MICROBIOLOGICAL QUALITY ATTRIBUTES OF NILE PERCH *LATES NILOTICUS* FISH FILLETS TREATED WITH AQUEOUS EXTRACT OF *LEPIDIUM SATIVUM L.* CRESS SEEDS

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Abstract

The antimicrobial activity of the aqueous and methanol extracts of *Lepidium sativum* cress seeds at various concentrations (1, 2 and 3 %) against four strains of gram-negative bacteria including *Escherichia coli, E. coli 157, Salmonella typhimurium* and *Kelbsiella pneumonia*, four strains of gram–positive bacteria including *Staphylococcus aureus*, *Bacillus subtilus, Bacillus megaterium* and *Listeria moncytogenes* serotype, two yeasts including *Candida albicans* and *Sacchromyces cerevisiae* and one fungi (*Aspergillus niger*) was assessed. Also, the effect of spraying with aqueous cress seeds extract on microbiological attributes and shelf life of Nile perch fish fillets was also studied. The results indicated that all concentrations of aqueous extract were active against all tested microorganisms except *Klebsiella pneumonae*. On the other hand, the methanol *Lepidium sativum* extract at 1 and 2 % had no effect on the growth of all gram-negative bacteria. Meanwhile, the above mentioned concentrations of methanol extract had detectable effects on the gram–positive bacteria, yeasts and fungi. Also, the antimicrobial effect of aqueous and methanol extracts was increased with increasing the concentrations. The results also showed that the distilled water was better than methanol for extracting antimicrobial substances from this seeds. Therefore, spraying of Nile perch fish fillets with various concentrations (1, 2 and 3%).of the aqueous extract was the suggested treatment to reduce the microbial load and extend the shelf life.

INTRODUCTION

Nile perch *Lates niloticus* is the most important commercial fish species in Egypt, it is often displayed for sale to consumers' in the form of fillets. According to professionals in fish sales, the consumption of fillets will continue to increase in the future, due to new lifestyle habits as people have less time to cook, and the younger generations' increasing habit of consuming prepared products (GIAUC, 2006). Fish is an important source of high-quality proteins for humans (Tidwell and Allan, 2001). However, it is highly susceptible to both microbiological and chemical deterioration due to its high water activity, neutral pH, relatively large quantities of free amino acids

and presence of autolytic enzymes (Jeyasekaran, *et. al.,* 2006). Cold storage and freezing are the normally employed methods for fish preservation, but they do not completely inhibit the quality deterioration of fish (Jeon *et. al.,* 2002).

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are part of essential oils, tannins, terpenoids, alkaloids, and flavonoids. These metabolites have been found in vitro to have antimicrobial properties. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. Several reports are available in literature regarding the antimicrobial activity of plant crude extracts and the procedure for the bioassay-guided fractionation to yield active principles from these plants (Parekh and Chanda, 2008).

Lepidium sativum L., (LS) cress, locally known as Rashad, is a fast-growing annual tall herb with an erect stem belonging to the Brassicaceae family. *Lepidium sativum* plant and seeds are considered one of the popular medicinal herbs used in many countries. A number of recent studies pointed out the traditional uses of *Lepidium sativum* seeds extract in controlling many clinical problems (Eddouks *et. al*., 2002). Also, in controlling of many microorganisms, it was found that petroleum ether, methanol and water extracts of *Lepidium sativum* seeds had antimicrobial activity against five bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and one yeast (*Candida albicans*) using 2.5, 5 and 10% concentrations (Adam *et. al.,* 2011). They showed that the petroleum ether was the best solvent for extracting antimicrobial substances from this plant compared to methanol and water. Also Akrayi and Tawfeeq (2012) studied the antibacterial effect of ethanolic and aqueous extracts of *Lepidium sativum* against some gram negative and gram positive bacteria such as (*Klebsiella pneumoniae, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus mutans*)**.** The results revealed that, the extracts had an inhibitory effect on all the bacteria under study except *Klebsiella pneumoniae*.

