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Scolicidal Activity of *Eriobotrya japonica* and *Lonicera japonica* Extracts Against Hydatid Cysts of *Echinococcus granulosus*

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ABSTRACT

Background and Objective: Nowadays, cystic echinococcosis is one of the parasitic zoonosis caused by Echinococcus. Surgery is the main treatment for this problem, but because of the possibility of spreading its content (protoscolices) during the surgery, finding a better way to prevent the production of secondary infection is always a remarkable subject. This study tried to investigate the Scolicidal effect of methanolic extract of *Eriobotrya japonica* and *Lonicera japonica* against hydatid cyst protoscolices *in vitro*. **Materials and Methods:** Three concentrations of *Eriobotrya japonica* and *Lonicera japonica* (25, 50, 75 mg mL^{-1}) were used over different times of exposure to 5, 10, 20 and 30 min. Then, the eosin stain method was used to determine the percentage of viability. **Results:** After 30 min of exposure to 75 µg mL^{-1} of *Eriobotrya japonica*, the mean mortality rate was the highest amount (89.21%) than other samples. So *Lonicera japonica* was the most potent Scolicidal agent in the present study than *Eriobotrya japonica*. However, both extracts at the same time and concentration resulted in a higher mortality rate than the negative control group. **Conclusion:** As the results showed the Scolicidal effect of both extracts at three different concentrations was considerable and it has been increased over time. Based on these results, *Lonicera japonica* and *Eriobotrya japonica* can be considered candidates for the development of green Scolicidal.

KEYWORDS

Eriobotrya japonica, *Lonicera japonica*, *Echinococcus granulosus*, methanolic extract, ultrasonic

INTRODUCTION

Hydatidosis or hydatid disease is a major zoonotic disease caused by the *Echinococcus granulosus*[1-3]. *Echinococcus granulosus* is a tapeworm parasite with a worldwide distribution[4].

After eating eggs by the definitive host (like dogs), parasites can penetrate the intestines and spread through the bloodstream to the liver, lungs and, sometimes, other tissues such as the kidney, heart, thyroid, breast etc.[5]. Feces of dogs are another way to spread infectious eggs into the environment[6]. Humans, cattle, sheep, pigs and horses are intermediate hosts that are infected by ingesting the infectious

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eggs, which results in the growth of hydatid cysts containing microscopic larvae or protoscolices in different parts of the body, especially the liver and lungs[7]. The symptoms of this disease depend on the affected organ, the size of the cyst and its exact location in the affected part as well as the reactions between the cyst and the affected organ[8].

Eriobotrya japonica is a large evergreen tree originating in Southeastern China. It is native and cultivated for about 2000 years in China and is now found in more than 30 countries worldwide and belongs to the family Rosaceae[9-11]. The *Eriobotrya japonica* is an anti-inflammatory, antioxidant, antitumor, antiviral, antimutagenic, that has hypoglycemic, cytotoxic, chronic bronchitis, nephropathy, nuclear factor-kB (NF Kappa B) inhibitor and hypolipidemic activities[12]. *Eriobotrya japonica* is a plant with a high medicinal value that has been known as herbal medicine for thousands of years. *Eriobotrya japonica* extract has been suggested to prevent skin abnormalities, cough, chronic bronchitis (CB), phlegm, inflammation, ulcer, diabetes and cancer in Chinese traditional medicine. *Lonicera japonica* is belonging to the family of Caprifoliaceae that spreads by seeds and has rhizomes and runners. *Lonicera japonica* has been used for treating fever, cholera, dysentery, inflammatory diseases, arthritis and infectious diseases[13]. Now-a-days, plant-derived compounds are remarkable as ingredients to prevent spreading the protoscoleces widely. However, several types of research have demonstrated the Scolicidal activity of selected essential oils (EO) against protoscoleces of *Echinococcus granulosus*[1,14,15]. Based on reports about the toxicity effect of some herbal medicine on pathogens and vectors, we selected *Eriobotrya japonica* and *Lonicera japonica* as new potential sources that can be used to produce Scolicidal drugs. Herein, the methanolic extract of both plants under different concentrations and times of exposure to protoscolices were evaluated *in vitro* for toxicity effect and their scolicidal activity again *Echinococcus granulosus*.

