Current Drug Discovery Technologies
Year 2020
ISSN: 1875-6220 (Online)
ISSN: 1570-1638 (Print)

In Vitro and Ex Vivo Evaluation of *Capparis Spinosa* Extract to Inactivate Protoscoleces During Hydatid cyst Surgery

Hossein Mahmoudvand[1], Amal Khudair Khalaf [2] and Mania Beyranvand[3,*]

[1]Razi Herbal Medicines Research Center, Lorestan University of Medical sciences, Khorramabad, Iran; [2]Department of Microbiology, College of Medicine, Thiqar University, Thiqar, Iraq; [3]Department of Surgery, Lorestan University of Medical Sciences, Khorramabad, Iran

Abstract: ***Background*:** Hydatidosis is one of the most dangerous zoonosis diseases in the world caused by the larval stage of the broad-worm or Echinococcus granulosus parasite. Today, cysts' rupture or content leakage during surgery and involvement of organs adjacent to the organ involved, and consequently secondary cysts, are the major concern for hydatid cyst surgeons. Therefore, using scolicidal substances such as hypertonic saline 20%, silver nitrate and formalin has been considered to reduce the risk of protoscoleces spread and recurrence of disease in recent years. The current work designed to assess the antiparasitic effects of *Capparis spinose L.* extract against hydatid cyst protoscoleces.

***Methods*:** Collected protoscoleces from liver fertile hydatid cysts of infected sheep were exposed to the different concentrations of the essential oil (150, 300, 600 mg/mL) for 5-60 min *in vitro* and *ex vivo*. Then by using the eosin exclusion assay the viability of protoscoleces was studied. The primary phytochemical analysis of the *C. spinosa* extract was done to assess the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and glycosides.

***Results*:** *C. spinosa* extract had a powerful protoscolicidal activity *in vitro* so that at the 300 and 600 mg/ml entirely eliminates the parasite after 10 and 5 minutes; whereas at lower doses demonstrated weak protoscolicidal activity. *Ex vivo* assay, no similar effect with *in vitro* was observed, so that requiring a more time to show a potent protoscolicidal activity. *C. spinosa* extract at the concentrations of 300 and 600 mg/mL after exposure time of 20 and 12 min, killed 100% of protoscoleces within the hydatid cyst, respectively. The findings of primary phytochemical screening of the *C. spinosa* extract demonstrated the existance of flavonoids, tannins, terpenoids, glycosides and alkaloids in this plant.

***Conclusion*:** The obtained results *in vitro* and *ex vivo* exhibited that potent protoscolicidal effects of C. spinosa extract particularly at the concentrations of 600 and 300 mg/ml which entirely eliminates the parasite after 5-20 min exposure. However, more and supplementary works are required to verify these findings through assessing in animal models and clinical subjects.

Keywords: Cystic echinococcosis, *Echinococcus granulosus*, protoscoleces, Eosin test, surgery, mortality.

1. INTRODUCTION

Hydatidosis is one of the most dangerous zoonosis diseases in the world caused by the larval stage of the broad-worm or *Echinococcus granulosus* parasite [1]. The disease, which is seen in most parts of the world, is classified as hyper-endemic in

*Address correspondence to this author at the Department of Surgery, Lorestan University of Medical Sciences, Khorramabad, Iran; E-mail: manbeiran@gmail.com

Iran, especially in rural areas of the north and west [2, 3], and could potentially cause significant damage to the communities involved [4]. Dogs and Canidae are the ultimate hosts of the parasite, and humans are infected as a random host by drinking water, consuming vegetables and food contaminated with the parasite's eggs [1]. Although the adult form of the parasite in the main host is not life-threatening, its larvae in the host intermediate may cause severe illness even death by causing cysts in various tissues such as the liver, lung, and brain [5].

The onset of the disease does not have any specific symptoms, but depending on the location and size of the cyst and over time, the clinical symptoms appear [6]. In small and inactive cysts, using medications of the family of benzimidazole is an optimal treatment option, but in the treatment of large and active cysts, surgery is considered as the preferred strategy [6,7].

Today, cysts' rupture or content leakage during surgery and involvement of organs adjacent to the organ involved, and consequently secondary cysts, are the major concern for hydatid cyst surgeons [7]. Therefore, using scolicidal substances such as hypertonic saline 20%, silver nitrate and formalin has been considered to reduce the risk of protoscoleces spread and recurrence of disease in recent years [8]. However, various studies have shown that these substances are not risk-free and can cause complications such as biliary fibrosis, hepatic necrosis, and cirrhosis [9, 10]. Therefore, the need to find a new protoscoleces with greater efficiency and lower complications has always been of interest to researchers.