The objectives of this study were to estimate the antimicrobial activity of methanol and aqueous extracts of Egyptian cress seeds at various concentrations (1, 2 and 3%) against numerous microorganisms using the Disc Assay Procedure, and then evaluate the effect of spraying with above mentioned concentrations of aqueous cress seed extract on the microbiological quality attributes and shelf life of Nile perch

fish fillets during displaying under conditions resembling that in the fish market (6 hrs) at room temperature and through cold storage at 4±1°C up to spoilage.

MATERIALS AND METHODS

Materials

Fish

Fresh Nile perch fish *Lates niloticus* was purchased from the private sector shop in the local fishery market at Giza, Egypt. It was transferred to the laboratory of Meat and Fish Res. Dept., FTRI using an ice box within 45 min.

Cress seeds

Cress *Lepidium sativum* seeds were obtained from a local market in Giza, Egypt.

Bacterial strains

Eight strains of bacteria representing gram- negative bacteria including *Escherichia coli,* NRRL B-210*, E coli 0157*, , *Salmonella typhimurium*, and *Kelbsiella pneumonnia* ATTCC700603 and gram – positive bacteria including *Staphylococcus aureus*, NRRL B-313*, Bacillus subtilus* NRRL B-543, *Bacillus megaterium* NRRL B-1366 and *Listeria moncytogenes* serotype NRRL 3105 in addition to two yeast including *Candida albicans* NRRL Y-477 and *Sacchromyces cerevisiae* NRRL Y-12632 and one fungi (*Aspergillus niger* NRRL-3) were obtained from Department of Chemistry of Natural and Microbial product, National Research Center. These microorganisms were kept in our laboratory in the frozen state until used.

Methods

Preparation of aqueous and methanol cress seeds extracts

Various concentrations of the aqueous and methanol extracts (1, 2 and 3 %) were prepared as the fowling: The aqueous extracts were prepared by boiling 1, 2 and 3 g of dried powdered seeds of *L. sativum* separately in distilled water for 10 min and left for 15 min to infuse. Thereafter, the extracts were cooled and filtered to remove particulate matter (Patel *et. al.,* 2009). For the methanol extracts, the cress seeds powder was steeped in methanol for 3 days and then filtered using filter paper (Whatman No.1) and centrifuged at 3000 rpm for 10 minutes. The supernatants were collected separately and stored in sterile bottles and stored at 4±1°C (Akrayi and Tawfeeq, 2012).

Fish fillets and treatments

Fish were washed with tap water, beheaded, gutted, washed again with tap water, and then filleted. The fillets were divided to four groups. The first group left without any treatment, meanwhile, other groups (second, third and fourth) were

sprayed with various concentrations of aqueous cress seeds extract (1, 2 and 3 %), respectively. All groups were kept at room temperature up to 6 hrs, after that stored in refrigerator at 4±1^{o}C up to spoilage. Analyses were carried out at zero, 6 hrs at room temperature and 3, 6, 9 and 12 day of refrigerated storage.

Microbiological methods

Determination of antimicrobial activity of different concentrations of aqueous and methanol cress seeds extracts

All microbial cultures were taken out of the freezer and kept at room temperature for one hour in order to get thawed. Two loopfuls of each culture were transferred aseptically into sterile agar slants specific for each organism. They were incubated at 37ºC/ 24hr for bacteria strains and at 25ºC/ 4 days for yeast and fungi strains, then, they were kept in the refrigerator until used. To start work, cultures were activated by transferring two loopfuls from each agar slant into 9 ml of the broth specific for each microorganism. All inoculated broth media were incubated at 37ºC/ 24hr for bacteria strains and at 25ºC/ 4 days for yeast and fungi strains, 0.1ml of each culture was inoculated into 9 ml of its respective broth medium and incubated at 37ºC/ 24hr for bacteria strains and at 25ºC/ 4 days for yeast and fungi strains. The count of each culture was determined, followed by applying the Paper Disc Plate method as reported by Loo *et. al.,* (1945).

