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IN VITRO EVALUATION OF THE PROTOSCOLICIDAL EFFECT OF EXTRACTS PREPARED FROM THE LEAVES OF *LAURUS NOBILIS* ON THE PROTOSCOLICES OF *ECHINOCOCCUS GRANULOSUS*

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ABSTRACT : Hydatid cyst is also known as a larval cestode infection of *Echinococcus granulosus* in the intermediate hosts such as herbivorous animals and humans. It is worth mentioning that the surgery is ideal treatment when the vital organs are infecting with enlarged cysts or when there is a risk of bursting the cyst but the option of surgery is still risky. The attention turned to the plant and herb extracts as alternative materials without side effects and with low costs as some currently used chemical materials may cause many side effects. This study was designed to assess protoscolicidal activity of *Laurus nobilis* against protoscoleces of *Echinococcus granulosus in vitro*. The Viability of isolated protoscoleces was tested and the protoscolicidal activity was determined by preparation three concentrations of the crude aqueous extract of *Laurus nobilis* (2.5, 5, 10 mg/ml). Based on the results, the highest percentage of mortality was observed at 10 mg/ml of the extract and the lowest mortality level was found at 2.5 mg/ml. The mortality rate increased with increasing the incubation period. The present study concluded that the dry leaf extract requires long incubation time, which can be minimized by increasing the concentration of the extract.

Key words : *Echinococcus granulosus*, plant extract, mortality rate.

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INTRODUCTION

Hydatid cyst is caused by the metacestode stage of *Echinococcus granulosus*. Adult tapeworm of *E. granulosus* inhabits the small intestine of some domestic and wild carnivores that belong to canine family (Barzinji *et al*, 2009). In the intermediate hosts (herbivorous animals and humans), it causes a disease called hydatidosis, a chronic and complex zoonosis disease that effect both humans and their economic animals (Shahnazi *et al*, 2016; Ibtesam *et al*, 2021). It is an epidemic disease, especially in developing countries leading to a high annual loss in health and economy. This disease still of huge medical importance in Iraq (Abed and Al-bayati, 2019; El-Bahy *et al*, 2019). Unfortunately, the hydatid cyst affects the vital organs mainly liver followed by lungs then brain and other organs silently and it grows inside these organs untill it becomes large in size to cause serious symptoms (Basak and Candan, 2013). It is worth mentioning that the surgery is still the ideal way of treatment when the vital organs are infected with enlarged cysts or when there is a risk of bursting the cyst but the option of surgery is still risky for the burst of the cyst or seepage of its contents, which may lead to anaphylactic shock (Jamous *et al*, 2017). Therefore, it is necessary to look for an alternative method in order t to reduce the risk of leakage of the cysts' fluid and its protoscoleces during surgery that may disseminate into nearby tissues and starts a new infection (Shahnazi *et al*, 2016). However, the thing that be unavoidable are the side effects of these disseminated materials in addition to the development of resistance to the anthelmintics that may be used in some cases (Rezaeifar *et al*, 2018). Based on these facts, it became necessary to find new scolicidal agents with minimal side effects, lower cost and higher effectiveness (Ballesteros *et al*, 2016). So the attention focused on the plant and herbal extracts as alternative materials with less side effects and with low costs. *Laurus nobilis* is a plant known since ancient times and it was used as medicine, food, and as a cosmetic (Dharmaratne *et al*, 2015). It exhibits antioxidant, antifungal, antibacterial,

anthelmintic and insecticidal effects due to its bioactive contents in different parts, especially in leaves (Nasukhova *et al*, 2017). Thus, this study was designed to assess the protoscolicidal activity of *Laurus nobilis* against protoscoleces of E. granulosus under in *vitro* conditions.

