

Antibacterial Activity of Some Plant Extracts Against Fish Foodborne Spoilage Bacteria and Their Effects on the Quality of Mullet Fish

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ABSTRACT: The objective of this study was to look into the effect of plant extracts on the microbial contamination of mullet fish. Antimicrobial activities of three plant extracts (olive leaves, garlic heads, and *aloe vera* gel) against spoilage organisms of samples collected from Bardawil lagoon were assessed by measuring inhibition zones, using the plate diffusion method and *aloe vera* gel gave the best inhibition effect. The aqueous extracts (1%) of plants were used to make ice to study their antimicrobial effect against natural microbial fish isolates. The results indicated that the microbial contamination has increased during storage time in all treatments, however the preservation of fish in ice made with plants' extracts, especially *aloe vera* gel, decreased the numbers of TBC, Psychrophilic and *Vibrio* counts significantly ($P < 0.05$) compared to the control. The sensory evaluation test indicated that the best results have achieved with fish samples preserved in ice made with *aloe vera* gel. The results of this study constitute an indicator of possible bacteriological contamination of mullet fish. This study confirms the effectiveness of some plant extracts as natural antimicrobials and recommends the possibility of employing them in fish preservation where spoilage is caused mainly by microbial activity.

Keywords: Antimicrobial activity, plant extracts, fish spoilage, fish preservation.

INTRODUCTION

Fish is considered an important source of food for humans and it is a very perishable product. After the death, the quality of fish decreases for many reasons such as; the chemical reactions which lead to changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine. Additionally, microbiological contamination is another important source of fish spoilage. Such factors will lead to the loss of freshness and quality of fish over a time period. (Sikorski *et al.*, 1990; Parisi *et al.*, 2002; Özogul *et al.*, 2006). Fish contain many bacteria in their digestive system such as gram-negative *Vibrio*-like organism ($\sim 10^7$ CFU/g) and by the time these bacteria may cause a violent autolysis post mortem and a microbiological spoilage (Larsen *et al.*, 1978; Cakli *et al.*, 2007). The most common bacteria that cause fish spoilage and shorten shelf life are; *pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibronaceae*, *Aeromonadaceae*, *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, *Corynebacterium*, and *Photobacterium Phosphorenm*. Fish spoilage with this microorganisms may be increased when the total bacterial counts in fish increase up to 10^5 - 10^8 CFU/g which results in the production of off-odors and flavors which mainly caused by bacterial metabolites (Cutting and Spencer 1968; Gram and Dalgaard, 2002). This quality of fresh fish is largely dependent on the microbial contamination during handling, marketing and during storage (El-Deen *et al.*, 2007), especially that fish muscles contain a low amount of glycogen in addition to the high post-mortem pH which makes the fish meat more susceptible to microbial attack (Black *et al.*, 1962). Care in fish handling is vital because the unnecessary damage can hasten fish spoilage by providing access to the spoilage bacteria through cuts and wounds and penetrate the flesh via the collagen fibers when the fish were stored at

higher temperatures ($> +8^\circ\text{C}$) and as a result the fish must be chilled quickly and kept chilled (Graham *et al.*, 1992).

The main aim of fish preservation is to delay, reduce or inhibit the microbial spoilage. Fresh fish can be preserved by chilling to about $0-8^\circ\text{C}$ in wet ice (1:1). However, there are some disadvantages of using water ice such as; drip loss, textural toughness, nutrient loss, and decreased protein extractability and these disadvantages can be avoided by rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and storage (Lehane and Olley, 2000; Jeyasekaran *et al.*, 2004). However, the freshness loss during storage in ice depends on the species and ambient temperature (Lauzon *et al.*, 2010).

Plant extracts can be used to preserve fresh food from spoilage due to their antimicrobial activity. According to Samya and Ez-Elrigal, (2006) minced garlic could extend the shelf life of fish during cold storage. However, if plant oils and/or extracts will be used for food preservation, the issues of safety and toxicity should be considered (Reynolds, 1996). *Aloe vera* extract has antifungal and antimicrobial effects (Valverde *et al.*, 2004). The *Aloe* gel could inhibit the growth of *Trichophyton mentagrophytes* (20.0 mm), while the leaf inhibited the growth of both *Pseudomonas aeruginosa*, *Candida albican*, *Shigella flexneri* and *Streptococcus pyogenes*. In contrast, *Aloe vera* extracts could not inhibit the growth of *Xanthomonas* species (Satish *et al.*, 1999; Ferro *et al.*, 2003; Agarry *et al.*, 2005). This antimicrobial effect of *Aloe vera* may be due to the Acemannan which consider a strong anti-microbial against bacteria through its ability to stimulate phagocytic leukocytes (Lawless and Allan, 2000; Pugh *et al.*, 2001; Alemdar and Agaoglu, 2009).

Olive leaf extract is a natural product and consider as a potent natural antibiotic that can fight a great variety of resistant microorganisms with no side effects. Olive leaf extract has Oleuropein which has been shown to have antimicrobial activities against different microorganisms such as viruses, retroviruses, bacteria, yeasts, fungi, molds and other parasites since it interferes with amino acid's production pathways necessary for viruses and bacteria to grow (Benavente-García *et al.*, 2000; Furneri *et al.*, 2002; Cioffi *et al.*, 2010).

Garlic is one of the edible plants that has some materials such as allicin which has antiviral and antibacterial effects and its mechanism of antimicrobial action is through the interaction with thiol-containing enzymes, including cysteine proteases and alcohol dehydrogenases since these enzymes are essential for bacterial nutrition and metabolism (Rabinkov *et al.*, 1998; Kathi and Kemper, 2000; Ward *et al.*, 2002). Garlic extracts could prevent the formation of *Staphylococcus* enterotoxins A, B, and C1 and also thermonuclease (Gonzalez-Fandos *et al.*, 1994).