Paper Disc Plate method

1.0 ml of the above incubated culture (average counts for all strains 1.0 – 7.0 × 10^{6} cfu /ml) was inoculated into 15 ml of sterile agar (45 – 50ºC) specific for each organism. The inoculated agar was poured aseptically into sterile Petri plates. The agar was allowed to solidify. A sterile filter paper of 0.5 cm diameters was saturated with each concentration of both aqueous and methanol cress seed extracts for 30 sec, and then it was placed in the center of each Petri plate containing the inoculated specific agar. The plates were incubated at 37ºC/ 24hr for bacteria strains and at 25ºC/ 4 days for yeast and fungi strains. The diameter of each inhibition zone was determined in mm.

Microbial load of fish fillets

Sample preparation

Ten gram of a representative fish fillets sample were mixed with 90 ml of sterile peptone water (10 g peptone / 1 L distilled water) in a blender, under sterile conditions, to give 1/10 dilution. Serial dilutions were prepared to be used for counting total bacteria count, coliform bacteria, proteolytic bacteria, psychrophilic bacteria, *Staphylococcus aureus* and yeast and mold counts.

Bacteriological methods

Total bacterial count (TBC), *Staphylococcus aureus*, coilform bacteria, proteolytic bacteria, Psychrophilic and yeast & mold counts of fish were determined by using nutrient agar, Baird-parker, MacConkey, nutrient agar medium plus 10% sterile skim milk, nutrient agar and Potato dextrose agar media, respectively according to the procedures described by Difco Manual (1984). Incubations were carried out at 37ºC / 48 hrs for TBC, at 37ºC / 24 hrs for *Staphylococcus aureus* and coliform bacteria, at 30 ºC for 3 days for proteolytic bacteria, at 7ºC / 10 days for psychrophilic and 25ºC / 5 days for yeasts & molds counts.

RESULTS AND DISCUSSION

Antimicrobial effect of aqueous and methanol extracts of cress seeds

The antimicrobial effect of aqueous and methanol extracts of cress seeds at various concentrations (1, 2 and 3%) against four strains of gram negative bacteria, *Escherichia coli*, *Escherichia coli 157, Salmonella typhimurium, Klebsiella pneumonae,* four strains of gram positive bacteria, *Staphylococcus aureus, Bacillus subtilus, Bacillus megaterium, Listeria moncytogenes* and two yeasts strains, *Candida albicans, Sacchromyces cerevisiae* as well as one fungi, *Aspergillus niger* are summarized in Table (1). The results indicated that various concentrations (1, 2, and 3%) of aqueous extract of *Lepidium sativum* were active against all tested microorganisms except *Klebsiella pneumonae*, which was high resistant for the above mentioned concentrations, this may be attributed to the presence of a capsule in *K. pneumoniae* structure that protects it from the effect of plant extract or prevents the entrance of these extracts to inner of the cell (Akrayi and Tawfeeq, 2012). Also, the inhibition zone for all studied microorganisms was increased with the increasing the concentrations of aqueous *Lepidium sativum* extract. This might be attributed to the presence of some chemical components which had antimicrobial activity such as flavonoids, alkaloids, strerols and/or triterpens, tannins and glucosinolaters (Brotonagoro and Wiharti, 2001).

The concentrations of methanol *Lepidium sativum* extracts (1, 2, and 3%) showed different effects against tested microorganisms, whereas, 1 and 2% concentrations had no effect on the growth of all gram negative bacteria. On the other hand, the same concentrations had detectable effects on gram positive bacteria, yeast and fungi consequently the latter microorganisms were more sensitive than gram negative bacteria. Also, from the same Table, it could be observed that the highest inhibitory action of methanol *Lepidium sativum* extract against all gram

negative bacteria strains except *Klebsiella pneumonae* was recorded at 3% concentration.

