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Effect of pomegranate and moringa extracts on *E.coli* load in *Nile tilapia*

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ABSTRACT

Pomegranate and *Moringa oleifera* are useful food of great interest and they have multiple useful effects on human health. Pomegranate and *Moringa oleifera* are used to increase the nutritional and hygienic quality of food by using their extracts. The antibacterial action of ethanolic extract of (1% pomegranate, 1% *Moringa oleifera* and 1%mixturefrom both) were tested against *E.coli* (O128:H2) artificially inoculated into fresh Tilapia fillet and the inoculated samples were stored at 4 ± 1°C then analyzed for their sensory characteristics and *E. coli* count. The obtained results showed that the sensory attributes of all treated and control samples were acceptable for all judgment members either fresh or during storage. The combinations of 1% Pomegranate peels and 1%*Moringaoleifera*had the highest inhibitory effect against *E.coli* population (p<0.05) during the storage periods. It was concluded that the ethanolic extracts of both Pomegranate peels and *Moringa oleifera* leaves could be used for fish preservation where it improve its quality and increase its shelf life.

1. INTRODUCTION

Fish safety as food is an extremely vital part essential to protect fish consumers and warranty the sustainability of the industry (Mchazime and Kapute, 2018).

Nile tilapia (*Oreochromis niloticus*) is the most usually cultured species among tilapia in many countries in the world (Salem, 2015).

The level of bacterial pathogens in fish flesh is related to environment and handling procedures in food market and restaurants which could be transferred to persons in contact and result in foodborne sickness(FPB, 2017).Non-pathogenic *E. coli* strains can cause disease if they extend outside from the intestine to other organs. The pathogenic strains of *E. coli* may cause diarrhea by releasing toxins (called enterotoxigenic *E. coli* or ETEC) and may be the cause of food decomposition in fish (Soliman et al., 2010).

The medicinal plants were used as food additives in aquaculture due to their ability of enhancing the fish immune system (Van Hai, 2015).

"Moringa" tree is measured as one of the world's most beneficial trees, as almost every part of the Moringa tree can be used for food or has some other valuable properties. It contains extra vitamins than carrots, extra calcium than milk, higher iron than spinach, higher vitamin C than oranges, and more potassium than bananas; the protein quality of Moringa leaves even as that of milk and eggs(Devendra et al., 2011).

The Bio-chemical presence of phytochemicals in "*Moringa oleifera*" has presented positive result for tannin, flavonoid and alkaloid. These phytochemicals have capability to fight against microorganisms or inhibit the growth of microorganisms (Kruti et al., 2018).

Pomegranate plant *(Punicagranatum L.)* is a fruit which is rich source of phytochemicals such as tannins and other phenolic. Pomegranate peel extracts were informed to have notable antioxidant, antibacterial, anti-inflammatory and hypolipidemic bioactivities (Panichayupakaranant et al., 2010).The phytochemical analysis of pomegranate proved thatit is a rich source of antioxidant has revealed the presence of flavones, luteolin, glycosides, alkaloids, organic acids and tannins. The pomegranate extract has vital polyphenols as gallotannins, ellagitannins, punicalagin and punicalin(Nisha et al., 2015).

Therefore, the purpose of this study was to examine the inhibitory effect of ethanolic extracts of pomegranate peels PPE1%,*Moringa oleifera* MOE1% and mixture of both against *E.coli* (O128:H2) artificially inoculated into fresh tilapia fillet stored at 4°C.

2. MATERIAL AND METHODS

2.1. Plant materials:

Pomegranate peel extract (PPE)and *Moringa oleifera extract*(MOE)were purchased from Animal Health Research Institute (AHRI), Egypt.