MATERIALS AND METHODS

Extract preparation : The leaves of *Laurus nobilis* were obtained from a local market in Baquba city, Diyala Province, Iraq. After grinding, 100 g of powdered leaves were dissolved in 1000 ml of boiling distilled water and the mixture was boiled for 15 minutes and then left to cool down. After that, the mixture was filtered through a filter paper (Whatman No. 1) and the supernatant was collected. The supernatant was centrifuged at 1500 rpm for ten minutes. The liquid extract was evaporated to dryness by using a rotary evaporator. The solid extract was stored at 4°C until used.

Isolation of protoscolices : The liver and lungs collected from animals infected with hydatid cysts were collected from Baqupa city butcher shops and immediately transferred to the laboratory at the College of Education for Pure Sciences, Diyala University. The cysts surfaces were sterilized by 70% ethanol. By using sterile syringes, the fluid of the cyst was drained and placed in tubes. The fluid left until the protoscolices were deposited, then the supernatant was removed and a thick deposited liquid containing protoscolices was obtained and used in the experiments.

Viability test : The viability of protoscolices was determined as follow: the infective livers with cysts were kept at refrigerator immediately after arrival to the laboratory. A mixture of 0.01 ml solution of pooled protoscolices and 0.01 ml of 0.1% aqueous eosin stain were mixed together on a clean glass slide, and viability was evaluated by low power microscopy after 5 min. Vitality was measured every day after the first reading and for three weeks. Stained protoscolices were considered as dead while unstained protoscolices were considered as viable. When the percentage of viability of protoscolices was 95% or more in the sediment, they were considered to be appropriate for experiments. The percentages of viable and dead protoscolices were determined by counting an average of 500 protoscolices (as a ratio of number of viable protoscolices to total protoscolices). All experiments were performed in triplicate.

Antiprotoscolex activity : Antiprotoscolex was determined by preparation three concentrations of the crude aqueous extract of *Laurus nobilis* (2.5, 5, 10 mg/ml). For the preparation of *L. nobilis* extract solution at concentrations mentioned above, 0.025, 0.05, 0.1 g of dried extract was dissolved in 10 ml of distilled water, respectively. One ml of each concentration of *L. nobilis* was placed in a test tube, then a drop of protoscolex sedimentation liquid (with at least 500 protoscolices) was added. The content of each tube was incubated for 5,10, 15, 30 and 60 min after they were gently mixed. Each tube was centrifuged for 1 min at 300 rpm, at the end of each time of incubation, the supernatant was discarded. One milliliter of 0.1% eosin stain was added and mixed gently. Later, protoscolices deposited was drained and smeared on a glass slide, then the glass slide was covered with a cover glass and examined under a light microscope. The percentages of viability of protoscolices were determined as above mentioned. All experiments were performed in triplicate. The saturated and normal saline were used as positive and negative control groups, respectively, in this study according to Houshmand *et al* (2019) and Mahmoudvand (2014).

Statistical analysis : For each concentration/ time, the numbers of live and dead protoscolices were counted to enable percentages for each sample and the mean percentage across the three samples to be calculated. SPSS software was used to analyze data and LSD was performed to compare the means of the different concentrations for each time period and concentration examined. Significance was set at $P<0.05$.

RESULTS

The majority (83.3%) of hydatid cysts were recovered from the livers and the rest from the lungs (16. 7%) of infected animals. About 90% of the collected hydatid cysts (27 cysts out of the 30) showed viable protoscolices. The viability of the protoscolices was stable during the first two weeks of examination and then the viability was gradually declined (Figs. 1 and 2).