Table 1. Antimicrobial activity as diameters inhibition zone (mm) of aqueous and methanol extracts of cress seeds at various concentrations

Tested Organisms	Aqueous extract			Methanol extract		
	1.0 %	2.0 %	3.0 %	1.0 %	2.0 %	3.0 %
Escherichia coli,	9	11	14	-	-	9
Escherichia coli 157	8	10	13	-	-	7
Salmonella typhimurium	11	14	15	-	-	5
Kelbsiella pneumonnia	-	-	-	-	-	-
Staphylococcus aureus	15	16	18	7	8	10
Bacillus subtilus	12	14	17	7	9	12
Bacillus megaterium	9	11	15	5	7	10
Listeria moncytogenes	14	16	17	9	11	13
Candida albicans	9	11	14	5	9	11
Sacchromyces cerevisiae	12	15	18	8	10	15
Aspergillus niger	10	14	16	6	8	12

From the above mentioned results, it could be illustrated that the antimicrobial effects of aqueous and methanol *Lepidium sativum* extracts were more pronounced on gram positive bacteria than gram negative bacteria. In this concern, several studies showed that gram negative bacteria had higher resistance to plant extracts than gram positive bacteria (Kudi *et. al.,* 1999 and Palombo and Semple, 2001). This can be as a result of the variation in the cell wall structures of gram positive bacteria and gram negative bacteria. More specifically, gram negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substrances including antibiotics (Palombo and Semple, 2001). The results also showed that the distilled water was the best solvent for extracting antimicrobial substances from *Lepidium sativum* seeds compared to methanol. This was shown by its high inhibitory effect against all tested microorganisms at the studied concentrations. These results were in agreement with findings of Adam *et. al.* (2011) and Akrayi and Tawfeeq (2012).

Microbial load of Nile perch fish fillets as affected by various concentrations of aqueous seeds extract

1- Total bacterial count

The data presented in Table (2) shows total bacterial count (TBC) of fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and cold-storage at 4±1^{o}C up to spoilage. It was observed that the initial total bacterial count of fresh fish fillets was 5.1 × 10^3 cfu / g for control (untreated), this value slightly decreased to 2.48 × 10^3, 2.43 × 10^3 and 1.80 × 10^3 cfu / g after 30 min from treatment of Nile perch fillets by spraying with aqueous cress seeds extract at 1, 2 and 3% concentrations, respectively. After leave the fish fillets either untreated or treated for 6 hrs at room temperature, it was found that the total bacterial count increased markedly for untreated fillets than treated fillets, this may be due to the cress seeds contain some chemical compounds which had antimicrobial effect as reported by Al-Yahya *et. al.,* (1994) and Adam *et. al.,* (2011).

With cold storage period at 4±1^{o}C, the total bacterial count showed an increasing trend for all samples. The increase was higher for untreated sample compared to treated samples. This indicates the effectiveness of aqueous cress seeds extract for inhibiting the growth of microorganisms. Also, TBC of fish fillets was decreased with increasing the aqueous cress seeds extract concentrations at any time of cold-storage. The control sample (untreated fillets) reached 5.44 × 10^6 cfu / g in the 6th day of cold-storage. It exceeds the maximal permissible limit of 10^6 cfu / g for the total bacterial count in chilled-fish (Egyptian Standard, 2005), while the TBC of treated samples reached about 10^6 in the 12th day of cold-storage. Therefore, the treatment of Nile perch fish fillets with aqueous cress seeds extract was more effective to maintain high microbiological quality and extending the shelf life during the displaying of fillets in the fish markets under normal conditions and during cold-storage.