2.2. Fish:

Accurately 1.250 g fresh tilapia fillets were obtained from the supermarkets in EL-Gharbia governorate. Fish was divided into 5 groups for control positive, control negative, PPE 1%, MOE 1% and mixture of both(Each group 250 g)

2.3. Bacterial strain:

E. coli strain O128: H2was reference strain obtained from Animal Health Research Institute, Egypt. The preparation

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of *E.coli* inoculum was done according to Abdallah(2016)as follows**:** *E. coli* was sub-cultured from stock cultures in sterile bottles containing nutrient broth incubated over-night at 37°C. Directly, prior to the experiment, fresh microbial cultures were adjusted to 0.5 McFarland to be equivalent to about 10^6 CFU/ml. The initial load after 30 min was 10^9 CFU/ g.

2.4 Sensory examinations:
A nine-point Hedonic scale was used to score samples for eye color, odor/smell, skin feel/texture, skin color and overall acceptability according to Sugri et al. (2010)**.**

2.5 Escherichia coli count:
E. coli count was determined on (EMB) agar (Oxoid) according to ISO (2007). Which were then incubated at 37 °C for 24 hrs. Suspected colonies of green metallic color with dark purple center was counted.
The experiment was repeated 3 times individually.

2.6 Statistical analysis:
The data were analyzed and the mean and Standard Error (S[) were calculated by one-way ANOVA test according to Statistical Analysis System Users Guide SAS (2004) software and probability of ($p<0.05$) (SAS Institute Inc. 2004).

3. RESULTS

Table (1)showed that MOE1% acceptability till 5th day. While PPE 1%showed overall acceptability extended to 6th day of cold storage. PPE 1%plusMOE 1%showed acceptability till 7th day While, control negative showed acceptability till 3rd day. In contrast, the control positive groups showed overall acceptability till 2th days.
Table (2)showed that MOE 1%reduced *E. coli* count that inoculated in fish during storage to 7.21±0.05, 6.82±0.05, 6.35±0.03, 5.38±0.03, 4.68±0.01and 5.33±0.1with reduction percent reached to 49.29% after 4 days storage.
Table (3)showed that PPE 1% reduced *E. coli* count that inoculated in fish during storage to 4.62±0.03with reduction percent reached to 49.94% after 5 days storage. While, PPE 1% plus MOE 1%reduced *E. coli* count that inoculated in fish during storage to 4.25±0.13with reduction percent reached to 53.95% after 6 days of storage.

Table 1 Sensory evaluation of control and Pomegranate peel and *Moringa oleifera* extracts on counts of *E.coli* (log cfu/g) artificially inoculated into Tilapia fish fillet during cold storage at 4ºC

Groups	Zero day	1st day	2ndday	3rd day	4th day	5th day	6th day	7th day
Control-VE	8	7	6	R	-	-	-	
Control+ VE	8	6	R	-	-	-	-	
MOE 1%	8	7	7	6	5	R	-	
PPE 1%	8	8	8	7	6	6	5	R
MOE 1%plus PPE 1%	8	8	8	7	7	6	6	5

Score System for Sensory Evaluation (Sugri et al., 2010): 9: Excellent. 8: Very very good. 7: Very good. 6-good. 5: Medium. 4: Fair. 3: Poor. 2: Very poor. 1: Very very poorR: rejecter

Table 2 The effects of Pomegranate peel and *Moringa oleifera* extracts on counts of *E.coli* (log cfu/g) artificially inoculated into Tilapia fish fillet during cold storage at 4ºC.

Group	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control –VE	-	-	-	-	R			
Control +VE	7.42±0.30^a	7.56±0.02^a	7.85±0.06^a	R	-	-	-	-
MOE1%	7.21±0.05^a	6.82±0.05^a	6.35±0.03^b	5.38±0.03^a	4.68±0.01^b	5.33±0.1^b	R	-
PPE 1%	7.00±0.05^a	6.68±0.03^a	6.23±0.03^a	5.89±0. 2^b	4.77±0.03^b	4.62±0.03^b	4.93±0.16^b	R
MOE 1%plus PPE 1%	6.51±0.2^b	5.91±0.06^b	5.33±0.3^c	5.13±0.11^c	4.35±0.02^b	4.22±0.16^b	4.25±0.13^a	4.4±0.21^b

Initial load of *E.coli* at zero time= 9. 23±0.33log cfu/g. The values represent Mean ± SD of three experiments. Means within a column followed by different letters are significantly different ($P < 0.05$).