The results showed that the mortality rate increases significantly with increasing the concentration of the selected medicinal plant and with increasing the time of in vitro incubation (Fig. 3 and Table 1). It can be seen from Table 1 that the highest percentage of mortality was observed at 10 mg/ml of *L. nobilis* extract and the lowest mortality level was at 2.5 mg/ml. As shown in Fig. 3, 98% mortality rate with aquatic extract was observed at the concentrations of 10mg/ml after 60 min of incubation while in the 5, 10, 15, 30 and 60 min the rates of mortality were 43.3, 60.0, 81. 7, 88.3% and 98.0, respectively. The mortality rate of the extract of *L. nobilis* at concentration of 5 mg/ml was 33.333, 49.667, 52.333, 91.333 and 97.667% after 5, 10, 15, 30 and 60 min of incubation,

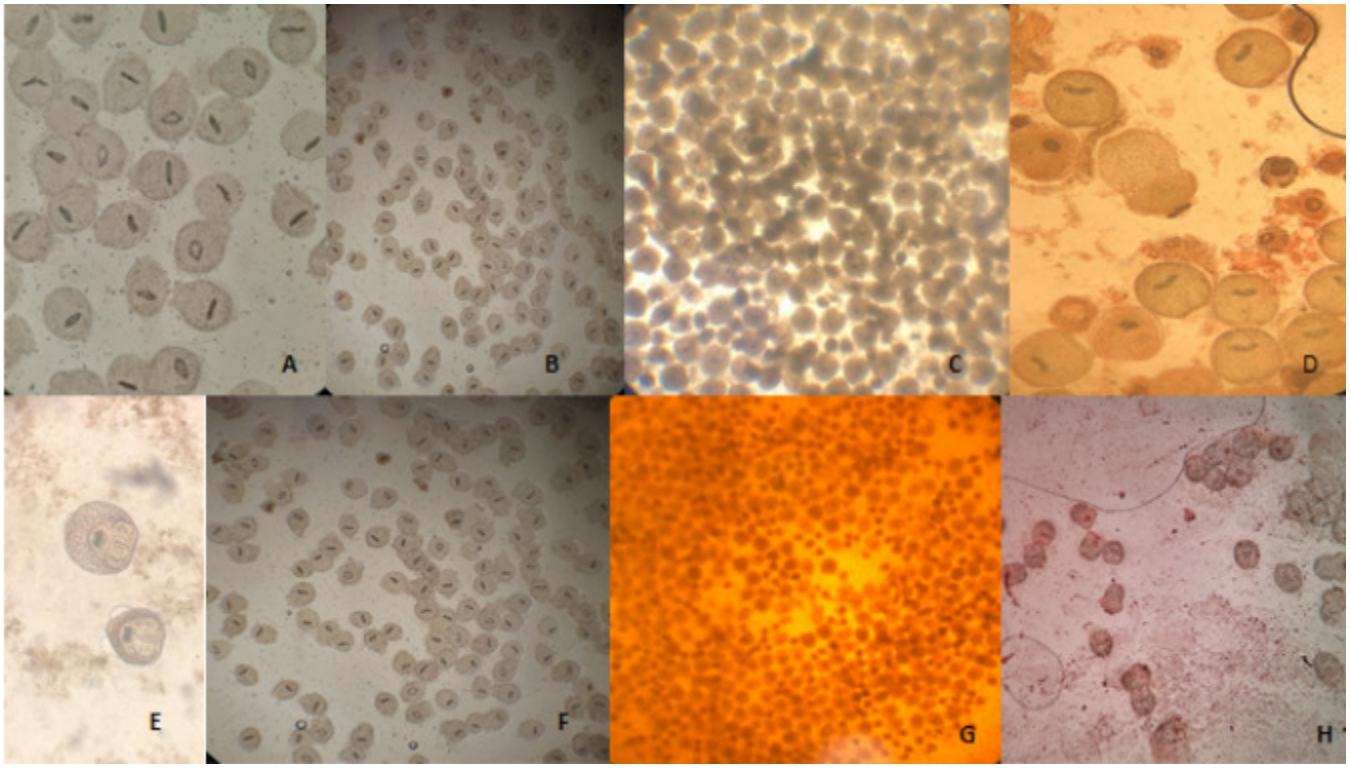

Fig. 1 : Protoscolecs of *E. granulosus*. A: On the first day of experiment 40x; B: At the end of first week (4x); C: At the end of the second week (4x); D: Dead protoscolecs at the end of the experiment (40x). E: Alive protoscolecs (40x); F: Alive protoscolecs in plant extract experiments (4x); G: Dead protoscolecs (4x); H : Dead protoscolecs in plant extract experiments (10x).

