied. A control to which no crude extracts or thiazolidinone derivatives were added was investigated in parallel to that containing the agents (crude extract or thiazolidinone derivatives).

In liquid cultures, crude extracts exhibited inhibitory effects of different degrees against different microbes.

As shown in Fig.1 the highest inhibitory effects against the growth of *Aeromonas hydrophila* was that caused by the extract of *Anagallis arvensis*, followed by *Brassica tournefortii*, *Urtica urens*, *Rumex dentatus*. The lowest effects was recorded the extract of *Convolvulus arvensis*. Figure 2 shows the inhibitory effects exhibited by different plant extracts upon the growth of *Pseudomonas aeruginosa*. No clear differences were observed between the effects of different extracts i.e. all the extracts were inhibitory to the *Pseudomonas aeruginosa* by approximately the same potency. In regard to *Pseudomonas putida* the growth curves as shown in Fig. 3 indicate that the organisms were affected by extracts of *Urtica urens* followed by *Brassica tournefortii* and *Convolvulus arvensis*. The inhibitory effects of *Anagallis arvensis* and *Rumex dentatus* were very low. The growth curves of *Pseudomonas stutzeri* shown in Fig.4 show that extracts of *Anagallis arvensis* and *Brassica tournefortii* have approximately the same and the highest effects followed by the extracts of *Urtica urens*, *Rumex dentatus* and *Convolvulus arvensis*.

The inhibitory effects of different thiazolidinone derivatives against *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* are represented by the growth curves in figures 5,6,7 and 8 respectively. Figure 5 shows that all the derivatives were of moderate inhibitory effects against the growth of *Aeromonas hydrophila*. The highest effects were recorded to the thiazolidinone derivatives (2d) and (1d) respectively followed by the derivative (4a) and the lowest effect was recorded to the derivative (4d).

Growth curves of *Pseudomonas aeruginosa* shown in figure 6, reflect the inhibitory effects exhibited by different thiazolidinone derivatives upon the growth of the organisms. The highest inhibition was that caused by the derivative (2d) followed by the derivative (1d). Moderate to limited inhibitory effects caused by the derivatives (4d) and (4a) respectively. Figure 7 shows that the highest inhibitory effect against the growth of *Pseudomonas*

putida was that of thiazolidinone derivative (2d) followed by the derivative (1d), while the derivative (4a) showed a limited inhibitory effect. No observable inhibitory effect was recorded to the derivative (4d) against the growth of *Pseudomonas putida*.

Table (1): The identification criteria of the *Aeromonas* isolates A1, A2 and A3 according to Bergey(1984).

Criterion	**Isolates no.**		
Colonies on nutrient agar:	**A_1**	**A_2**	**A_3**
Color	White	White	White
Shape	Circular	Circular	Circular
Elevation	Convex	Convex	Convex
Margin	Entire	Entire	Entire
Cell morphology:			
Cell shape	Straight rod	Straight rod	Straight rod
Cell length	2.5µm	2.5µm	2µm
Cell diameter	0.5µm	0.5µm	0.5µm
Gram reaction	-ve	-ve	-ve
Capsule formation	-	-	-
Motility	+	+	+
Flagella	One (Polar)	One (Polar)	One (Polar)
Optimum temperature	28˚C	28˚C	28˚C
Oxidase test	+	+	+
Lipase production	+	+	+
Sodium required	-	-	-
Growth at 37˚C in nutrient broth	+	+	+
Production of :			
Gas from glucose	+	+	+
H_2S from cystiene	+	+	+
Fermentation of mannitol	+	+	+
Fermentation of sucrose	+	+	+
Reduction of NO_3 to NO_2	+	+	+
Utilization of :			
D-mannitol	+	+	+
L-histidine	+	+	+
L-arginine	+	+	+
L-arabinose	+	+	+

Table (2): Identification criteria of the *Pseudomonas* isolates P1-P11

Criterion	Isolates no.		
	P_1,P_5,P_6 & P_8	**P_2,P_7,P_9 & P_{10}**	**P_3,P_4 & P_{11}**
Colony color	White	White	White
Cell shape	Rod	Rod	Rod
Cell length	3µm	2.5µm	2.3µm
Cell diameter	0.8µm	0.8µm	0.7µm
Gram reaction	-ve	-ve	-ve
Aerobiosis	+	+	+
Motility	+	+	+
No. of flagella	1	taft	1
Oxidase test	+	+	+
Catalase test	+	+	+
Levan formation	-	-	-
Starch Production	-	-	-
Starch hydrolysis	-	-	+
Lecithinase	-	-	-
Denitrification	+	-	+
Fluerscent Pigment	+	+	-
Diffusable pigment	-	-	-
PHB accumulation	-	-	-
Growth at 4˚C	-	+	-
Growth at 41˚C	+	-	+
Optimum temperature	37˚C	30˚C	36˚C
Utilization of sorbitol	-	+	-
Utilization of mannitol	+	+	+
Utilization of N-butanol	+	+	+
Utilization of D&L-alanin	+	+	+
Utilization of sucrose	-	+	-
Utilization of lactate	+	+	+
Utilization of acetate	+	+	+
Utilization of pyruvate	+	+	+
Utilization of succinate	+	+	+
Utilization of L-arabinose	-	+	-