Table 2. Total bacterial count (cfu / g) of Nile perch fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4°C up to spoilage

Treatments / Storage	Control	1.0 %	2.0 %	3.0 %
Zero time	5.1×10^3	2.48×10^3	2.43×10^3	1.80×10^3
6 hrs	2.25×10^4	8.89×10^3	6.15×10^3	3.51×10^3
3 days	2.88×10^5	6.54×10^4	1.0×10^4	5.5×10^3
6 days	5.44×10^6	1.06×10^5	8.11×10^4	6.98×10^4
9 days	1.45×10^7	9.21×10^5	5.05×10^5	2.42×10^5
12 days	7.65×10^7	6.45×10^6	3.63×10^6	1.04×10^6

2- Coilform bacteria

Data in Table (3) shows coilform bacteria counts of fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4±1°C up to spoilage. At zero time and after 6 hrs of storage at room temperature, it was noticed that coilform bacteria appeared in control and fish fillets treated with1.0 % aqueous cress seeds extract, but the latter sample had lower coilform bacteria than control (untreated), meanwhile coilform bacteria not detected in fish fillets treated with 2 and 3 % aqueous cress seeds extracts at zero time and during cold-storage, this indicates that the treatment of fish fillets with 1.0 % aqueous cress seeds extract led to partially inhibition for coilform bacteria, while concentrations 2.0 and 3.0 % had complete inhibition for this bacteria, this may be due to 2 and 3 % of aqueous cress seeds extracts had higher some chemical compounds having antimicrobial activity than 1% concentration. During cold-storage at 4±1°C, coilform bacterial count increased for both control and that treated with 1.0 % aqueous cress seeds extract, but the rate of increase was lower for the samples treated by aqueous cress seeds extract, this may be due to that the cress seeds have antimicrobial activity as reported by Al-Yahya *et. al.,* (1994) and Adam *et. al.,* (2011). The control sample reached 3.34×10^3 cfu / g in the 9th day of cold-storage. Meanwhile fish fillets treated with 1.0 % not reached 10^3 at the same day, this may be attributed to the inhibitory effect of cress seeds extract.

Table 3. Coilform bacteria (cfu / g) of Nile perch fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4°C up to spoilage

Treatments / Storage	Control	1.0 %	2.0 %	3.0 %
Zero time	3.5×10^1	2.5×10^1	ND	ND
6 hrs	6.5×10^1	4.0×10^1	ND	ND
3 days	1.28×10^2	8.5×10^1	ND	ND
6 days	6.3×10^2	1.27×10^2	ND	ND
9 days	3.34×10^3	8.25×10^2	ND	ND
12 days	5.12×10^3	2.14×10^3	ND	ND

ND = Not Detected

3- Proteolytic bacteria

Proteolytic bacterial counts of fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4±1°C up to spoilage are presented in Table (4). At zero time, it was observed that proteolytic bacteria appeared in all fish fillets samples, but the counts in treated samples with various concentrations were slightly lower than control sample, this reflex the inhibitory effect of aqueous cress seeds extract. The population of proteolytic bacteria was decrement with increment aqueous cress seeds extract concentrations not only at zero time, but also at any time of cold storage. From the same Table, it could be noticed that, proteolytic bacteria grew fast in control sample compared to fish fillets treated with various concentrations of aqueous cress seeds extract especially (2.0 and 3.0 %) during storage. Proteolytic bacteria of control sample rapidly increased from the initial count 9.5×10^1 to 4.96×10^2 cfu / g by the end of displaying time at room temperature up to 6 hrs and reached 9.05×10^5 cfu / g by the end of cold storage (12^{th} day). On the other hand, treatment of fish fillets with various concentrations of aqueous cress seeds extract lowered the population of proteolytic bacteria. The percentage of decrement after 6 hrs at room temperature was 64.71, 81.85 and 86. 89 % of fish fillets treated with concentrations of 1.0, 2.0 and 3.0 % of aqueous cress extracts respectively, compared with control sample. These percentages were increased to 91.85, 96.06 and 97.81 %, respectively when compared with control sample at the end of cold storage.