Table 3 Reduction % of *E. coli* (log cfu/g) artificially inoculated into Tilapia fish fillet during cold storage at 4ºC.

Group	Zero day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control +VE	19.60	18.09		-	-	-	-	-
MOE1%	21.88	26.11	31.20	41.71	49.29	42.25	-	-
PPE1%	24.16	27.62	32.50	36.18	48.32	49.94	46.58	-
MOE 1%plus PPE 1%	29.46	35.96	42.25	44.42	52.87	54.27	53.95	52.32

4. DISCUSSION

Results given in table (1) indicated that treatment with PPE 1%plus MOE 1% had the highest score among other treatments and control fish. While MOE 1% had lower score among other treatments and control fish. On the other hand, all treatments had about 60% (6/10) of total scores at the end of storage and were accepted.
All treated and control fish were acceptable for all judgment members either it was fresh or stored. This means that the PPE 1%plus MOE 1% can be used in fish preservation. These results were in contract with those reported by Anwar and Bhanger(2003) and Topuz et al.(2014), who showed that *Moringa oleifera* had antibacterial activity and therefore it can be used for fish preservation. Also saucing of anchovy marinades with Pomegranate sauce can delay the adverse quality changes, retard the lipid oxidation and increase the sensory properties. Further,Ibrahim et al., (2018) found fish fillet group treated with PPE showed somewhat acceptable improvement of sensorial characters and extended the shelf life till the 4th day compared with control group during storage period. On the other hand, Gökoğlu et al., (2009)

reported that the reducing of sensory scores is in relation with the increasing of storing time.

Results recorded in tables 2 and 3 showed that MOE 1% reduced *E. coli* count inoculated in fish during storage to 5.38± 0.03with reduction percent of 49.29% after 4 days storage.

The primary screening of the extracts of *Moringa oleifera* found the presence of alkaloids, flavonoids and saponins. The most liable organisms to the antibacterial action of *Moringa oleifera* were *E. coli.* The same rate obtained from the chloroform extract and aqueous extracts on *E. coli* and *S. typhi*. (Abalaka et al.,2012).Also, acetone extract yielded very good antibacterial activity by inhibiting 86% isolates of *E. coli.* Chloroform extracts exhibited poor antibacterial activity and inhibited only *E. coli* (23% isolates)as reported byPatil and Jane (2013). However, Adeyemi et al., (2013) found all Moringa Marinade 1%, 2% and 3% (w/v) solutions on Smoke African catfish (*Clarias gariepinus*) showed antibacterial potency. Also, Onyuka et al.(2013)revealed that *Moringa oleifera*n-hexane extract concentration and time increase; there was decrease in bacterial load in *O. niloticus*.

Water-based MOE strongly inhibited (halo > 13 mm) the growth of *S. aureus*, and *E. coli* isolated from tilapia and shrimp, samples, mainly at 150 and 200 µL/dish, and also were inhibited by ethanol-based moringa extracts at all the volumes tested(Vieira et al., 2010). Also, Manoj et al. (2014)use the Moringa seed coat and pod husks as antimicrobial materials or as a medicinal plant in shrimp.

Active compounds of *Moringa oleifera* have been shown to cause distraction of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other elements of several microorganisms such as *E. coli*(Moreno et al ., 2006). Therefore, the mode of action by the phytochemical ingredients of *Moringa oleifera*is the reason for antibacterial activity nearby causing bacterial enzyme inhibition such as the sortase inhibitory effect, DNA replication, and bacterial toxin causing the lysis of bacterial cells. It had been submitted that pterygospermin acts by the inhibition of the transaminase enzyme and through cell membrane distresses (Onyekaba et al., 2013).