Table (3): Diameters (mm) of inhibition zones around the filter paper discs saturated by different test plant extracts on the growth plates of different *Aeromonas* and *Pseudomonas* species. Data presents the mean values ± Standard errors.

Test Plant	*A. hydrophila*	*P. aeruginosa*	*P. putida*	*P. statzeri*
Anagallis arvensis	22±2.00	9.33±0.67	0±0.00	6±0.00
Brassica tournefortii	18±1.53	8±1.15	8±1.15	10±1.15
Convolvulus arvensis	0±0.00	0±0.00	6±0.00	0±0.00
Rumex dentatus	15±1.00	10±1.15	0±0.00	0±0.00
Urtica urens	16.67±0.67	0±0.00	11±1.00	8±0.00

Table (4): Diameters of inhibition zones (mm) produced around the filter paper discs saturated by 1d, 2d, 4a and 4d derivatives of thiazolidonine on the growth plates of different test *Aeromonas* and *Pseudomonas* species. The solvent DMSO (12.5% in water) used as a control. Data presents the mean values ± Standard errors.

Thiazolidonine derivative	*A. hydrophila*	*P. aeruginosa*	*P. putida*	*P. stutzeri*	Control
1d	20.67±0.67	10.67±0.67	6.67±0.67	0±0.00	0±0.00
2d	24±2.31	8±1.15	0±0.00	7.33±0.67	0±0.00
4a	8±1.15	0±0.00	0±0.00	0±0.00	0±0.00
4d	6±0.00	0±0.00	8±0.00	6.67±0.67	0±0.00

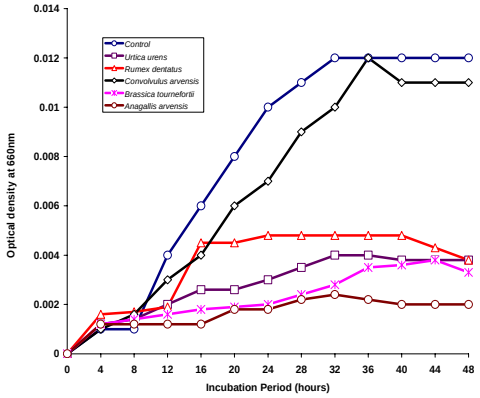

Fig.1: The growth curves of *Aeromonas hydrophila* growing in liquid media containing the plant extracts. Control is free from plant extract.

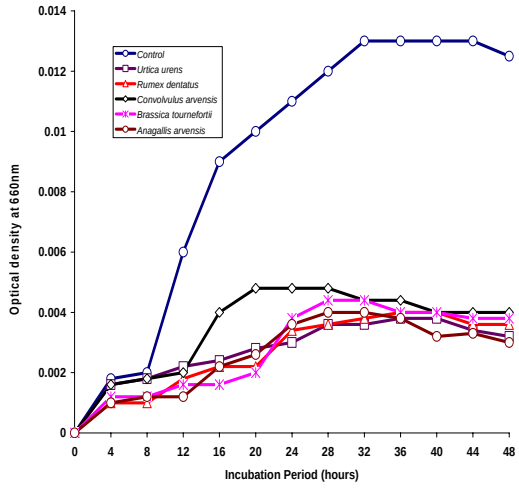

Fig.2: The growth curve of *Pseudomonas aeruginosa* growing in liquid medium containing the plant extracts. Control is free from plant extracts.

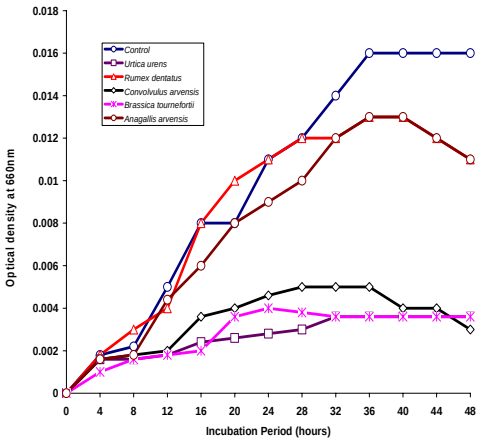

Fig.3: The growth curve of *Pseudomonas putida* growing in liquid medium containing plant extracts. Control is free from the plant extracts.