Table 4. Proteolytic bacteria (cfu / g) of Nile perch fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4°C up to spoilage

Storage \ Treatments	Control	1.0 %	2.0 %	3.0 %
Zero time	9.5×10^1	6.0×10^1	4.5×10^1	3.0×10^1
6 hrs	4.96×10^2	1.75×10^2	9.0×10^1	6.5×10^1
3 days	2.76×10^3	8.17×10^2	4.3×10^2	2.03×10^2
6 days	1.65×10^4	6.85×10^3	2.7×10^3	9.45×10^2
9 days	8.98×10^4	2.04×10^4	8.62×10^3	3.91×10^3
12 days	9.05×10^5	7.38×10^4	3.57×10^4	1.98×10^4

4- *Staphylococcus aureus*

Data presented in Table (5) show *Staphylococcus aureus* counts of fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and during cold storage at 4±1°C. up to spoilage. From these results it could be noticed that, *Staphylococcus aureus* appeared in all fish fillets samples except fish fillets treated with 3 % aqueous cress seeds extract at zero time. The initial *Staphylococcus aureus* count of the untreated sample was 6.5×10^1 cfu / g, which reduced to 2.5×10^1 and 2.0×10^1 cfu / g for samples treated with 1 and 2 % aqueous cress seeds extract. *Staphylococcus aureus* not detected in fish fillets treated with 3 % aqueous cress seeds extract either at zero time or along cold storage period. With advancement of storage, the counts of *Staphylococcus aureus* showed an increasing trend. The increase was higher for control compared to treated samples. This indicates the effectiveness of aqueous cress seeds extract in inhibiting the growth of *Staphylococcus aureus*. Also the counts of *Staphylococcus aureus* in fish fillets were decreased with increasing with of aqueous cress seeds extract concentrations at any time of storage. The control sample reached 3.18×10^3 cfu / g in the 9th day of cold-storage, it was exceed the maximal permissible limit of 10^3 cfu / g for *Staphylococcus aureus* in chilled fish (Egyptian Standard, 2005), while *Staphylococcus aureus* of fish fillets treated with 1 and 2 % aqueous cress seeds extract (9.03×10^2 and 5.73×10^2 cfu / gm, respectively) was not exceeded the maximal permissible limit. Moreover, at the end of cold storage (12th day), concerning *Staphylococcus aureus*, fish fillets which treated

with 2 % aqueous cress seeds extract, not exceeded the maximal permissible limit which reported by (Egyptian standard, 2005).

Table 5. Staphylococcus aureus (cfu / g) of Nile perch fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4oC up to spoilage

Treatments / Storage	Control	1.0 %	2.0 %	3.0 %
Zero time	6.5×10^1	2.5×10^1	2.0×10^1	ND
6 hrs	8.5×10^1	3.5×10^1	3.5×10^1	ND
3 days	3.4×10^2	9.0×10^1	5.0×10^1	ND
6 days	9.65×10^2	3.55×10^2	1.62×10^2	ND
9 days	3.18×10^3	9.03×10^2	5.73×10^2	ND
12 days	7.0×10^3	2.41×10^3	8.97×10^2	ND

5- Psychrophilic bacteria

Microorganisms which grow in food at refrigeration temperature have usually been called psychrophilic. The gram- negative psychrophilic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Ibrahim, 2007). Psychrophilic bacteria of fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4±1°C. up to spoilage are presented in Table (6). The initial psychrophilic bacteria count of control was 6.17×10^2 cfu / g which reduced to 2.61×10^2 , 7.0×10^1 and 5.5×10^1 cfu / g for fish fillets treated with 1, 2 and 3 % aqueous cress seeds extract, respectively. The above mentioned initial psychrophilic bacteria count was lower than the values reported by Ojagh *et. al* (2010) for fresh trout fillet which found to be 3.85 $\log_{10}$ cfu / g.

During cold-storage, psychrophilic bacteria count gradually increased for all fish fillets samples, but the rate of increase was lower for the samples treated by aqueous cress seeds extract than control sample. This may be due to the cress seeds contain some photochemical compounds which have antimicrobial activity as reported by Al-Yahya *et. al.,* (1994) and Adam *et. al.,* (2011). The data of psychrophilic bacteria of fish fillets as affected by using various concentrations of aqueous cress seeds extract during storage period is found in Table (6). From the results, it could be observed that, psychrophilic bacteria of control reached1.87×10^6 cfu / g by the end of cold storage (12^{th} day), meanwhile, reached 5.18×10^5, 8.65×10^4 and 2.53×10^4 cfu / g for fish fillets treated with 1, 2 and 3 % aqueous cress seeds extract. These

results are partially in agreement with those obtained by Hernandez, *et. al.*, (2009) who found that psychrophilic aerobic count in meagre fillets was 2.82 log cfu / g at zero time and reached to 10.8 log cfu / g after 18 days in ice storage.