Since plant sources contain beneficial compounds that are compatible with the body and have no unwanted side effects, they can be considered as a good alternative to chemical drugs [11, 12].

Capparis spinosa is a plant of the Capparidaceae family. It is a thorny shrub that is almost in the shape and size of an oak and has a very firm root with thick skin. It is a native Mediterranean plant and is found in humid and warm weather in some parts of Europe, North Africa and some parts of Asia, especially Iran [13].

In traditional medicine, this plant's skin has a calming, diaphoretic, soothing effect and is effective in relieving cough, asthma attacks and excreting worms and also used to treat skin granules. It is also said to be effective in strengthening the spleen and brightening the complexion and eliminating and skin discoloration and spots [13, 14]. New studies have shown that different parts of this plant are effective in lowering blood sugar in patients with type 2 diabetes. It also has anti-obesity, weight-loss, and anti-inflammatory properties. It is used in the treatment of colon and breast cancer due to its high antioxidant properties. Antifungal and antibacterial properties are among other features of it [14, 15]. Given the biological activity, especially antimicrobial activity of *C. spinosa*, we aimed to investigate the *in vitro*, *ex vivo* anti-parasitic effect of *C. spinosa* extract on hydatid cyst protoscoleces for the first time.

2. MATERIALS AND METHODS

2.1. Ethics

This study was approved by ethical committee of Lorestan University of Medical Sciences, Korramabad, Iran (Ethics code: IR.LUMS.REC.1398.013).

2.2. Preparation of *Capparis spinosa*

We collected the *C. spinosa* fruit from the Khorramabad forest areas and transferred it to the Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran) for botanical identification.

2.3. Preparation of extract

Air-dried and pulverized *C. spinosa* fruit were put into a cellulose cartridge and extracted in a Soxhlet extractor (Germany) with 200 mL methanol. On a rotatory evaporator at 40 °C, the solvent was evaporated and, until the analysis, the residues were kept at 4 °C [17].

2.4. Phytochemical Analysis

The primary phytochemical analysis of the both *C. spinosa* extract were done to assess the presence of tannins, alkaloids, flavonoids, saponins, terpenoids and glycosides via following reagents and chemicals [18]: alkaloids with Mayer and Dragendorff's reagents, flavonoids with the use of Mg and HCl, tannin with 1% gelatin and 10% NaCl solutions, terpenoids with chloroform and conc. sulphuric acid, glycosides with FeCl2 and H2SO4, and saponin with the ability of producing suds.

2.5. Collection and Preparation of Protoscoleces

The livers of sheep infected with hydatid cyst were used to obtain protoscoleces. These infected livers were collected from Khorramabad slaughterhouse and transferred to the parasitological laboratory, Lorestan University of Medical Sciences, Iran. After washing the surface of the cyst with alcohol 70% of the hydatid fluid in the cyst containing protoscoleces was aspirated with a 50 ml sterile syringe and carried into a flask and left to set for 30min for protoscoleces to settle down and then the protoscoleces were collected after disposing of the supernatant; then they were washed twice with PBS (pH 7.2) solution. The number of protoscoleces/mL was adjusted to 5×10^3 protoscoleces in a 0.9% NaCl solution with an at least 90% viability rate by eosin exclusion text.

2.6. *In vitro* protoscolicidal activity

In this study, we first added the extract (0.2 ml) of *C. spinosa* at concentrations of 150, 300 and 600 mg/mL to 0.2 ml of the washed protoscoleces (5×10^3 protoscoleces/mL) for 5, 10, 20 and 30 min at 37°C. After this time, 50µL of 0.1% eosin stain (Sigma-Aldrich, St. Louis, MO, USA) was added to the protoscoleces and the were placed on a glass slide and tested under a light microscope. The results were reported in the percentage of dead and live protoscoleces with counting of 300 protoscoleces [19].

2.7. Dye exclusion test

Eosin exclusion assays was used to determine the viability of protoscoleces [19]. In this test, flame cell motility and impermeability to 0.1% eosin solution (1 g of eosin powder in 1000 mL of distilled water) used to evaluate the viability rate protoscoleces. After staining the live protoscoleces do not absorb color and displayed characteris¬tic muscular movements and flame cell activity; while in dead protoscoleces eosin enter the cell and protoscoleces become red.