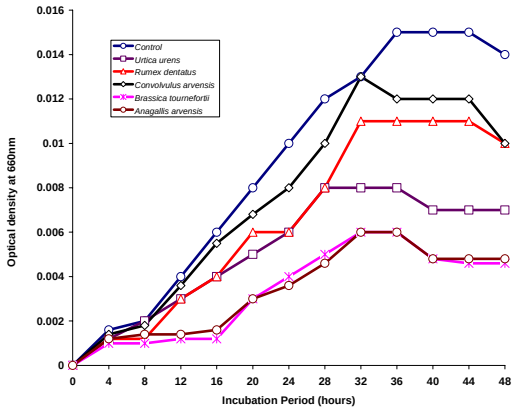

Fig.4: The growth curve of *Pseudomonas stutzeri* growing in liquid media containing plant extracts. Control is free from plant extracts.

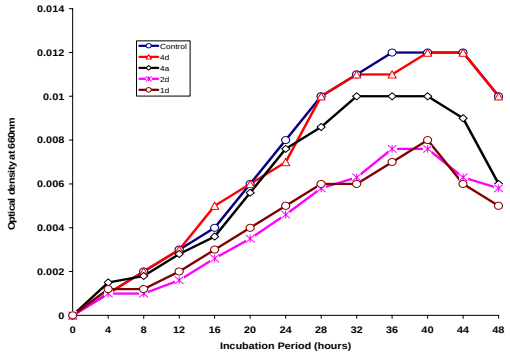

Fig.5: The growth curve of *Aeromonas hydrophila* growing in liquid medium containing the thiazolidinone derivative 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

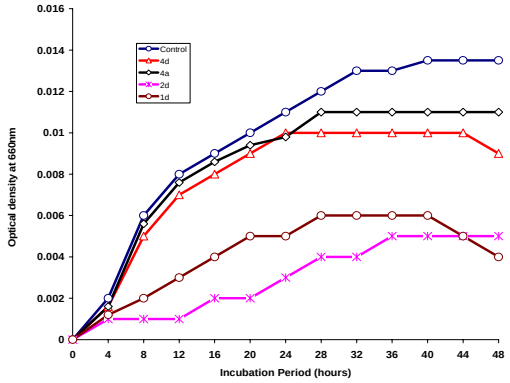

Fig.6: The growth curve of *Pseudomonas aeruginosa* growing in liquid medium containing thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% water).

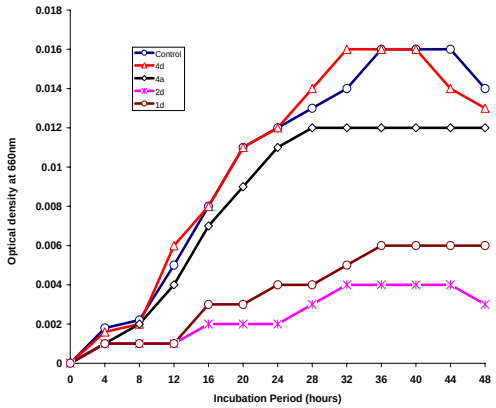

Fig.7: The growth curve of *Pseudomonas putida* growing in liquid medium containing the thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

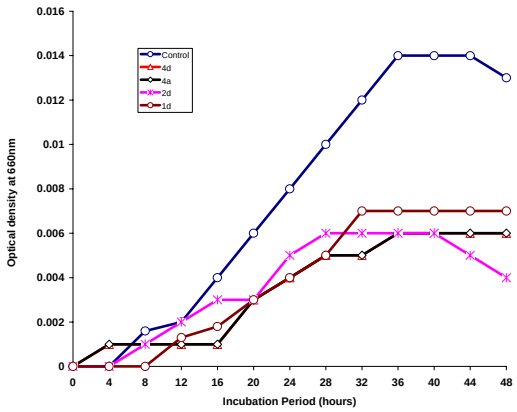

Fig.8: The growth curve of *Pseudomonas stutzeri* growing in liquid medium containing the thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

4. Discussion

This study dealt with bacterial isolates (three *Aeromonas* isolates and eleven *Pseudomonas* isolates) isolated from contaminated fish foods. These contaminated foods are used by some fishermen in waters of Nile River to attract fish to traps and barriers, caused bacterial contamination of water and fish surfaces (**Alne-na-ei and Shaaban, 2003**).

Aeromonas isolates and *Pseudomonas* isolates investigated in this study belong to two important genera in regard to their high frequency as fish food contaminants, their high frequency in fresh water and their pathogenicity to many species of fish (**Nedoluha and Westhoff, 1993**).