MATERIAL AND METHODS

Preparation of ice with natural additives:

The ice was made from sea water mixed with aqueous extract of powdered plants. The materials, (Olive tree leaves, Garlic and *Aloe* gel) were individually used to prepare aqueous extracts containing the polar active compounds as following; 10 g of powdered (dried olive leaves, dried garlic) (1 %, w/v) and (500 ml) *Aloe* gel were also mixed individually with 500 ml of sea water (1%, as total soluble solids, TSS), respectively. After maceration for 48 hours, the extracts were filtered through filter paper (5.5 mm.). These filtrates were then frozen and kept at -20 °C until used.

Bacteriological examinations:

Preparation of the sample:

Each fish sample was placed on its side over sterile plate hold by sterile forceps. The skin was sterilized by burning with ethyl alcohol, and then removed. Ten grams of fish muscle were transferred to a sterile blender Jar containing 90 ml of sterile 1% peptone water, the contents were homogenized for 2 minutes at 4000 R.P.M. and the mixture was allowed to stand for 5 minutes at room temperature. One ml of homogenate was transferred into separate tubes, each one contains 9 ml peptone water, from which 10-fold serial dilutions up to 10^{-6} were prepared (APHA, 1992).

Enumeration of Aerobic Plate Count (APC):

Total Aerobic plate count (APC) was determined as recorded by APHA (1992) as follow: One ml from each of the previously prepared dilutions was transferred into tow separated serial Petri dishes to which approximately 15 ml of sterile melted and tempered plat count agars (45°C) were added. After thorough mixing, the inoculated plates were allowed to solidify before being incubated at 37°C for 24 hours. The Aerobic plate count (APC) per gram was calculated on plates containing 30 - 300 colonies and each count was recorded separately.

Enumeration of total psychrophiles count:

Total psychrophiles count was determined according to the method mentioned by APHA (1992) as follow: On each duplicate standard plate count agar, 0.1 ml from each previously prepared dilution was plated by using surface plate technique. The inoculated plates were incubated at 7°C for 5 to 7 days and the total psychrophiles count was expressed as CFU/g.

Enumeration of total coliform count (ICMSF, 1996):

The same technique of the previous pour plate method was applied using Violet Red Bile agar medium. The plates were incubated at 37 °C for 24 hours. All dark red colonies measuring 0.5 mm or more in diameter on uncrowded plates were then counted and the average numbers of colonies were determined. The coliform count per gram was calculated, and expressed as CFU/g.

Enumeration of total *Vibrio* count species:

Preparation of the sample:

Ten grams of examined sample were transferred aseptically into a sterile homogenizer flask containing 90 ml of peptone containing 3 % NaCl and homogenized for one minutes at 8.000 rpm. The contents were allowed to stand for few minutes (A.P.H.A., 1992).

Isolation of *Vibrio* species:

One ml of prepared sample was inoculated into 9 ml of peptone water containing 3% NaCl. The inoculated tubes were incubated at 37°C overnight. Loopfuls from the inoculated tubes were streaked on Thiosulfate Citrate- Bile salts Sucrose (TCBS) agar and inoculated at 37°C for 24 hours. Rounded colonies 2-3 mm in diameter, green or blue colour were considered to be *Vibrio parahaemolyticus*, while *V. alginolyticus* and *V. vulnificus* colonies were large and yellow colored (A.P.H.A., 1992).

RESULTS AND DISCUSSION

Effect of handling and storage at the microbiological contamination of fish:

Total bacterial count (TBC):

Fish usually have bacteria on its gill, skin and intestine when it is alive, but can not attack the fish mucle at this time. But once the fish dies the bacteria can penetrate into the flesh muscle of the fish. This normal bacterial flora of fish may include antibiotic resistance genes even in the absence of exogenously applied antibiotics (Shah *et al.*, 2012). The data presented in Figure (1) indicated that the total bacterial count has increased gradually during cold storage of bori fish under market and laboratory conditions. However, the total bacterial counts in fish stored under laboratory condition were significantly lower than those of fish stored under market condition ($P < 0.05$). The TBC for the market samples were increased from 4.28 to 4.76 $\log_{10}$ CFU/gm during storage period. While it increased from 3.98 to 4.08 $\log_{10}$ CFU/gm in fish stored under laboratory condition. Those low numbers of TBC in laboratory samples copmapare to the market ones may be due to the proper handling and storage of fish under laboratory conditions which extend its shelf life

time by slowing the action of enzymes and bacteria, and the chemical and physical processes that can affect quality. It should be noted that there is no correlation between the total bacterial counts and presence of any bacteria of public health significance (Gram, 1989). The enough amount of ice reduces the rate of bacterial penetration into the flesh muscle, while in market samples fish spoilage occurs quickly when the bacterial enzymes diffuses into the flesh muscle quickly (El-Deen *et al.*, 2007; Ocano-Higuera *et al.*, 2009; Aberoumand, 2010). Our results agree with those reported by Thanana (1984), El-Marrakchi *et al.*, (1990), Bennour *et al.*, (1991) and Mosilhy (1995).

