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Impact of using some essential oils on sensory acceptability and Aeromonas hydrophila contamination of Nile tilapia fish fillet during refrigeration storage

Ghada A. K. Kirrella[*1], Nader Y. Moustafa[1], Dina M. Kishk[2], Reda Abdallah[1]

[1]Department of Food Control, Faculty of Veterinary medicine, Kafrelsheikh University, Kafr El Sheikh, 33516, Egypt.

[2] M.V.Sc, Faculty of Veterinary medicine, Kafrelsheikh University.

[*]**Correspondence: ghada.karala@vet.kfs.edu.eg**

Abstract

Background/aim: Fresh fish is highly susceptible to spoilage with shorter shelf life especially during refrigeration storage. This study aimed to evaluate the effect of essential oils (EOs) of Thyme (*Thymus vulgaris*), Cinnamon (*Cinnamomic zeylanicum*), and Garlic (*Allium sativum*) with concentrations of 0.5 and 1% of each on sensory acceptability of Nile tilapia (*Oreochromis niloticus*) fish fillet during refrigeration storage at 4 ±1˚C and their ability to increase shelf life. The impact of these EOs on *Aeromonas hydrophila* contamination was also investigated.

Results: The results of the sensory evaluation showed that the overall acceptability of control samples significantly decreased as compared to EOs-treated samples. There was a higher significant (P< 0.05) difference in the overall acceptability among different EOs, with the lowest scores in garlic EOs and the highest in 1% thyme oil. There was a positive effect on the shelf-life extension of fish fillet samples which differed according to type and concentration of EOs. The shelf life of samples treated with thyme oil was 12 days in the refrigerator and still fit for human consumption. In addition to reduction percentage of *Aeromonas hydrophila* count with significance difference (P< 0.01) among different EOs showed better results for thyme oils compared to other EOs.

Conclusion: Using EOs reduced *Aeromonas hydrophila* growth without any adverse effect on the sensory attributes of the treated Nile tilapia fillets samples.

Keywords: Essential oils; Shelf life; Nile tilapia, sensory acceptability, Aeromonas hydrophila

1. Introduction

Fish is an excellent nutritive value food having high-quality protein and a large variety of vitamins and minerals (Pal et al., 2018). In contrast, due to its high moisture and nutrient content with a tendency for higher pH, fish is highly susceptible to spoilage even during refrigerator storage with shorter shelf life (Li et al., 2012). This spoilage is responsible for the initial loss of freshness affecting the sensory characters of fish. Fish act as a source of foodborne pathogens, including Aeromonas species that have been known as emerging foodborne pathogens of serious threat to a public health concern (Igbinosa et al., 2012). Sensory testing plays an important role in food quality evaluation where the ultimate criterion for eating quality of any food is a human response (Mlian et al., 2017).

Various studies reported that usage of chemical and/or synthetic food additives can lead to intoxications, degenerative diseases, and even cancer. This generates the need to look for alternative methods to extend shelf life and cover some antimicrobial properties. Recently, food preservation techniques have shown considerable interest in utilizing essential oils of natural substances extracted from medicinal and aromatic plants due to their ability to control the growth of pathogenic microorganisms (Aminzare et al.,2016).

Many trials attempt to reduce the incidence of Aeromonas species that essential oils can be added to fish flesh. It was noticed in comparison to several other mild preservation procedures like smoking, low dose irradiation, the addition of protective cultures, or high-pressure treatment that essential oils are inexpensive and uncomplicated methods of extending shelf-life of fish flesh (Mejlholm and Dalgaard, 2002). These natural preservatives could perfectly meet the increasing consumer demands for clean-label products that are fresh and free of chemical or synthetic additives therefore, using natural essential oils to preserve fish quality and extend shelf life are required (Hassoun and Çoban, 2017).

As consumers tend to adopt towards eating food containing no chemicals; essential oils come to fulfill part of the increasing demand for products of “green image" being obtained from natural sources (Holley and Patel, 2005). It was confirmed by in vitro experiments that Thyme, Cinnamon, garlic, and other spices inhibited the growth of both Gram-positive and Gram-negative food-borne pathogens (Guerino et al., 2019). They also prevent spoilage bacteria, yeast, and mold (Hoque et al., 2008). Using disk diffusion assay, the greatest zones of inhibition against Aeromonas species were obtained by many essential oils like Cinnamon, Oregano, Lemongrass, Thyme, and Garlic (Peyghan and Motamedi 2012; Starliper et al., 2015).