MATERIALS AND METHODS

Study area: The current study was carried out from November, 2019 to May, 2020. The leaves of *Eriobotrya japonica* and *Lonicera japonica* were collected from Gwan's Village in Soran District in November, 2019 (36°39°N, 44°32°E, Iraq). After collecting the leaves of these plants, then they were dried in the dark condition at room temperature.

Preparation of plant extract: The dried leaf of *E. japonica* and *L. japonica* were powdered using mortar. One gram of leaf powder was added to 10 mL of solvent (methanol) and mixed gently for 10 min using a magnetic stirrer plate[16]. To better extraction, an ultrasonic cell disruption system was used. The conditions of ultrasonic extraction were electric power of 190 W, at a frequency of 100 kHz, a liquid to solid ratio of 10 (mL g^{-1}) and at room temperature for 40 min. The solution was stirred again and filtered and then the solvent was removed by evaporation in a rotary evaporator system (IKA® RV 05 Basic, Werke, USA). The extracts were then held at a temperature of 4°C in a refrigerator[17-19].

Collection of protoscolices and viability assay: Hydatid cysts from livers of naturally infected sheep were obtained from an abattoir in the Kurdistan-Soran District. The hydatid fluid was transferred into a large glass beaker and left to set for 20-30 min. The protoscolices settled down at the bottom of the large beaker. The supernatant was removed and the gained protoscolices were washed three times with normal saline[20]. Each time, fluid containing protoscolices was centrifuged at 2,000 rpm for 5 min and the supernatant was discarded leaving just a trickle of fluid at the bottom of the tube. To be considered that just normal saline (as supernatant) should be removed after centrifugation, care must be taken not to discard protoscolices with normal saline[21]. Then the protoscolices in the sediment were assessed by microscopic examination on a slide by eosin stain method to determine the percentage of viability[16]. The effect of the dye on protoscolices was observed at intervals at 5, 10, 20 and 30 min following dye application and taking acquisition the dye was considered as criteria for viability, otherwise recorded as dead.

Scolicidal assay: In this study, to prepare three different concentrations (25, 50 and 75 mg mL^{-1}) 0.5, 1 and 1.5 g of the extract were respectively dissolved in 20 mL distilled water[22]. In each experiment, 0.1 mL of the extract was poured into the test tube, afterwards, 0.1 mL of protoscoleces-rich sediment was added. Then the contents of the tubes were gently mixed. The tubes were then incubated at 37°C for 5, 10, 20 and 30 min in an incubator. At the end of each incubation time, the content of each tube was taken from each well and poured on a scaled glass slide. Mortality rates were noted by eosin staining (0.1%) and the number of viable and dead protoscolices was recorded under a light microscope. Positive control comprised 5% sodium chloride, while 0.9% sodium chloride was used as a negative control. All the experiments were repeated three times for each tested concentration[23].

Statistical analysis: Differences between the means of mortality rate in different exposure times in each concentration of tested extracts were analyzed by ANOVA followed Bonferroni *post hoc* test. Data analysis was done using SPSS statistical package. Data were expressed as (Mean±SD).

RESULTS

The Scolicidal effects of different concentrations of *E. japonica* and *L. japonica* leave extract against the protoscolices of hydatid cyst over different times of exposure to 5, 10, 20 and 30 min (Fig. 1-4). In the present study, the Scolicidal effect of *E. japonica* and *L. japonica* extract significantly compared to the control groups ($p<0.01$). Time and concentration as the main parameters had a significant effect on the mortality rate of the protoscoleces of hydatid diseases ($p<0.01$), but their interaction was not significant. *L. japonica* showed greater Scolicidal efficacy on the protoscolices of hydatid cysts (89.2%) than *E. japonica* extract (83.6%) when the extracts were applied at the concentration of 75 mg mL^{-1} for an exposure time of 30 min. It was while, the death rate of negative and positive controls was 23.9 and 89%, respectively.

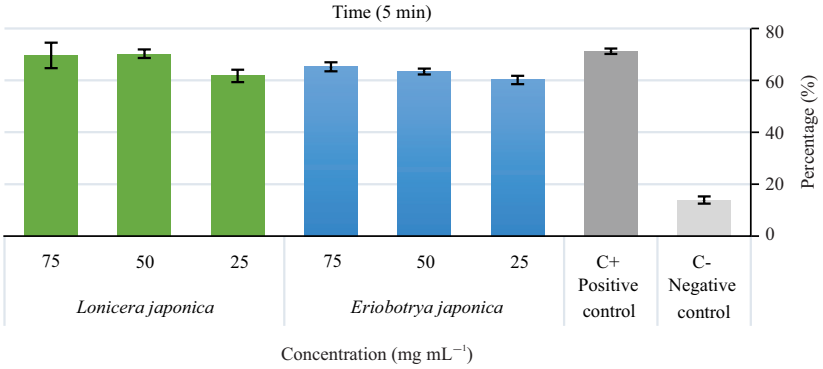

Fig. 1: Scolicidal effect of *Eriobotrya japonica* and *Lonicera japonica* extract at concentrations of 25, 50 and 75 mg mL^{-1} after 5 min of application