Total psychrophilic count:

The data presented in Figure (2) shows that there was a considerable increase in psychrophilic bacterial counts during the cold storage under both laboratory and market conditions. However, those numbers of bacteria were quite low in fish stored under laboratory condition compared to those stored under market condition ($P < 0.05$). The highest number of psychrophilic bacterial count was $3.87 \log_{10}$ CFU/gm in fish stored under market, where it was $3.49 \log_{10}$ CFU/gm in fish stored under laboratory condition. These results are in agreement with those reported by Frazier (1967), Franz (1970) and Mosilhy (1995).

Total *Vibrio* count:

The total *Vibrio* count of mullet fish during storage in ice under market and laboratory conditions are present in Figure (3). It was clear that the total *Vibrio* counts were increased with storage time and the total *Vibrio* counts of mullet fish stored under laboratory condition were significantly ($P < 0.05$) lower than those of mullet fish stored under market condition. The initial *Vibrio* count of market samples increased from 2.94 to $3.15 \log_{10}$ CFU/gm. Whereas in laboratory samples it increased from 2.05 to $2.74 \log_{10}$ CFU/gm. Similar results have been reported by Huq and Colwell, (1995) and Yongling *et al.*, (2011).

Total coliform group:

The mullet fish samples which stored under laboratory and market conditions have been examined for the presence of coliform group and the results were negative which means that there was no contamination at all. Several pathogenic bacteria may either be present in the environment or contaminate the fish during handling.

Effect of plant extracts and storage at the microbiological contamination of fish

The data in Table (1) shows the inhibition zones of the aqueous extracts of different plant materials (olive

leaves, 0.5 and 1 % w/v; garlic heads, 0.5 and 1 % w/v; and *aloe vera* gel, 0.5 and 1 % as TSS) against natural microbial fish isolates. The data indicated that, the inhibition zones were 12 , 15 and 16 (mm) for olive leaves, garlic heads and *aloe vera* gel aqueous extracts respectively when 0.5% of extracts has been used while they were 15 , 19 and 20 mm when 1% extract (w/v) has been used. *Aloe vera* gel aqueous extract gave the best inhibitory result and this may be due to the antimicrobial effect of *Aloe vera* because of the presence of the Acemannan, a polysaccharide component from *Aloe vera*, which consider a strong anti-microbial against bacteria through its ability to stimulate phagocytic leukocytes (Pugh *et al.*, 2001; Alemdar and Agaoglu, 2009). Additionally, olive leaf extract has Oleuropein which has been shown to have antimicrobial activities against different pathogens because it can interfere with the bacterial amino acid's production pathways (Privitera 1996; Benavente-García *et al.*, 2000). The antibacterial effect of garlic is due to the Allicin which reacts very rapidly with free thiol groups, via thiol-disulphide exchange and, therefore, it is thought that its main mechanism of antimicrobial action is through interaction with thiol-containing enzymes, including cysteine proteases and alcohol dehydrogenases (Ankri, *et al.*, 1997; Rabinkov, *et al.*, 1998). The results indicated that 1% concentration of plant materials had the best inhibitory effect. Therefore, this concentration was used to prepare the ice used to cool fish during this study.

Total bacterial count (TBC):

Total bacterial count has continuously increased during cold storage of bori fish under market and laboratory conditions (Figure 4). The initial TBC in mullet fish stored with crushed ice without any additives (control) was $3.97 \log_{10}$ CFU/g at zero time of storage time then increased during storage period to be $4.19 \log_{10}$ CFU/g at the end of storage period. Whereas, the other fish samples that treated with crushed ice with additives (olive leaves, garlic heads and *aloe vera* gel extracts) showed lower numbers of TBC during storage time as compared to the control. *Aloe vera* gel showed the highest preservative effect during storage period. The results showed a significant difference ($P < 0.05$) between the samples treated with *aloe vera* gel ($3.68 \log_{10}$ CFU/g) and control ($4.9 \log_{10}$ CFU/g) at the end of storage period. Such results indicated that plant extracts, such as *aloe vera* gel, live leaf and garlic extracts, have anti-bacterial effect and can extend the shelf life period of fish stored in ice (Heggers *et al.*, 1995; Ferro *et al.*, 2003). These results are in agreement with those results reported by Thanana (1984) and Gram and Huss, (1996).

Table (1): Antimicrobial activity of olive leaves extract, garlic heads extract and *aloe vera* gel aqueous extract on the growth of TBC isolates.

Aqueous extracts	Diameter of inhibition zone (mm)	
	0.5 %	1 %
Olive leaves (w/v)	12	15
Garlic heads (w/v)	15	19
Aloe gel (as TSS)	16	20

Total psychrophilic count:

The psychrophilic bacterial count during cold storage of bori fish with crushed ice made with some plant extracts has increased during the cold storage (Figure 5). At the zero time of storage period, the initial TPC for the control sample was $3.46 \log_{10}$ CFU/g then increased at the end of storage period to be $3.83 \log_{10}$ CFU/g. Whereas, the examined mullet fish samples covered with crushed ice made from sea water mixed with olive leaves aqueous extract was increased from 2.92 to $3.73 \log_{10}$ CFU/g during storage period and samples covered with crushed ice made from garlic extract was increased from 3.34 to $3.62 \log_{10}$ CFU/g. Interestingly, the mullet fish samples that cooled with ice mixed with *Aloe vera* gel increased slightly as compared to other treatments (from 3.13 to $3.50 \log_{10}$ CFU/g). These results are similar to those reported by Franz (1970) Singh and Balange, (2005). This high contents of psychrophilic bacteria during storage period may be due to the presence of psychrophilic spore forming bacteria which grow during storage, and these results are in agreement with those obtained by Suvanich *et al.*, (2000).