Table 6. Psychrophilic bacteria (cfu / g) of Nile perch fish fillets treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) during storage at room temperature up to 6 hrs and at 4°C up to spoilage

Storage \ Treatments	Control	1.0 %	2.0 %	3.0 %
Zero time	6.17×10^2	2.61×10^2	7.0×10^1	5.5×10^1
6 hrs	8.19×10^2	4.96×10^2	1.10×10^2	9.0×10^1
3 days	5.17×10^3	1.27×10^3	8.5×10^2	4.61×10^2
6 days	6.75×10^4	9.85×10^3	3.76×10^3	1.21×10^3
9 days	4.79×10^5	8.12×10^4	2.68×10^4	8.5×10^3
12 days	1.87×10^6	5.18×10^5	8.65×10^4	2.53×10^4

6- Yeast sand molds counts

All fish fillets samples whether the untreated or treated with various concentrations of aqueous cress seeds extract (0.0, 1.0, 2.0 and 3.0 %) were completely free from yeasts sand molds either at zero time or during storage period.

CONCLUSION

From the above study, it was concluded that the aqueous cress seeds extract with various concentrations (1.0, 2.0 and 3.0 %) has antimicrobial activity against many microbial strains. Also, treatment of Nile perch fish fillets with this extract led to maintain high microbiological quality and extending the shelf life

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خصائص الجودة الميكروبيولوجية لشرائح سمك قشر البياض المعامل بالمستخلص المائي لبذور حب الرشاد

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أجري هذا البحث بهدف التعرف على النشاط المضاد لنمو الميكروبات للمستخلص المائي والميثانولي لبذور حب الرشاد المحضر بتركيزات المختلفة (1، 2، 3 %) لكل منهما حيث أجريت الدراسة على أربع سلالات من البكتريا السالبة لجرام (Escherichia *coli,* E. *coli 157, Salmonella* (*typhimurium* and *Kelbsiella pneumonia*. وأربع سلالات أخري من البكتريا الموجبة لجرام (*Staphylococcus aureus*, *Bacillus subtilus, Bacillus megaterium*, *Listeria moncytogenes* serotype) وسلالتين من الخمائر (*Candida albican, and Sacchromyces cerevisiae*) وسلالة فطر (*Aspergillus niger*). كذلك تم تقييم التركيزات المختلفة للمستخلص المائي لبذور حب الرشاد على خصائص الجودة الميكروبيولوجية لشرائح سمك قشر البياض في ظروف تماثل عرضها للبيع على درجة حرارة الغرفة وكذلك أثناء تخزينها بالتبريد على درجة حرارة 4 ْم حتى الفساد.

وبصفة عامة أشارت النتائج إلي أن المستخلص المائي بتركيزاته المستخدمة له تأثير مضاد لنمو جميع الميكروبات بدرجات مختلفة تبعا لنوع الميكروب باستثناء بكتريا *Kelbsiella pneumonia* التي أظهرت مقاومة عالية لجميع التركيزات للمستخلص المائي والميثانولي. كما أوضحت النتائج أن تركيز 1 و 2 % من المستخلص الميثانولي له تأثير مضاد لنمو جميع الميكروبات باستثناء البكتريا السالبة لصبغة جرام بينما تركيز 3 % له تأثير مضاد لنمو جميع أنواع الميكروبات تحت الدراسة. كما أشارت النتائج إلي أن معاملة شرائح سمك قشر البياض بالتركيزات المختلفة للمستخلص المائي أدت إلي تقليل الحمل الميكروبي وزيادة مدة صلاحيتها.