Essential oils can be added to fish flesh by dipping or spraying as one of the main methods of the natural preservatives as potential alternatives to the familiar preservatives (Khalafalla et al., (2015). The use of essential oils is a potentially novel approach to treat fish bacterial infections with a lower risk of bacterial resistance (Guerino et al., 2019). However, few studies are investigating the effect of these essential oils against fish bacteria as Aeromonas hydrophila and its impact on sensory acceptability for consumers. Therefore, this work aimed to determine the effect of some essential (Thyme, Garlic, and Cinnamon) oils on sensory characters of Nile tilapia (*Oreochromis niloticus*) fish fillet during refrigeration storage with minimum concentrations which still maintains the inhibitory effect on Aeromonas hydrophila count and has a positive effect on sensory characters trying to increase the shelf life.

2. Materials and methods

2.1. Collection of samples

A total number of 35 samples of Nile tilapia (*Oreochromis niloticus*) fish fillet with an average weight of 100 g were collected from fish markets in Kafr El-Sheikh city, Egypt. The samples were identified and packaged separately in a sterile plastic bag under aseptic conditions. The samples were transferred directly with a minimum of delay to the laboratory Food control department, Faculty of Veterinary Medicine, Kafrelsheikh University to apply the protocol of treatment. For purpose of in vitro examination of antimicrobial activity of essential oils on the fish fillet, standardized *Aeromonas hydrophila* strain ($5×10^7$ CFU/g) was obtained from Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt.

2.2. Preparation of fish samples

Essential oils of Thyme (*Thymus vulgaris)*, Garlic (*Allium sativum)*, and Cinnamon *(Cinnamomum zeylanicum)* with concentrations of 0.5 and 1%, for each were purchased from the oil extraction unit, National Research Center, Egypt.

2.3. Preparation of treated samples

The samples were divided into 7 groups; each group contain 5 samples with fixed weight (100 g/fillet) and all samples were inoculated with *Aeromonas hydrophila* strain ($5×10^7$ CFU/g). This was followed by dipping the sample in treated extract for 10 minutes. (Khalafalla et al., 2015). The 7 groups were divided as follow: The 1st group (untreated control) group; the 2nd group dipped in Thyme extract 0.5%; the 3rd group dipped in Thyme extract 1%; the 4th group dipped in Garlic extract 0.5%; the 5th group dipped in Garlic extract 1%; the 6th group dipped in Cinnamon extract 0.5%; and the 7thgroup dipped in Cinnamon extract 1%.

2.4. Packaging and storage

Treated and untreated fish fillets were labeled and aerobically packaged inside fiber dishes, then stored at 4 ± 1 °C inside the refrigerator. The treated groups were subjected to sensory evaluation and Aeromonas counts at day zero (within 2 h after treatment) then periodically every three days until decomposition.

2.5. Sensory evaluation

The sensory assessment was performed on the raw fish fillet using the scoring test Fan et al., (2008). Sensory evaluation was based on a five-point scale to determine: odor (5, extremely desirable; 1, extremely unacceptable); color discoloration (5, no discoloration; 1, extreme discoloration); texture (5, firm; 1, very soft); and general appearance (5, extremely desirable; 1, extremely unacceptable) of the samples. When the sensory attributes declined below 4.0, the samples were rejected.

2.6. Aeromonas hydrophila strain inoculation

Aeromonas hydrophila strain was subculture on Brain Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. The cells were harvested by centrifugation (3000×g, 15 min), washed twice and resuspended with saline (NaCl, 0.85%, w/v) (Tassou et al.,1995). For inoculation of the *Orechromis niloticus* fillets, 1 ml of the dense suspension $5×10^7$ /g for *Aeromonas hydrophila* was employed.

2.7. Aeromonas hydrophila count

One ml of homogenized sample was aseptically transferred into the surface of specific media of Aeromonas species (Aeromonas Agar Media, Lab M) and then was spread by a sterile glass spreader. Inoculated plates were incubated at 37°C for 18-24 hours. All confirmed colonies (translucent, pale green colonies 0.5-3.0 mm diameter) were counted as Aeromonas according to this formula: Aeromonas count/g = No. of colonies × dilution factors × 10.