Total *Vibrio* species:

The data showed that there was a considerable increase in *Vibrio* counts in control samples during the cold storage from $2.05 \log_{10}$ CFU/g at zero time to $5.10 \log_{10}$ CFU/g at the end of storage period. While, the examined sample which treated with crushed ice made from seawater mixed with plant extracts increased slightly. The lowest number of *vibrio* spp. has observed at the end of experiment with mullet fish cooled in ice made with *aloe vera* gel ($3.50 \log_{10}$ CFU/g). Samya and Ez-Elrigal, (2006) showed that 3% concentration of minced garlic kept silver carp fish fillets in good condition for 6 days under cold storage period when compared with the control sample. These results were in

agreement with those reported by Ólafsdóttir *et al.*, (1997) and Özogul *et al.*, (2006). Such contamination with *Vibrio* can cause gastroenteritis food poisoning (Faruque and Nair, 2008).

Total coliform group:

No coliform species have been detected in any of the samples throughout the storage period, which indicated that there was no contamination with sewage or human faeces. The contamination after pre-harvest with pathogens from the animal/human reservoir may pose a risk. These results indicated that Bardawil lagoon is a clean lagoon and produce high quality fish.

Sensory evaluation:

The results presented in Table (2) indicated that chilling of bori fish in crushed ice made from seawater mixed with plant aqueous extracts extended the shelf life of fish and reduced the appearance that lead to the spoilage. The results indicated that control samples lost its freshness faster than samples that treated with crushed ice made from seawater mixed with plant aqueous extracts. The best results have achieved with fish samples preserved in ice made with *aloe vera* gel. These antioxidant and antimicrobial effects of *Aloe vera* gel could be involved in the action of the gel on maintaining the quality and safety of fish (Valverde *et al.*, 2004).

It has been reported by earlier studies that microbial metabolites such as peptides or amino acids, derived from protein hydrolysis, contribute significantly to undesirable sensory changes in seafood products Rodríguez *et al.*, (2003). Therefore, these results showed that treated ice with aqueous extracts of olive leaves, garlic heads and *aloe* gel decreased the total count of microorganisms as well as the microbial metabolites as a result of the antimicrobial activity of these plant extracts.

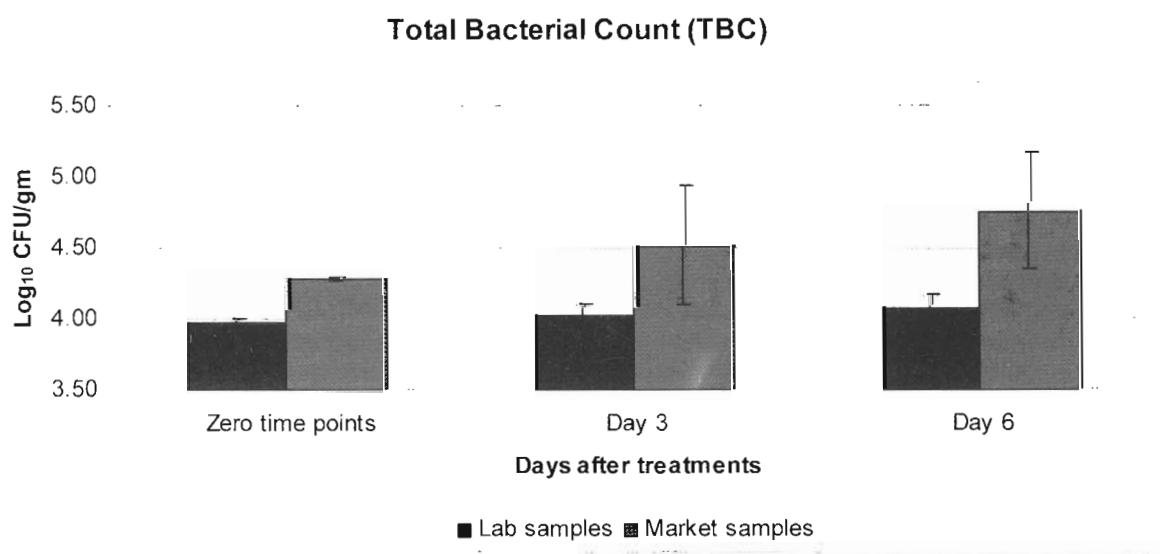


Figure (1): Changes in total bacterial counts of examined mullet fish during storage under laboratory and market conditions.

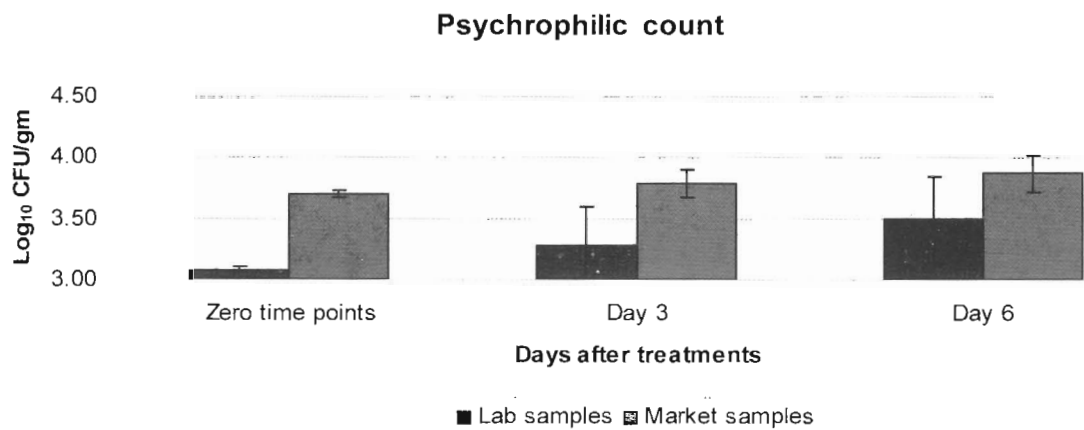


Figure (2): Changes in total psychrophilic bacterial counts of examined mullet fish during storage under laboratory and market conditions.