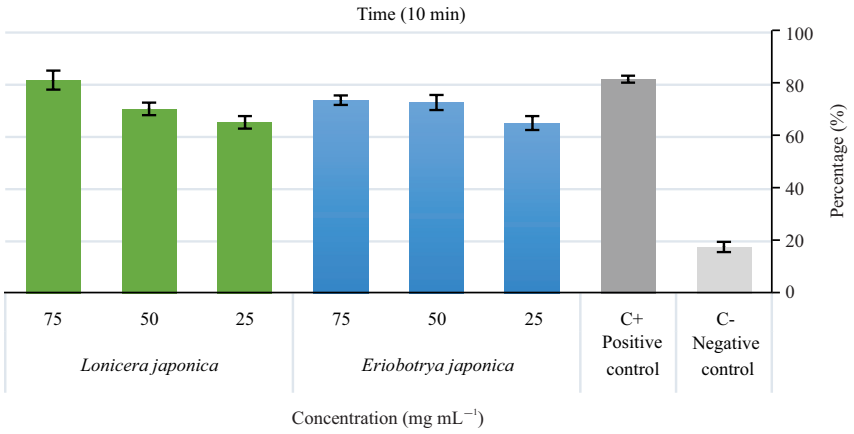

Fig. 2: Scolicidal effect of *Eriobotrya japonica* and *Lonicera japonica* extract at concentrations of 25, 50 and 75 mg mL^{-1} after 10 min of application

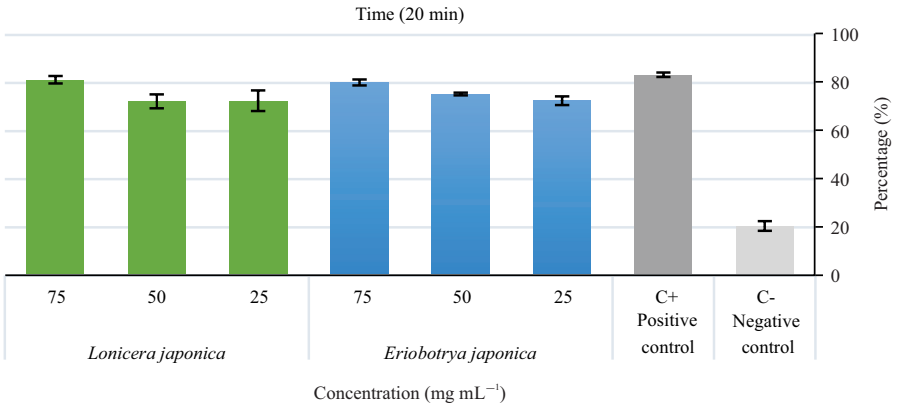

Fig. 3: Scolicidal effect of *Eriobotrya japonica* and *Lonicera japonica* extract at concentrations of 25, 50 and 75 mg mL^{-1} after 20 min of application

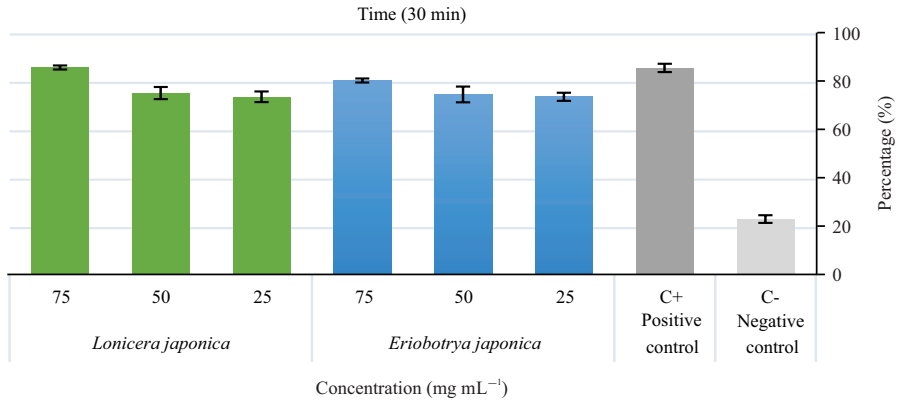

Fig. 4: Scolicidal effect of *Eriobotrya japonica* and *Lonicera japonica* extract at concentrations of 25, 50 and 75 mg mL^{-1} after 30 min of application