2.8. Statistical analysis

The evaluation and interpretation of obtained results were carried out using one-way ANOVA and Chi-squared test to detect the significant difference in the obtained results.

3. Results

3.1. Sensory evaluation

As shown in table (1), the mean values of essential oils effects on odor, color, texture, and general appearance (5 points, for each) of Nile tilapia fillets samples which concluded by the mean values of overall acceptability (20 points) on sensory acceptability provided by panelists of control, thyme, garlic and cinnamon oils (0.5 and 1%) group samples at different refrigerator storage time. Where, the mean values of overall acceptability of control samples were 18.51±0.51 and 9.13±2.13 after the first 2 hours and the 3rd day, respectively and the mean values of thyme 0.5% group samples were 18.18±0.18, 15.54±2.15, 13.06±1.33, and 9.48±1.44 after the first 2 hours, 3rd, 6th and 9th day, respectively. While the mean values of thyme 1% group samples were 17.6±2.17, 15.28±4.15, 13.54±0.40, 11.36±1.33, and 7.94±1.77 after the first 2 hours, 3rd, 6th, 9^{th,} and the12th day, respectively.

Moreover, the mean values of garlic 0.5% group samples were 15.5±1.55, 11.8±3.11, 8±1.80 after the first 2 hours, 3rd and 6th day, respectively. As well as garlic 1% group samples were 13.95±1.34, 10.65±5.16, 7.9±1.77 after the first 2 hours, 3rd and 6th day, respectively. In addition to the mean values of cinnamon 0.5% group samples were 18.65±1.65, 14±4.11, and 10.45±1.44 after the first 2 hours, 3rd and 6th day, respectively. While cinnamon 1% group samples were 18.04±1.64, 14.83±3.18, 11.9±1.89, 7.4±1.17 after the first 2 hours, 3rd, 6th and 9th day, respectively.

These results indicated that there was a high significance difference of mean values of overall acceptability of samples ($P < 0.05$) among different essential oils extract. Whereas it was noticed after the first 2 hours of applying the treatment in thyme and cinnamon (0.5 and 1%) group samples were very good, while low scores were given to garlic (0.5 and 1%) group samples. Meanwhile, the mean values of overall acceptability of samples decreased rapidly in control samples. On the other hand, sensory acceptability takes time to decrease in treated samples according to the type and concentration of essential oil.

Table 1. Mean values of essential oils on sensory acceptability of fish fillets (n = 5).