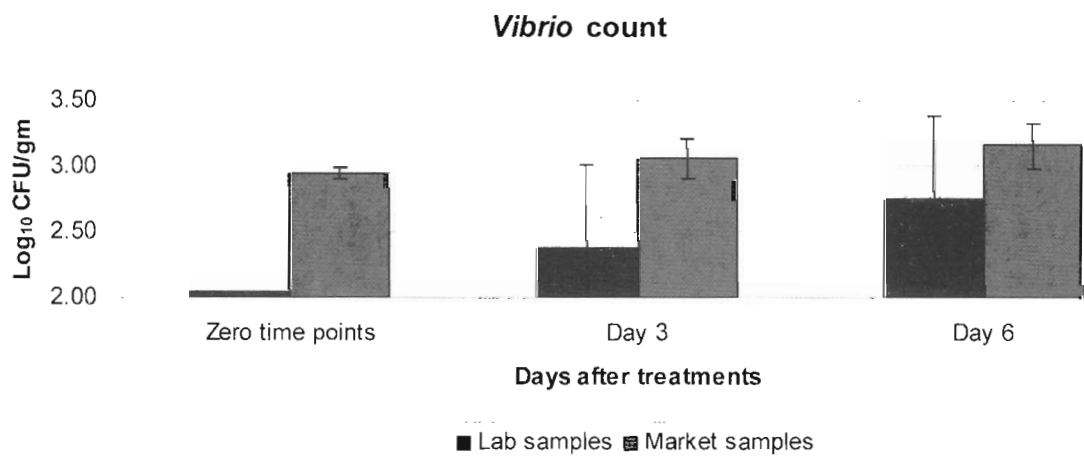


Figure (3): Changes in total *Vibrio* counts of examined mullet fish during storage under laboratory and market conditions.

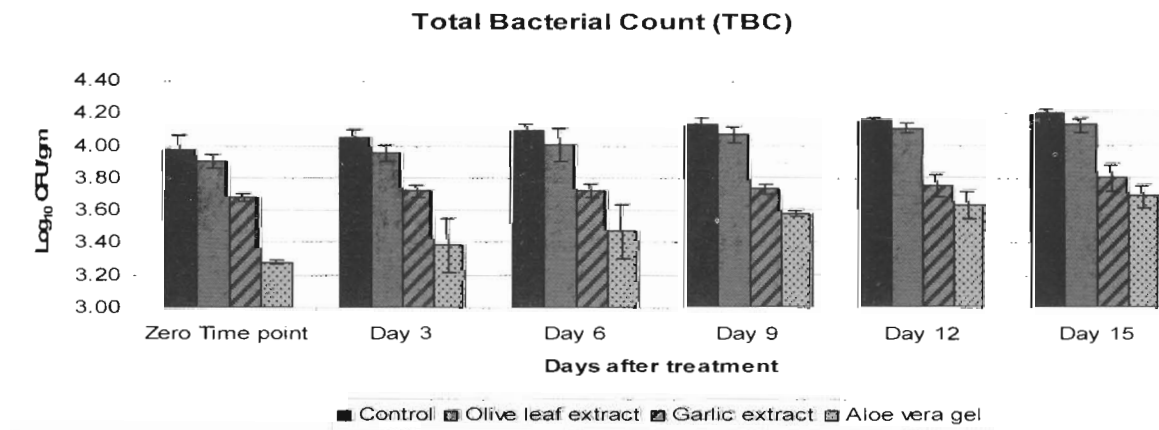


Figure (4): Changes in total bacterial count of examined mullet fish during storage under laboratory and market conditions.

Table (2): Changes in sensory quality of mullet fish during storage in ice which made from sea water mixed with some plant extracts.

Treatments		Control					Olive leaves (1% w/v)					Garlic heads (1% w/v)					Aloe gel (1% as TSS)						
Days	0	3	6	9	0	3	6	9	12	15	0	3	6	9	12	15	0	3	6	9	12	15	
General appearance	skin	10	7	4	2	10	8	5	4	3	1.5	10	8	7	6	4	2	10	8	7.5	6	3	2
	stiff	10	6	3	1	10	7	5	3	2.5	1	10	8	7	6	4	2	10	8	7	6	3	1
	Betty	10	7	3	1	10	7	5	3	2.5	1	10	7	7	5	4	1	10	8	7	6	3	1
	odour	10	6	3	1	10	6	5	4	3	1	10	7	7	6	3	2	10	7	7	5	3	2
Eyes	clarity	10	7	3	1	10	8	5.5	3	2.5	1.5	10	8	6	5	3	2	10	8	7.5	5	3	2
	shape	10	7	3	1	10	8	5	3	2.5	1.5	10	8	6	5	3	2	10	8	7.5	6	3	2
Gill	colour	10	6	3	1	10	7	5	3	2.5	1	10	8	6	6	3	1.5	10	8	7	5	4	1.5
	smell	10	6	3	1	10	7	5	3	2.5	1	10	8	6	6	3	1.5	10	8	7	5	4	1.5
Average		10	6.5	3.12	1.12	10	7.25	5.06	3.25	2.62	1.18	10	7.75	6.5	5.62	3.37	1.75	10	7.87	7.18	5.5	3.25	1.62
%		100	52	25	9	100	58	40.5	26	21	9.5	100	62	52	45	27	14	100	63	57.5	44	26	13