The pomegranate plant (*Punicagranatum*) has high amount of several important phytochemicals, these important phytochemicals make Pomegranate as important source of antioxidant (Nishaet al.,2015).Furthermore about 50% of the total fruit weight is the peel, which is important source of bioactive complexes as phenolic, flavonoids, ellagitannins (ETs), and proanthocyanidin compound.(Li et al.,2006).Also the palatable part of the Pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mostly fructose and glucose, and 1.5% pectin, organic acid such as ascorbic acid, citric acid, and malic acid, and bioactive complexes such as phenolic and flavonoids, principally anthocyanin(Tezcan et al., 2009).

Results recorded in tables 2 and 3 showed that PPE 1% extracts reduced *E. coli* count that inoculated in fish during storage to 4.62± 0.03with reduction percent reached to 49.94% after 5 days storage.

Our results was supported by Mahboubi et al., (2015) who found antimicrobial action of PPE and its elements showed a highly antibacterial activity against Gram positive (*S. aureus*) and Gram negative bacteria (*E. coli*) causing food poisoning and so this extracts can be used for inhibition of food borne diseases or as additive in food industry.Also, Reddy et al. (2007)presented that Pomegranate water and ethanolic and butanolic extracts has antimicrobial activity when tested against *E. coli.* Furthermore, Voravuthikunchai et al. (2004) studied antibacterial and antimicrobial properties of *Punicagranatum*. Extracts from the plant have been found to work against *E. coli.* Further, Pagliarulo, et al.(2015) extracted Pomegranate by-products in 50% (v/v) aqueous ethanol, to check antimicrobial activity against isolated human pathogenic microorganisms their results revealed that both Pomegranate aril and peel extracts have an active antimicrobial activity, as shown by the inhibitory effect on the bacterial growth of two significant human pathogens, mainly *S. aureus* and *E.coli*, which are often causes of food-borne illness.

Sweetie et al. (2010) studied the antimicrobial action of PPE against the common food-borne pathogens such as *E. coli* many concentrations of PE (0.01%, 0.05% and 0.1%) were used to set the minimum inhibitory concentration. Also, Öztürka, et al.(2018) found PPE showed antibacterial effect against L. monocytogenes, *E. coli*, and *S. aureus* ($P<0.05$). As a result of 13 days storage of mackerel fillets coated with alginate enriched with 2.5, 5, 7.5 and 10% PPE at 4 °C, the highest inhibition zone was obtained from 10% PPE and the zone diameters for *E. coli* was determined as 24mm.Furthermore,Martinez-Lorena et al.(2019)found Pomegranate extract late the lipid oxidation, stately as volatile compounds, and the microbiological decomposition in fish patties.

On the other hand, Gill and Holley (2006) suggested that antimicrobial components of the Pomegranate extracts (terpenoid, alkaloid and phenolic compounds) work together with enzymes and proteins of the microbial cell membrane initiating its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes required for amino acids biosynthesis. Also, Khan et al.(2011)proposed that the antimicrobial activity of Pomegranate peel against *E. coli* could most likely be related to its oxidizing property as the active compositions of pomegranate peel (phenolic acids) act as antimicrobial oxidizing agents.

Results recorded in tables 2 and 3 showed that PPE 1% plus MOE 1% reduced *E. coli* count that inoculated in fish during storage to 4.25±0.13with reduction percent of 53.95% after 6 days of storage.

Our results was supported by Elbagory et al. (2019),who explained that pomegranate is a food of great importance and it has a multiple valuable effects on human health, *Moringa oleifera* leaves have a high amount of essential amino acids, iron protein and Vitamin B complex so the Pomegranate and *Moringa oleifera* were used to improve the nutritive and sanitary quality of food products by using their extracts.

5. CONCULSION

From the present study we concluded that Pomegranate and *Moringa oleifera* can be used as natural preservative in fish and fish product as it decrease pathogenic microorganism like *E. coli* and improve sensory quality of fish during cold storage.

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