Sensory attributes	Samples	Storage periods					
		Zero day After 2-hour treatment	3rd day	6th day	9th day	12th day	15th day
Odor (5 points)	Control	4.9±0.9^{a}	2.6±0.20^{e}	0.12±02^{e}	-	-	-
	Thyme 0.5%	4.5±0.5^{b}	4.2±0.4^{a}	3.5±0.30^{a}	2.6±0.12^{b}	1.28±0.11^{b}	-
	Thyme 1%	4.0±0.2^{c}	3.6±0.3^{c}	3.2±0.30^{b}	2.8±0.18^{a}	2.1±0.11^{a}	1.15±0.11^{a}
	Garlic 0.5%	3.3±0.3^{d}	2.9±0.3^{d}	2.0±0.10^{c}	0.62±0.10^{e}	-	-
	Garlic 1%	2.2±0.20^{e}	1.8±0.3^{f}	1.2±0.11^{d}	0.36±0.10^{f}	-	-
	Cinnamon 0.5%	4.95±0.20^{a}	4.0±0.20^{b}	3.3±0.13^{b}	1.15±0.10^{d}	-	-
	Cinnamon 1%	4.0±0.12^{c}	3.55±0.50^{c}	3.2±.12^{b}	2.2±0.12^{c}	1.22±0.11^{c}	-
Color (5 points)	Control	4.75±0.5^{b}	2.45±0.5^{f}	0.41±0.1^{f}	-	-	-
	Thyme 0.5%	4.6±0.6^{b}	3.6±0.3^{b}	3.0±0.10^{b}	2.4±0.10^{b}	0.78±0.1^{b}	-
	Thyme 1%	4.7±0.7^{b}	3.9±0.3^{a}	3.54±0.4^{a}	2.86±0.6^{a}	2.3±0.10^{a}	0.74±0.10^{a}
	Garlic 0.5%	4.4±0.6^{c}	3.0±0.2^{e}	2.1±0.2^{e}	0.7±0.10^{d}	-	-
	Garlic 1%	4.35±0.4^{c}	3.35±0.3^{c}	2.4±0.10^{d}	1.13±0.1^{c}	-	-
	Cinnamon 0.5%	4.5±.5^{c}	3.1±0.3^{d}	2.35±0.30^{d}	0.6±0.10^{d}	-	-
	Cinnamon 1	4.82±0.2^{a}	3.84±0.3^{a}	2.88±0.2^{c}	1.8±0.10^{c}	0.3±0.01^{c}	-
Texture (5 points)	Control	4.36±0.36^{b}	2.26±0.2^{c}	0.34±0.03^{d}	-	-	-
	Thyme 0.5%	4.72±0.70^{a}	4.0±0.20^{a}	3.2±0.20^{a}	2.2±0.10^{a}	0.45±0.20^{b}	-
	Thyme 1%		3.98±0.30^{a}	3.5±0.30^{a}	3.0±0.21^{a}	1.54±0.15^{a}	0.68±0.11^{a}
	Garlic 0.5%	4.6±0.40^{a}	3.2±0.20^{b}	2.0±0.10^{c}	0.54±0.21^{c}	-	-
	Garlic 1%	4.38±0.40^{b}	3.1±0.30^{b}	2.7±0.22^{b}	0.57±0.15^{c}	-	-
	Cinnamon 0.5%	4.2±0.22^{b}	3.7±0.30^{a}	2.6±0.21^{b}	0.31±0.11^{c}	-	-
	Cinnamon 1	4.8±0.30^{a}	3.7±0.12^{a}	2.9±0.20^{b}	1.8±0.11^{b}	0.37±0.10^{b}	-
		4.7±0.23^{a}					
General appearance (5 points)	Control	4.5±0.4^{a}	1.82±21^{c}	0.5±0.02^{e}	-	-	-
	Thyme 0.5%	4.36±0.30^{a}	3.74±14^{a}	3.36±0.03^{a}	2.28±0.12^{b}	0.62±0.20^{b}	-
	Thyme 1%	4.3±0.30^{a}	3.8±31^{a}	3.3±0.30^{a}	2.7±0.12^{a}	2.0±0.10^{a}	0.55±0.11^{a}
	Garlic 0.5%	3.42±0.21^{a}	2.7±21^{b}	1.9±0.11^{d}	0.62±0.12^{d}	-	-
	Garlic 1%	3.2±22^{b}	2.4±21^{b}	1.6±0.11^{d}	0.3±0.13^{d}	-	-
	Cinnamon 0.5%	4.4±21^{a}	3.2±21^{a}	2.2±0.12^{c}	0.44±0.14^{d}	-	-
	Cinnamon 1	4.52±21^{a}	3.74±17^{a}	2.92±0.12^{b}	1.6±0.11^{c}	0.54±0.11^{b}	-
Overall acceptability (20 Points)	Control	18.51±0.51^{a}	9.13±2.13^{d}	1.37±0.2^{f}	-	-	-
	Thyme 0.5%	18.18±0.18^{a}	15.54±2.15^{a}	13.06±1.33^{a}	9.48±1.44^{b}	3.13±1.33^{b}	-
	Thyme 1%	17.6±2.17^{b}	15.28±4.15^{a}	13.54±0.40^{a}	11.36±1.33^{a}	7.94±1.77^{a}	3.12±1.14^{a}
	Garlic 0.5%	15.5±1.55^{c}	11.8±3.11^{b}	8.0±1.80^{e}	2.48±1.44^{d}	-	-
	Garlic 1%	13.95±1.34^{d}	10.65±5.16^{c}	7.9±1.77 ^{d}	2.36±1.38^{d}	-	-
	Cinnamon 0.5%	18.65±1.65^{a}	14.0±4.11^{b}	10.45±1.44^{c}	2.5±0.55^{d}	-	-
	Cinnamon 1	18.04±1.64^{a}	14.83±3.18^{b}	11.9±1.89^{b}	7.4±1.17^{c}	2.43±1.43^{c}	-