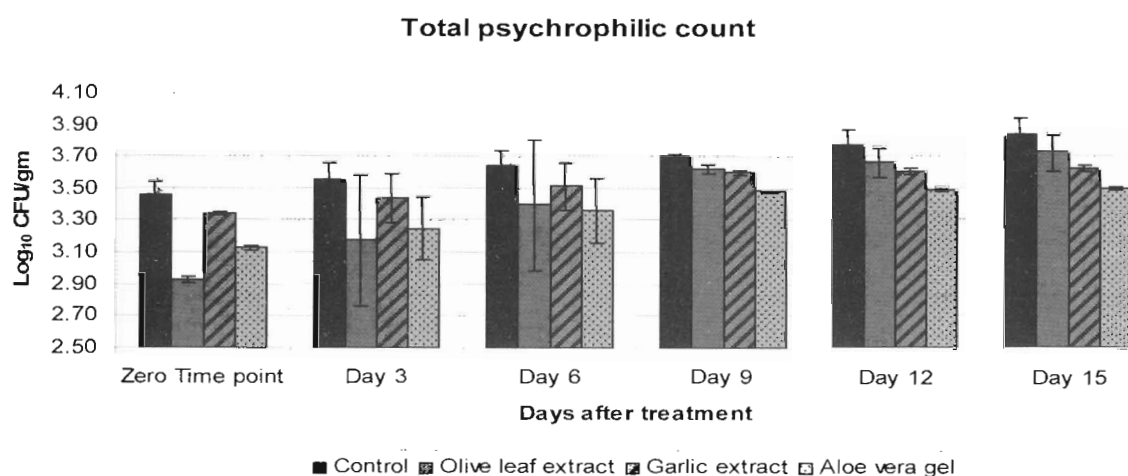


Figure (5): Changes in total psychrophilic count of examined mullet fish during storage under laboratory and market conditions.

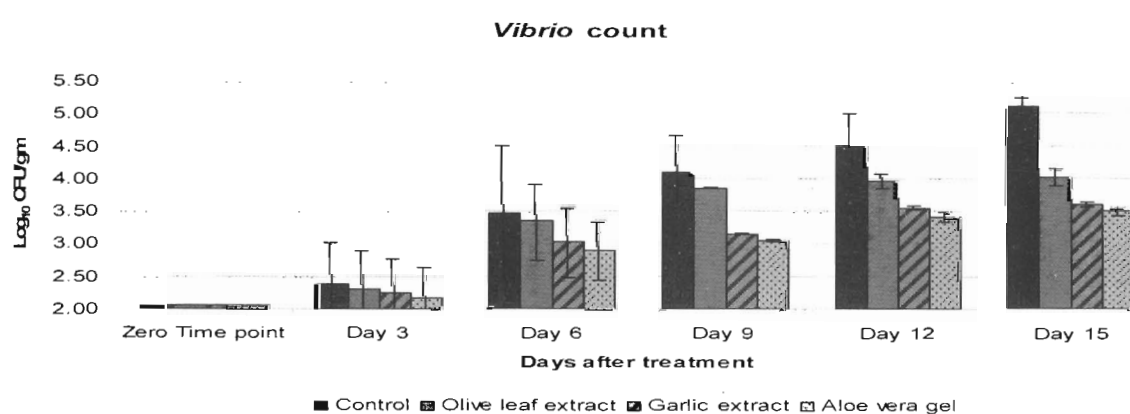


Figure (6): Changes in *Vibrio* spp. count of examined mullet fish during storage under laboratory and market conditions.

CONCLUSION

The microbial contamination has increased during storage time in all treatments; however the preservation of fish in ice made with plants' extracts, especially *aloe vera* gel, decreased the numbers of TBC, Psychrophilic and *Vibrio* counts significantly compared to the control. The best results of sensory evaluation test have recorded with fish samples preserved in ice made with *aloe vera* gel. This study confirms the effectiveness of some plant extracts as natural antimicrobials and recommends the possibility of employing them in fish preservation.

REFERENCES

- Aberoumand, A. (2010). Investigation of some microbiological and chemical parameters associated with spoilage of Cod fish. *World Journal of Fish and Marine Sciences* 2 (3): 200-203.
- Agarry, O., Olaleye, M. T. & Bello-Michael, C. O. (2005). Comparative antimicrobial activities of *Aloe vera* gel and leaf. *Afr. J. Biotechnol.* 4:1413-1414.
- Alemdar, S. & Agaoglu, S. (2009). Investigations of in-vitro antimicrobial activity of *Aloe vera* Juice. *J Anim Vet Adv* 8(1):99-102.
- Ankri, S., Miron, T., Rabinkov, A., Wilechek, M. & Mirelman, D. (1997). Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of *Entamoeba histolytica*. *Antimicrob Agents Chemother*; 41:2286-8.
- APHA. (American Public Health Association) (1992). In C. Vanderzant and D. F. Splittsloesser (Eds.), *Compendium of methods for the microbiological examination of foods* (3rd Ed.). Washington, DC: APHA.
- Bennour, M., El-Marrakchi, A., Bouchriti, N., Hamama, A. & El-Ouadaa, M. (1991). Chemical and Microbiological Assessments of Mackerel