As can be seen in Fig. 1, after 5 min of treatment, *E. japonica* at 25, 50 and 75 mg mL^{-1} killed 60.1, 63.3 and 65.2% of the protoscoleces respectively while *L. japonica* at the same concentrations killed 61.7, 70.2 and 69.6% of the protoscoleces respectively. On the other hand, *L. japonica* at the same dose and time point was more toxic to protoscoleces. The mean difference between the mortality rate of protoscoleces at 50 mg mL^{-1} concentration of *E. japonica* and *L. japonica* was significant ($p>0.01$) but in other doses, the two extracts were not significant. Meanwhile, the mortality rate of the protoscolices was 13.8 and 71.2% for negative and positive control, respectively.

The mortality rate of protoscoleces to *L. japonica* extract at a concentration of 25, 50 and 75 mg mL^{-1} were 65.6, 70.7 and 81.7%, after 10 min of application. While for concentrations of 25, 50 and 75 mg mL^{-1}, the mortality rates of *E. japonica* were 65.3, 73.2 and 74.1%, at the same time in Fig. 2. The mean mortality rate of protoscoleces after 10 min exposure to *E. japonica* between 50 and 75 mg mL^{-1} concentration didn't show any significant difference ($p>0.01$) but between 25 and other concentrations (50 and 75 μg mL^{-1}) were significant. While the mean mortality rate between concentrations of *L. japonica* was significant ($p>0.01$). Also, the comparison of two extracts in the same concentration of 75 mg mL^{-1} showed a significant difference in mortality rate but in the other two concentrations, there were no significant differences. In comparison to control, all samples showed a significant difference with positive control except 75 mg mL^{-1} of *L. japonica*, meanwhile, all showed significant difference with the negative control.

The percentage of protoscoleces morality after 20 min exposure to *E. japonica* and *L. japonica* extracts at the concentration of 75 mg mL^{-1} was higher than 80% in Fig. 3.

The mean mortality rate of protoscoleces at the concentrations of 25 and 50 mg mL^{-1} of *L. japonica* after 20 min exposure didn't show any significant difference ($p>0.01$). But a difference of 75 mg mL^{-1} with the other two concentrations (25 and 50 mg mL^{-1}) significant was observed ($p<0.01$). The difference between the mortality rate of three different concentrations of *E. japonica* was significant. Also, a significant difference wasn't observed between the effect of both extracts on the mortality rate at the same concentrations after 20 min of exposure time. The samples showed significant differences between positive and negative controls ($p<0.01$).

The Scolicidal effect of both extracts at three different concentrations of 25, 50 and 75 mg mL^{-1} was considerable and it has been increased over time in Fig. 4. While the death rate in the negative control group was 23.9%, the effective rate of *E. japonica* was 76.6, 77.6 and 83.6% at concentrations of 25, 50 and 75 mg mL^{-1}, respectively. Also, the effective rate of *L. japonica* in comparison to negative control was considerable, 76.6, 78.2 and 89.2% at concentrations of 25, 50 and 75 mg mL^{-1}, respectively. The highest mortality rate of protoscoleces (89.2%) has belonged to *L. japonica* extract at the concentration of 75 mg mL^{-1} after 30 min exposure. It means the efficacy of *E. japonica* on the mortality rate of protoscoleces at a concentration of 75 mg mL^{-1} was very close to the positive control group, 89%.