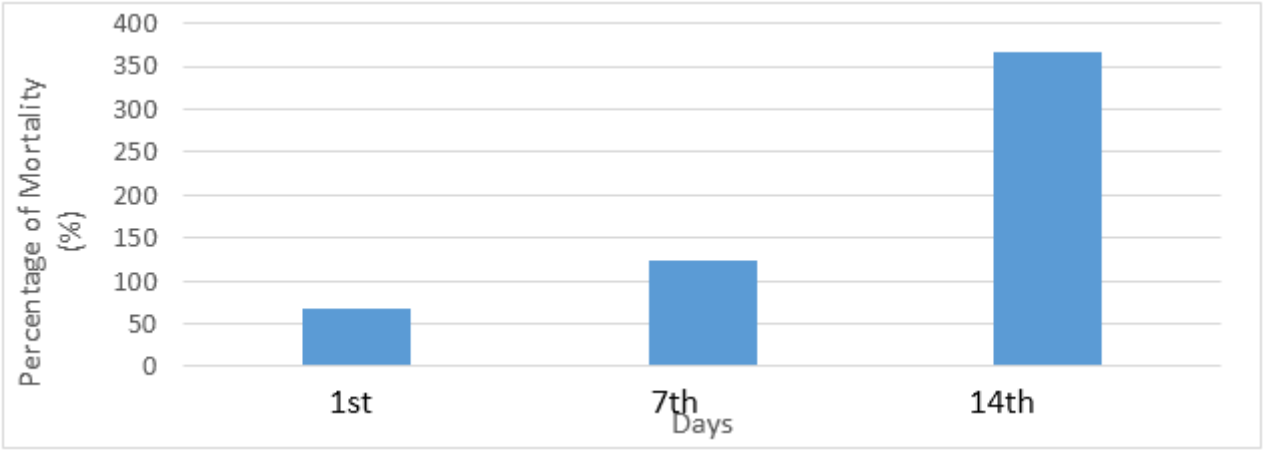

Fig. 2 : Percentage mortality of the protoscolices.

Table 1 : The mean of Mortality of protoscolces according to time and concentration.

Time (min) / Concentration (mg/ml)	Mortality (%)				
	5	**10**	**15**	**30**	**60**
2.5	17.0	30.0	72.7	80.0	94.7
5	33.3	49.7	52.3	91.3	97.7
10	43.3	60.0	81.7	88.3	98.0
Positive control	100	100	100	100	100
Negative control	0	0	0	0	0