- (Scomber Scombrus) stored in ice. *Journal of Food Protection*, 54, (10) 789-792.
- Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A. & Del Río, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chemistry*, 68, 457-462.
- Black, E. C. (1962). Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish. Res. Board Can.*, 19(3):409-36.
- Cioffi, G., Pesca, M. S., De Caprariis, P., Braca, A., Severino, L. & De Tommasi, N. (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. *Food Chemistry*, 121, 105-111.
- Cutting, C. L. & Spencer, R. (1968). Fish and fish products. Quality control in the food industry. Vol.2. Academic press, London, New York.
- El-Deen, G., El-Shamery, M. R. (2007). Studies on contamination and quality of fresh fish meats during storage. *Egypt. Acad. J. biolog. Sci.*, 2(2): 65-74.
- El-Marrakchi, A., Bennour, M., Bouchriti, A., Hamama, A. and Tagafait, H., (1990). Sensory, chemical and microbiological assessments of Moroccan sardines (sardine olichardus) stored in ice. *J. Food Prot.*, 53 (7): 600-605.
- Faruque, S. M., Nair, G. B. (2008). *Vibrio cholerae: Genomics and Molecular Biology*. Caister Academic Press. ISBN 978-1-904455-33-2.
- Ferro, V. A., Bradbury, F., Cameron, P., Shakir, E., Rahman, S. R., & Stimson, W. H. (2003). In vitro susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. *Antimicrob Agents Chemother* 47, 1137-1139.
- Franze, E., (1970). Plant hygienic aspects of the fish processing industry, *Allgemeine fish Wirtschaftszeitung* 22 (1) 123 - 126.
- Furneri, P. M., Marino, A., Saija, A., Uccella, N. & Bisignano, G. (2002). In vitro antimycoplasmal activity of oleuropein. *Int. J. Antimicrob. Agents* 20, 293-296.
- Gonzalez-Fandos, E., Garcia-Lopez, M. L., Sierra, M. L. & Otero, A. (1994). Staphylococcal growth and enterotoxins (A-D) and thermonuclease synthesis in the presence of dehydrated garlic. *J. Appl. Bacteriol.* 77: 549-552.
- Graham, J. W. A., Johnston, F. J. & Nicholson, (1992). Ice in fisheries. Torry Research Station . Aberdeen, United Kingdom. ISBN 92-5-103280-7.
- Gram, L. (1989). Identification, characterization and inhibition of bacteria isolated from tropical fish. Ph.D. Thesis. The Technological Laboratory of the Danish Ministry of Fisheries and The Royal Veterinary and Agricultural University.
- Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*, 13: 262-266.
- Heggers, J. P., Kucukcelebi, A., Stabenau, C. J., Ko, F., Broemeling, L. D., Robson, M. C., & Winters, W. D. (1995). Wound healing effects of *Aloe* gel and other topical antibacterial agents on rat skin. *Phytother. Res.* 9:455-457.
- Huq, A. & Colwell, R.R. (1995). A microbiological paradox: viable but nonculturable bacteria with special reference to *Vibrio cholerae*. *J. Food Protect*, 59, 96-101.
- Jeyasekaran, G., Ganesan, P., Shakila, J. R., Maheswari, K. & Sukumar. D. (2004). Dry ice as a novel chilling medium along with water ice for short-term preservation of fish Emperor Breams, lethrinus (*Lethrinus miniatus*). *Innovative Food Science and Emerging Technologies* 5:485-493.
- ICMSF. (1996). Microorganisms in foods. 5. Characteristics of microbial pathogens. International Commission on Microbiological Specifications for Foods (ICMSF) Blackie Academic & Professional, London.
- Kathi, J. & Kemper. (2000). Garlic (*Allium sativum*). Longwood Herbal Task Force. The Center for Holistic Pediatric Education and Research. MD, MPH.
- Larsen, J. L., Jensen, N. C. & Christensen, N. O. (1978). Water pollution and the ulcer- syndrome in the cod (*Gadus morhua*). *Vet. Sci. Commun.* , 2:207-16.
- Lauzon, H. L., Margeirsson, B., Sveinsdóttir, K., Guðjónsdóttir, M., Karlsdóttir, M. G., & Martinsdóttir, E. (2010). Overview on fish quality research. Impact of fish handling, processing, storage and logistics on fish quality deterioration. *Nýsköpun og neytendur, Skýrsla Matis* 39-10, Nóvember, ISSN 1670 – 7192.
- Lawless, J. & Allen, J. (2000). *Aloe vera- Natural wonder care*. Harper Collins Publishers, Hammersmith, pp 5-12.
- Lehane, L. & Olley, J. (2000). Review Histamine fish poisoning revisited. In: *International Journal of Food Microbiology* 58 : 1 -37.
- Mosilhy, S. H., (1995). Studies on handling and preservation of Bardawil lagoon fish, B.Sc. Thesis, Faculty of agriculture, Suez canal University.
- Ocaño-Higuera, V. M., Marquez-Ríos, E., Canizales-Dávila, M., Castillo-Yáñez, F. J., Pacheco-Aguilar, R., Lugo-Sánchez, M. E., García-Orozco, K. D., Graciano-Verdugo, A. Z. (2009). Postmortem changes in cazon fish muscle stored on ice. *Food Chemistry*, 116: 933-938.
- Olafsdóttir, G., Martinsdóttir, E., Oehlenschläger, P., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I. M., Henehan, G., Nielsen, J., & Nilsen, H. (1997a). Methods to evaluate fish freshness in research and industry. *Food Science and Technology*, 8: 258-263.
- Özogul, Y., Özogul, F., Kuley, E., Özkutuk, A. S., Gökbulut, C., & Köse, S. (2006). Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage. *Food Chemistry*, 99(4), 752 – 758.
- Parisi, G., Franci, O., & Poli, B. M. (2002). Application of multivariate analysis to sensorial and instrumental parameters of freshness in