DISCUSSION

Echinococcus granulosus tape-warm zoonotic disease that the condition caused by cysts containing larval stage and sometimes fatal, sometime hydatid disease also known as hydatidosis that to treat this disease in humans and remove cysts from the body, the surgical operation is considered as the most efficient method but always it has some risks. There is the probability of spreading the fluid contained in the cysts or parts of it, which causes the production of secondary cysts and even death of the patient might occur. Also, sometimes access to cysts and surgical activity is difficult and medication is another alternative. But the side effects of these drugs are problematic and can be associated with certain risks. A good Scolicidal substance should have properties like low toxicity, fewer side effects and high performance[24]. In this study, we investigated the Scolicidal effect of methanolic extract of *Eriobotrya japonica* and *Lonicera japonica* on the protoscolices of hydatid cyst, separately. It was revealed that the efficacy of the *L. japonica* extract outweighed *E. japonica* extract at different extract concentrations and different exposure times on the protoscolices of hydatid cysts. We found that both extracts had a toxicity effect on the protoscolices at 25, 50 and 75 mg mL^{-1} at different exposure times (5, 10, 20 30 min), but 75 mg mL^{-1} showed a strong Scolicidal activity at the same times. The most morality rate of protoscolices was 89.2% belonged to *L. japonica* at 75 mg mL^{-1} during 30 min incubation. Although in both extracts, the difference between concentrations of 50 and 75 mg mL^{-1} was not significant after 5 min of exposure, in *E. japonica* it was not also significant after 10 min of exposure. Of three exposure times, after 30 min it was seen more Scolicidal activity in both extracts which indicates the extracts require further time to show potent Scolicidal effects.

So far, many studies have been done on the Scolicidal effects of various herbs on hydatid cyst protoscolices. Abdel-Baki *et al.*[25] investigated the Scolicidal effect of ethanolic extract of *Salvadora persica*, their results showed that at 50 mg mL^{-1} concentration after 10 min, the mortality rate of the protoscolices was 81.4%. In another study, the obtained results indicated that *Quercus infectoria* extract in the concentration of 50 mg mL^{-1} was able to kill all protoscoleces during 20 min[26]. The finding of this study showed that Ginger extract showed the strongest Scolicidal effect (100%) after 20 min at a concentration of 30 mg mL^{-1} and 10 min at 50 mg mL^{-1}. The maximum Scolicidal effect of turmeric was 93.2% after 30 min at a concentration of 50 mg mL^{-1} [27]. The Scolicidal effect of methanolic extract of *Allium sativum* showed that, at 25 mg mL^{-1} concentration and 10, 20 and 30 min, the mortality rate of the protoscolices were 87.9, 95.6 and 96.8%, respectively. Moreover, the scolicidal activity of *A. sativum* extract at the concentration of 50 mg mL^{-1} was 100% after 10 min of application[28].

However, recent studies have proven new pharmacological properties of *L. japonica* including antibacterial, antiviral, anti-inflammatory, antipyretic, liver and gallbladder-protective and anti-oxidative, anti-fertility, anti-platelet aggregation, hypolipidemic, anti-allergic and immune regulatory effects[29,30]. It has been done some phytochemical studies on *L. japonica* and in these studies, several compounds have been isolated from it, which mainly include essential oil, organic acids, flavonoids, saponins, volatile oils[29,31] and phenolic acid[30]. In a study, it was revealed that between various triterpenes, sesquiterpenes, flavonoids, tannins and megastigmane glycosides in the leaves of *E. japonica*, cinchonine IIb, as well as flavonoid glycosides such as hyperoside, isoquercitrin, kaempferol glycosides, quercetin-rhamnoside, as well as two identified protocatechuic acid derivatives were the main substances responsible for the strong antioxidant activity[32]. Previous studies showed that some of these components have anti-tumour, antiviral, hypoglycemic, antioxidant and anti-inflammatory properties, in other research showed that triterpenes, ursolic acid, flavonoids and tannins have anti-parasite properties[33].

CONCLUSION

The findings of the present study demonstrated the Scolicidal activity of both methanolic extracts (*Lonicera japonica* and *Eriobotrya Japonica*) against protoscoleces of hydatid cysts. In *in vitro* studies, *Lonicera japonica* had a greater Scolicidal effect against hydatid cysts than *Eriobotrya Japonica*, so both of them can be considered the natural sources for the production of new Scolicidal agent. Also, these plants may be useful as an agent in the PAIR method for cystic echinococcosis because of their rapid and strong Scolicidal effects. However, more research is necessary to evaluate the mode of action and *in vivo* effects of these plant extracts and also possible side effects on animals and humans.

SIGNIFICANCE STATEMENT

This study discovered that herbal therapy could be a vast vision of modern, safe and powerful anti-Echinococcus herbal medicines with fewer side effects. The usage of plants can be beneficial for treatment and pre-surgery to prevent secondary cyst recurrent *in vivo*. This study will help the researchers to find more plants with this activity.

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