P value < 0.05, L.S.D for time = 6.236, L.S.D for concentration = 5.578

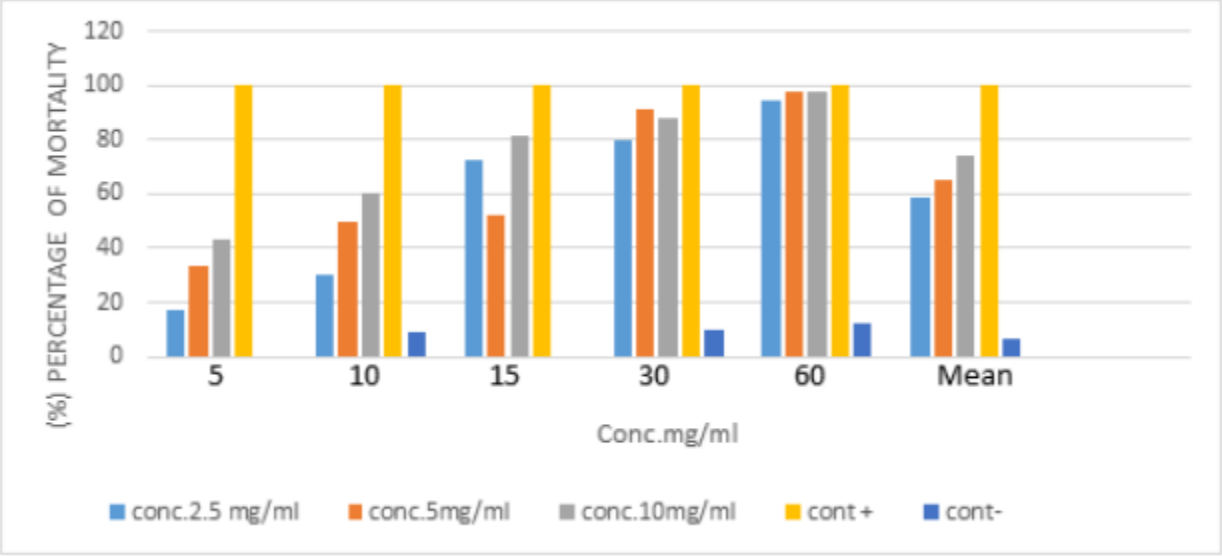

Fig. 3 : Comparison the effect of incubation period in mortality rate of protoscolecs.

respectively. These values for the concentration of 2.5 mg/ml were 17, 30, 72.667, 80 and 94.667%, respectively (Table 1). A significant difference in the per-centage of mortality was found be-tween the different concentrations of *L. nobilis* extract compared with positive and negative controls (P<0.05). Moreover, the protoscolicidal effect of saturat-ed saline as the positive control was 100% for all exposure times. The protoscolicidal effect of vari-ous concentrations of the extract, was extremely significant (P<0.05) compared to the negative control group (normal saline) at all exposure times (Table 1).

On the other hand, at that the extract requiring a further time and reducing the incubation period can be obtained by increasing the concentration. As shown in Fig. 4, according to the mean of mortality for each concentration. A significant difference in the percentage of mortality also occurred between the different treatment times with *L. nobilis* extract (P<0.05).

DISCUSSION

As the drug therapy has obtained low success, specialists have resorted for a surgery as a better option for treating hydatid cysts, however, this is not without the risk of cyst burst and occur of complications (Zibaei *et al*, 2012). Several chemical drugs have been used as scolicidal, but the majority of them may have side effects that limit their usage (El-Bahy *et al*, 2019). Given huge importance of hydatid cysts, researchers have been always looking for alternative methods, so they have tried to use extracts of herbs and plants as suitable, effective and safety way to control and treat the hydatid cyst (Niazia *et al*, 2019). Recently, several studies have been conducted on natural protoscolicidal products and many studies using different herbs and/or plants to study the thier effect on protoscolices that have yielded satisfactory results (Zibaei *et al*, 2012). In this study, we inves-tigated the effect of *L. nobilis* dry leafs extract on the viability of hydatid cyst protoscolices.

The findings of the present study showed that the mortality rate of different concentrations of *L. nobilis* extract, especially at the concentration 10 mg/ml, showed significant effect after 30 and 60 min of incubation indicating that the extract requiring a further time and reducing the incubation period can be obtained by increasing the concentration.

Laurus nobilis leaves have many components of essential oil, phenolic compounds and sesquiterpenic lactones as anactive substances (Kaurinovic *et al*, 2010; Kaurm *et al*, 2018). Fidan *et al* (2019) studied the chemical compound of *L. nobilis* and reported that the leaf Essential Oils were include 1,8-cineole (41.0%), α-terpinyl acetate (14.4%), sabinene (8.8%), methyl eugenole (6.0%), β-linalool (4.9%) and α-terpineol (3.1%). These materials exhibit a diverse activity such as antimicrobial (widely), anti-virus, antifungal anti-inflammatory, anti-diabetic and cytotoxic (anticancer) activities (Nasukhova *et al*, 2017). Previously, *L. nobilis* leaves were used against rheumatism, skin rashes, earaches and as stomachic, astringent, carminative, stimulant, emetic, abortifacient and insect repellent in addition to use it in cosmetic industry (Kaurinovic *et al*, 2010; Hassiotis and Evanthia, 2011).