- refrigerated sea bass (*Dicentrarchus labrax*) during shelf life. *Aquaculture*, 214, 153–167.
- Privitera, J. R. (1996). Olive Leaf Extract: A New/Old Healing Bonanza for Mankind, (Covina, CA: Nutriscreen, Inc.).
- Pugh, N., Ross, S. A., ElSohly, M. A., & Pasco, D. S. (2001). Characterization of Aloeride, a new high-molecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. *J Agric Food Chem* 49, 1030–1034.
- Rabinkov, A., Miron, T., Konstantinovski, L., Wichek, M., Mirelman, D. & Weiner, L. (1998). The mode of action of allic: trapping of radicals and interaction with thiol-containing proteins. *Biochim Biophys Acta*;1379:233–44.
- Reynolds, J. E. F. (1996). Martindale- the Extra pharmacopeia Royal Pharmaceutical Society of Great Britain, 3 Ist edn. London.
- Rodríguez, O., Barros-Velzquez, J., Ojea, A., Piñeiro, C., and Aubourg, S., (2003). Evaluation of sensory and microbiological changes and identification of proteolytic bacteria during the iced storage of farmed turbot (*Psetta maxima*). In: *Journal of Food Science*, 68(8), 2764–2771.
- Shah, S.Q.A., Colquhoun, D.J., Nikuli, H.L., Sørum, H., (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environmental Science and Technology*.
- Samya, I. A. H. & Ez-ELrigal, A. (2006). Effect of garlic and monosodium phosphate on some histological, microbiological, chemical and organoleptical properties of silver carp fish fillets during storage. *Egypt. J. Agric. Res.*, 84(1A); 277–294.
- Satish, S., Raveesha, K. A. & Janardhana, G. R. (1999). Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Letters in Applied Microbio.* 28: 145–147.
- Sikorski, Z. E., Kolakowska, K., & Burt, J. R., (1990). Post harvest, biochemical and microbial changes. In Z. E. Sikorski (Ed.), *Seafood: resources, nutritional composition, and preservation*. Boca Raton: CRC Press. Inc (pp. 55–75).
- Singh, R. & Balange, A., (2005). Characteristic of pink perch (*Nemipterus japonicus surimi*) at frozen temperature. *Journal of Food Processing and Preservation*, 29(1) 75–83.
- Suvanich, V., Marshall, D. L. & Jahncke, M. L., (2000). Microbiological and color quality changes of channel catfish frame mince during chilled and frozen storage. *J. Food Sci.* 65:(1) 151–154.
- Thanaa, M. H. E., (1984). Chemical, physical and histology studies on super chilled fish, Ph. D. Thesis, Faculty of Agriculture, Zagazig University.
- Valverde, J. M., Valero, D., Martínez-Romero, D., Guill'en, F., Castillo, S., & Serrano, M. (2004). Novel edible coating base don Aloe vera gel to maintain table grape quality and safety. *J. Agric. Food Chem.* 53(20):7807–7813.
- Yongling, S., Lei, L., Huixing, S., Juan, Y., & Yongkang, L. (2011). Effect of sodium alginate-based edible coating containing different antioxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). *Food Control*, 22, 608–615.
- Ward, P. M., Fasitsas, S., & Katz, S. E. (2002). Inhibition, resistance development, and increased antibiotic and antimicrobial resistance caused by neutraceutical. *Journal of Food Protection*, 65, 528–533.

نشاط بعض المستخلصات النباتية كمضادات لبكتريا فساد الاسماك وأثارها على جودة سمك البوري

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تهدف هذه الدراسة إلى التعرف على تأثير بعض المستخلصات النباتية على التلوث الميكروبي لأسماك العائلة البورية. تم دراسة النشاط الميكروبي لثلاثة مستخلصات نباتية (أوراق الزيتون ، رؤوس الثوم ، وجيل صبار الألوفيرا) على بكتريا الفساد المعزولة من عينات السمك التي تم تجميعها من بحيرة البردويل والتي تم تقييمها من خلال قياس قدرة تثبيط المستخلصات للميكروبات باستخدام طريقة الانتشار على سطح الاطباق ولقد أعطي جيل صبار الألوفيرا أفضل تأثير تثبيطي للبكتريا . تم استخدام مستخلص مائي (١%) لتصنيع الثلج وذلك لدراسة تأثير هذه المستخلصات ضد الميكروبات الطبيعية التي تم عزلها من الأسماك . أشارت النتائج إلى أن التلوث الميكروبي زاد خلال فترة التخزين في كل المعاملات، وبرغم ذلك فإن الاسماك التي تم حفظها في الثلج المصنوع من المستخلصات النباتية خاصة جيل صبار الألوفيرا حيث تم انخفاض أعداد البكتريا الهوائية وكذلك البكتريا المحبة للبرودة والملوحة بمعنوية (<0.05) مقارنة بعينات الكنترول. وأشار اختبار التقييم الحسي إلى أن أفضل النتائج التي تم تحقيقها كانت مع عينات الأسماك المحفوظة في الثلج المصنوع من جيل صبار الألوفيرا. نتائج هذه الدراسة تشكل مؤشرا على التلوث البكتريولوجي المحتمل لسمك البوري، كما تؤكد هذه الدراسة على فعالية بعض المستخلصات النباتية كمضادات بكتيرية طبيعية وتوصي بإمكانية توظيفها في حفظ الأسماك من الفساد الناتج بشكل رئيسي من النشاط الميكروبي.