Mean values within the same column of different letters are significantly different at ($P < 0.05$)

3.2. Impact of essential oils on *Aeromonas hydrophila*

Table (2) revealed the mean values of essential oils against *Aeromonas hydrophila* counts of fish fillets samples which were experimentally inoculated with *Aeromonas hydrophila* strain by intensity 5×10^{7} CFU/g at different refrigerator storage times. Whereas, the initial counts in control samples increased gradually during the refrigerator storage time starting with a mean value of $7.71\times10^{7} \pm 0.71\times10^{7}$ CFU/g after the first 2 hours. There was significant difference ($P<0.01$) between essential oils with high significant difference of thyme 1%. While cinnamon 0.5% was the least essential oil affect *Aeromonas hydrophila* count. Finally, as illustrated in Table (3) the reduction percentage of essential oils against *Aeromonas hydrophila* counts was calculated. Whereas it was. Whereas it was reported that the most reduction percentage was to thyme oil 1% which extend up to the 12th day of refrigerator storage time followed by Cinnamon 1% and Thyme 0.5%. Despite cinnamon 0.5% concentration did not present any reduction against *Aeromonas hydrophila* counts at the 9th and 12th day of storage. However, there was a low reduction percentage on the 3th and 6th day. As well as garlic 1 and 0.5% concentration did not present any reduction against *Aeromonas hydrophila* counts at the 9th and 12th day of storage but, nearly similar in reduction percentage as thyme and cinnamon.

Table2. Mean values of essential oils on *Aeromonas hydrophila* counts of fish fillets (n=5).

Time	Control	Thyme 0.5%	Thyme 1%	Garlic 0.5%	Garlic 1%	Cinnamon 0.5%	Cinnamon 1%
Zero day (after 2 h)	$7.71\times10^{7} \pm 0.71\times10^{7a}$	$1.19\times10^{7} \pm 0.21\times10^{7e}$	$1.43\times10^{6} \pm 0.41\times10^{7f}$	$4.00\times10^{7} \pm 0.44\times10^{7c}$	$2.77\times10^{7} \pm 0.22\times10^{7d}$	$6.40\times10^{7} \pm 0.44\times10^{7b}$	$2.60\times10^{6} \pm 0.57\times10^{7e}$
3rd day	$1.49\times10^{9} \pm 0.41\times10^{7a}$	$1.20\times10^{8} \pm 0.75\times10^{7c}$	$2.17\times10^{7} \pm 0.27\times10^{7f}$	$5.82\times10^{7} \pm 0.27\times10^{7d}$	$3.40\times10^{7} \pm 0.37\times10^{7d}$	$7.81\times10^{8} \pm 0.77\times10^{7b}$	$2.87\times10^{7} \pm 0.81\times10^{7e}$
6th day	Spoiled ^{a}	$1.26\times10^{8} \pm 0.27\times10^{7c}$	$1.09\times10^{8} \pm 0.29\times10^{7f}$	$1.38\times10^{8} \pm 0.33\times10^{7e}$	$2.37\times10^{8} \pm 0.37\times10^{7d}$	$9.24\times10^{8} \pm 0.27\times10^{7b}$	$1.00\times10^{8} \pm 0.28\times10^{7g}$
9th day		$1.45\times10^{8} \pm 0.47\times10^{7d}$	$1.61\times10^{8} \pm 0.61\times10^{7c}$	Spoiled ^{a}	Spoiled ^{a}	Spoiled ^{a}	$6.02\times10^{8} \pm 0.67\times10^{7b}$
12th day		Spoiled ^{a}	$1.87\times10^{8} \pm 0.87\times10^{7b}$				Spoiled ^{a}
15th day			Spoiled ^{a}				

Mean values within the same row of different letters are significantly different at ($P < 0.01$).