Aeromonas isolates A_1, A_2 and A_3 were identified according to **Bergey (1984)** as *Aeromonas hydrophila*, an *Aeromonas* species of great importance and activities in fresh water and fish ponds water, and effects on the biological aquatic life (**El-Gammal, 2000**). *Aeromonas hydrophila* is one of the virulent fish pathogens causing severe infections and mortal diseases to fresh water fish (**Shotts *et al.*, 1972; Plumb *et al.*, 1976; Boulanger *et al.*, 1977; Twiddy, 1995; El-Gammal, 2000**).

Pseudomonas isolates P_1, P_5, P_6 and P_8 were identified as *Pseudomonas aeruginosa*, a *Pseudomonas* species known for its great frequency in fresh and marine aquatic life, causing some mortal fish diseases (**Fluchter, 1979; El-Gammal, 2000**). The isolates P_2, P_7, P_9 and P_{10} were identified as *Pseudomonas putida* and the isolates P_3, P_4 and P_{11} were identified as *Pseudomonas stutzeri*. Both, the species *Pseudomonas putida* and *Pseudomonas stutzeri* were recorded as important species of the genus *Pseudomonas* found in substantial numbers as

free-living saprophytes in fresh water and marine environments causing many chemical transformations affecting the aquatic life (**Shaaban, 1992; Ghanem *et al*., 1993**).

The specific importance of the four identified species, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* (1- as fish food contaminants able to denaturize the food components, producing toxic substances, 2- as fish pathogens causing severe infections which may transfer to human beings) made it very important to look for natural and chemical nontoxic substances of antibacterial activities against these harmful bacterial species.

Substances chosen in this study included the crude extracts of five wild weeds grassed by animals i.e. have no toxicity to animals (*Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens*) and previously recorded as having antimicrobial activities (**Shaaban, 1988; El-Abyad *et al*., 1990**) and four new derivatives of thiazolidinone, synthesized compounds of no toxicity and potent antimicrobial activities against the growth of Gram +ve bacteria, Gram –ve bacteria and actinomycetes (**Abdel-Rahman *et al*., 2008; El-Sayed *et al*., 2008; 2009**).

It was found important to carry out the antimicrobial activities tests of the test substances on both the solidified nutrient agar plates and in liquid cultures, to avoid the disadvantage of each technique (**Egorov, 1985; Shaaban and El-Sherif, 2001**).

On solid media the results showed, in regard to the crude weed extracts, that, the crude extract of *Brassica tournefortii* was the most potent as antimicrobial agent inhibiting the growth of all the test organisms followed by the extract of *Anagallis arvensis*, which inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*. The crude extract of *Urtica urens* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri*, while the extract of *Rumex dentatus* was found inhibitory against the growth of two microbes only (*Aeromonas hydrophila* and *Pseudomonas aeruginosa*). The extract of *Convolvulus* was absolutely the weakest, inhibiting only the growth of *Aeromonas hydrophila* with an inhibition zone of 8mm diameter. Antimicrobial activities of plant extracts were investigated on solid media in many studies. Extracts of different plants showed different antimicrobial potencies against different bacterial species i.e. the effect is correlated to the chemical composition of the extract and susceptibility or resistant of the tested bacterial species (**Shaaban, 1988; El-abyad, 1990; Shaaban and El-Sharif, 2001; Khattab, 2009**).

Thiazolidinone derivatives (1d, 2d, 4a and 4d) showed also different inhibitory effects against the growth of the tested bacterial species. These derivatives and similar compounds showed high inhibitory effects against Gram +ve bacteria and actinomycetes, and weak inhibitory effects against the growth of Gram –ve bacteria (**Singh *et al*., 1981; Nasr *et al*., 2003; El-Sayed *et al*., 2009**).

The results obtained from the investigation of the antimicrobial potencies of either the plant extracts or the thiazolidinone derivatives in liquid cultures showed that most of the examined plant extracts and the tested thiazolidinone derivatives exhibited inhibitory actions against most of the bacterial species investigated. The results here may be not coincident with that obtained on the solid plate technique. Some substances showed inhibitory affects against a certain species in liquid cultures, while the same substance caused no inhibition zones on the plates planted by the same species. This could be explained on the bases of the correlation between inhibitory potency (inhibition zone formation, and its diameter) and the ability of the tested substance to diffuse in the solid growth medium (**Egorov, 1985; Shaaban and El-Sharif, 2001**).

5. Conclusion:

We can conclude that studies should be directed towards nontraditional antimicrobial agents from natural sources and safe cheap chemicals (**El-Sayed *et al*., 2009, Shaaban 2011; 2012**) to be added to the fish foods to prevent or minimize the bacterial contamination in these foods.

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