L. nobilis is a potential resource of anthelmintic to combat helminthic diseases (Jamous *et al*, 2017) and the *in vitro* and *in vivo* antioxidant activities of different extracts of laurel concluded that the examined extracts exhibited a certain protective effect (Speroni *et al*, 2011).

The information from previous studies has shown that *L. nobilis* contains a large variety of substances that possess antiparasitic activity for plant and animal parasites (El-Sherbiny and Al-Yahya, 2011; Dharmaratne *et al*, 2015; Jamous *et al*, 2017; Kaur *et al*, 2018). Porrini *et al* (2011) constituted the first report of antiparasitic activity *in vivo* of plant extracts against the microsporidian *Nosema ceranae* and postulated of *L. nobilis* as natural alternative substances for antiparasitic treatment, while Ballesteross *et al* (2016) suggested a possible association between anthelmintic activity against the gastrointestinal nematode *Teladorsagia circumcincta* and the amount of phenols in some extracts leaves (including *L. nobilis* extract). The present study concluded that the dry leaf extract requiring a further time and reducing the incubation period can be obtained by increasing the concentration but this finding requires more studies.

REFERENCES

Abed N D and Al-bayati N Y (2019) Effect of pulmonary metacestodes of *Echinococcus granulosus* (Hydatid cyst) infection on lung tissue of sheep and cow. *Biochem. Cell. Arch.* **19**(2), 3463-3467.

Ballesteros M E, González L C, Rojo V F A and Martínez V M L (2016) *In vitro* anthelmintic activity of plant extracts against *Teladorsagia circumcincta* and its relationship with total phenols. *Europ. Multicolloquium of Parasitol*. http://hdl.handle.net/10261/149571

Barzinji A K R, Mothana R A and Nasher A K (2009) Effect of leaf extracts of *Dendrosicyos socotrana* and *Jatropha unicostata* on the viability of *Echinococcus granulosus* protoscoleces. *EurAsia. J. Bio. Sci*. **3**, 122-129.

Basak S S and Candan F (2013) Effect of *Laurus nobilis* L. essential oil and its main components on α-glucosidase and reactive oxygen Species scavenging activity. *Iranian J. Pharm. Res.* **12**(2), 367–379.

Dharmaratne H R W, Jain S, Tekwani B L, Jacob M R and Nanayakkara N P D (2015) Antifungal and antiparasitic activities of *Laurus nobilis* (bay leaves). *Planta Med*. **81**, PK171. DOI: 10.1055/s-0035-1556295

El-Bahy N M, Abdelaziz A R and Khalafalla R E (2019) *In vitro* evaluation of *Nigella sativa* and *Punica granatum* effect on protoscolices of hydatid cysts. *Braz. J. Vet. Parasitol*. **28**(2), 210-214.

El-Sherbiny A A and Al-Yahya F A (2011) Inhibitory effect of extracts of *Alpinia officinarum*, *Laurus nobilis* and *Solenostemma argel* on egg hatching of the root-knot nematode, *Meloidogyne incognita* and their possible application in nematode control on tomato. *Alexandria Sci. Exch. J*. **32**(4), 479-488.

Fidan H, Stefanova G, Kostovam I, Stankov S, Damyanova S, Stoyanova A and Zheljazkov V D (2019) Chemical composition and antimicrobial Activity of *Laurus nobilis* L. essential oils from Bulgaria. *Molecules* **24**(4), E804. doi: 10.3390/molecules24040804.

Hassiotis C and Evanthia D (2011) The effects of laurel (*Laurus nobilis* L.) on development of two mycorrhizal fungi. *Int. Biodeterior. Biodegrad*. **65**, 628-634. DOI:10.1016/j.ibiod.2011.03.006.