Table 3. Reduction percentage of essential oils on *Aeromonas hydrophila count.*

Time	Control/ Thyme 0.5%	Control/ Thyme 1%	Control/ Garlic 0.5%	Control/ Garlic 1%	Control/ Cinnamon 0.5%	Control/ Cinnamon 1%
Zero day (after 2 h)	84.6%	98.1%	48.1%	64%	17%	96.6%
3rd day	91.9%	98.5%	96%	97.7%	47.6%	98%
6th day	91.5%	92.7%	90.7%	84%	38%	93%
9th day	90%	89%	-	-	-	60%
12th day	-	87.4%	-	-	-	-

Chi2 = 25.30** ** = Significant at ($P < 0.01$)

4. Discussion

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple, and provides immediate quality information (Reineccius, 1990). The most effective essential oils on overall acceptability was thyme with a concentration of 1% than 0.5% whereas the shelf life of samples extended till the 9th day with a concentration of 0.5% and the 12th day with a concentration of 1%. This may be attributed to the effective material thymol present in it which is characterized by strong flavor, pleasant aromatic odor, and strong antiseptic properties. For that reason, thyme essential oil is considered a flavoring agent in the food industry (Anžlovar et al., 2014). In addition, the cinnamon essential oil was used widely in coloration and deodorant in the food industry because it contains the cinnamaldehyde compound. That is why cinnamon maintains the sensory attributes better than garlic essential oil. While the garlic is characterized by the remarkable sulfur-containing compound present in it which gives the samples its distinctive smell and yellow discoloration. So, garlic can't maintain the sensory attributes for a long time compared to thyme and cinnamon essential oils (Peter, 2012). The obtained results agreed with Khalafalla et al. (2015) who concluded that using thyme essential oil with a concentration of 0.5% maintained the sensory parameters of the fish fillets samples until the 18th during refrigerated temperature and Zhang et al. (2017) who recorded that cinnamon essential oil with a concentration of 0.1% extended the shelf-life of vacuum-packaged common carp fillets by about 2 days.

Aeromonas species aren't found only in fresh fish, but also, they grow in refrigerated storage. For that, there were many attempts to reduce the incidence of Aeromonas species in fish samples during refrigerated storage (Hoel et al., 2019). Our results indicated that there was a highly significant difference in the mean values of Aeromonas counts ($P < 0.01$) among different essential oils. Whereas thyme 1% gave the better result in reducing the growth of Aeromonas species until the 12th day of the storage time in comparison to other treated samples. Thyme contained three compounds (linalool, thymol, and carvacrol) which showed the highest antimicrobial effects against Gram-negative and Gram-positive bacteria (Mahboubi et al., 2017). While thyme 0.5% needed more time to reduce the growth of

Aeromonas species and this may be attributed to the high concentration of 1% compared to 0.5%. Cinnamon 1% showed a high reduction against Aeromonas species due to the action of cinnamaldehyde which possesses the highest antimicrobial against both Gram-negative and gram-positive bacteria. While cinnamon 0.5% can't achieve the satisfactory antibacterial against Aeromonas species (Zhang et al., 2017). It was observed that garlic with both concentrations (0.5 and 1%) has a high reduction against Aeromonas species due to its complex chemical composition such as diallyl mono-, di- and tri-sulfide, which possess the high biological potential as an antimicrobial effect. On the other hand, it was noted that changes in sensory attributes and couldn't be able to extend the shelf life for a long time in comparison to thyme and cinnamon. The obtained results agreed with Khalafalla et al. (2015) who found that the dipping of Nile tilapia fillets into thyme extract (0.5%) before refrigeration can control the number of Aeromonas species found in the treated fish samples by retaining the quality attributes.

Conclusion

The addition of essential oils with antimicrobial effects, maintain fish quality and extend the shelf life of fish under refrigerator storage at 4 ± 1°C. The storage time differs according to the type and concentration of essential oil. Dipping fish fillets into thyme with 0.5 and 1% concentration for 10 minutes before refrigeration can retain the quality attributes and extend the shelf life for about 6 and 9 days, respectively more than control samples during refrigerator storage at 4 ± 1°C. Moreover, it reduced *Aeromonas hydrophila* growth without any adverse effect on the sensory attributes of the treated fillets samples.

Conflict of interest

The authors declare that they have no conflict of interest.

Research Ethics Committee Permission

This study was approved by the local Ethics and guides of the Faculty of Veterinary Medicine, Kafrelsheikh University University Egypt.

Authors' contribution

Ghada A. K. Kirrella and Nader Y. Moustafa designed and conducted the study. Dina M. Kishk performed the practical study. All authors collected and analyzed the data. Ghada A. K. Kirrella drafted the manuscript. The final version of manuscript was revised and approved by Ghada A. K. Kirrella and Reda Abdallah.

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