Houshmand E, Kamalifar H and Elmi H (2019) *In vitro* scolicidal effect of ginger (*Zingiber officinale* Roscoe) ethanolic extract against protoscolices of hydatid cyst. *Iran. J. Vet. Med.* **13**(1), 87-99. doi: 10.22059/ijvm.2018.263561.1004915.

Ibtesam Badday Hassan, Wasan Abdulateef Majeed Alazawi, Ali Abbas Abboud and Mareb N AL-Khalidi (2021) Single nucleotide gene polymorphism of interleukin-1B gene at position –3962 in type 1 diabetes of Iraqi patients. *Biochem. Cell. Arch*. **21**, 000-000. DocID: https://connectjournals.com/03896.2021.21.000

Jamous R M, Ali-Shtayeh M S, Abu-Zaitoun S Y, Markovics A and Azaizeh H (2017) Effects of selected Palestinian plants on the *in vitro* exsheathment of the third stage larvae of gastrointestinal nematodes. *BMC Vet. Res*. **13**, 308. DOI 10.1186/s12917-017-1237-7

Kaur M, Chahal K K, Kumar A, Kaur R and Urvashi M (2018) Nematicidal activity of bay leaf (*Laurus nobilis* L.) essential oil and its components against *Meloidogyne incognita* . *J. Entomol. Zool. Stud*. **6**(2), 1057-1064

Kaurinovic B, Popovic L M and Vlaisavljevic S (2010) *In vitro* and *in vivo* effects of *Laurus nobilis* L. leaf extracts. *Molecules* **15**, 3378-3390; DOI: 10.3390/molecules15053378

Mahmoudvand H, Fallahi S, Mahmoudvand H, Shakibaie M, Fasihi Harandi M and Saedi Dezaki E (2016) Efficacy of *Myrtus communis* L. to inactivate the hydatid cyst pro-toscoleces. *J Invest Surg*. **29**, 137-143. doi.org/10.3109/08941939. 2015. 1088601

Nasukhova N M, Logvinenko L A, Kharchenko A L and Konovalov D A (2017) Biologically active substances of the *Laurus nobilis* leaves. *Pharm. Pharmacol*. **5**(3), DOI:10.19163/2307-9266-2017-5-3-200-221

Niazia M, Sakib M, Sepahvanda M, Jahanbakhsha S, Khatamic M and Beyranvand M (2019) *In vitro* and *ex vivo* scolicidal effects of *Olea europaea* L. to inactivate the protoscolecs during hydatid cyst surgery. *Annal. Med. Surg.* **42**, 7–10.

Porrini M, Fernández N, Garrido P, Gende L, Medici S and Eguaras M (2011) *In vivo* evaluation of antiparasitic activity of plant extracts on *Nosema ceranae* (Microsporidia). *Apidologie* **42**, 700–707.

Rezaeifar M, Rezaeifar M, Rezaeifar M and Niazi M (2018) *In vitro* lethal effects of *Amygdalus eburnea* spach extract against hydatid cyst protoscoleces. *J. Res. Med. Dent. Sci*. **6**(5), 31-35.

Shahnazi M, Azadmehr A, Latifi R, Hajiaghaee R, Saraei M and Alipour M (2016) *In vitro* protoscolicidal effects of various concentrations of *Ziziphora tenuior* L. extract at different exposure times. *Avicenna J. Phytomed*. **6**(4), 376-382.

Speroni E R, Cervellati R, Dall'Acqua S, Guerra M, Greco E, Govoni P and Innocenti G (2011) Gastroprotective effect and antioxidant properties of different *Laurus nobili*s L. leaf extracts. *J. Med. Food* **14**, 499-504. DOI: 10.1089/jmf.2010.0084.

Zibaei M, Sarlak A, Delfan B, Ezatpour B and Azargoon A (2012) Scolicidal effects of *Olea europaea* and *Satureja khuzestanica* extracts on protoscolices of hydatid cysts. *Korean J. Parasitol*. **50**(1), 53-56.