2.8. *Ex vivo* protoscolicidal activity

In this study, the liver of sheep that were naturally infected with hydatid cyst was used to evaluate the protoscolicidal activity of *C. spinosa* extract. Initially, more than 50% of hydatid fluid was extracted from the cysts, and then *C. spinosa* extract was added to the cysts at concentrations of 150 , 300 and 600 mg/mL . The hydatid fluid was removed from the cyst after 5 , 10 , 20 , 30 min and stained with 0.1% eosin and tested under a light microscopy for counting [20].

2.9. Statistical analysis

All the tests were performed in triplicate in the present study. Data analysis was carried out using SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA). The one-way ANOVA and descriptive statistics such as frequency calculation were used for data analysis. and independent-samples t test was used for further analysis. $P<0.05$ was considered statistically significant.

3. RESULTS

3.1. Phytochemical Analysis

In this study, the findings referred to primary phytochemical screening of the *C. spinosa* methanolic extracts demonstrated the presence of tannins, flavonoids, terpenoids, glycosides and alkaloids in this plant.

3.2. Effect on Protoscoleces *In Vitro*

The protoscolicidal effects of various concentrations of *C.spinosa* extract on the protoscoleces of hydatid cysts over 5, 10, 20 and 30 min intervals are shown in Table **1**. The results show that the extract of *C. spinosa* at all concentrations had significant protoscolicidal effects compared to the control group ($p <0.001$) (Fig. **1**). The mortality of protoscoleces was 100% after 5 minutes of exposure to 600 mg / mL of *C. spinosa* extract. In addition, after 10 minutes of exposure to 300 mg/mL, the scolicidal activity of the extract was 100%. Likewise, the extract of *C. spinosa* with 150 mg/mL concentration exposed to protoscoleces killed 52.3%, 72.6%, 98.3%, and 100% of the protoscoleces at time intervals of 5, 10, 20 and 30 minutes. The results showed that with increasing exposure time of the extract of *C. spinosa* at all concentrations, the mortality rate increased significantly ($p <0.05$). The mortality rate of protoscoleces in the negative and positive control group was 2.3% and 100% after 30 and 5 minutes of exposure, respectively.

Table 1. In vitro protoscolicidal effects of *C. spoinosa* extract against protoscoleces of hydatid cyst at various concentrations following various exposure times.

Concentration (mg/mL)	Time (min)	Mean of Mortality (%)
	5	52.3 ± 2.51
	10	72.6 ± 3.15
150	20	98.6 ± 2.51
	30	100.0 ± 0.0
	5	72.3 ± 2.51
	10	100.0 ± 0.0
300	20	100.0 ± 0.0
	30	100.0 ± 0.0

	5	100.0 ± 0.0
600	10	100.0 ± 0.0
	20	100.0 ± 0.0
	30	100.0 ± 0.0
	5	
		0.0 ± 0.0
Normal saline +Tween 20%	10	0.0 ± 0.0
	20	1.5 ± 0.5
	30	3.3 ± 0.15
Ag-nitrate	5	71.6 ± 2.88
	10	100.0 ± 0.0
	20	100.0 ± 0.0
	30	100.0 ± 0.0

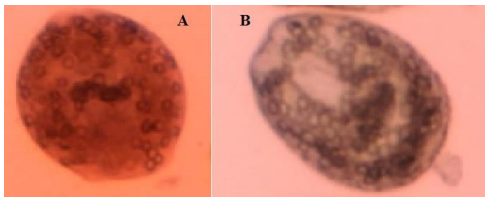

Fig. (1). Dead (A) and live (B) protoscoleces after exposure with *C.spinosa* extract.

3.3. *Ex Vivo* Effect on Protoscoleces

The results showed that after adding *C. spinosa* extract at concentrations of 150, 300, 600 mg/ml into hydatid cysts, the extract showed potential protoscolicidal effects at these concentrations. However, the results showed that this extract needs a longer time to eliminate protoscoleces in vivo. So that, at the concentration of 600 mg/ml after 12 minutes, all protoscoleces were destroyed (Table **2**).

Table 2. Ex vivo protoscolicidal effects of C. spinosa extract against protoscoleces of hydatid cyst at various concentrations following various exposure times.

Concentration (mg/mL)	Time (min)	Mean of Mortality (%)
	5	43.6 ± 2.15
	7	93.3 ± 3.51
600	12	100 ± 0.0
	20	100 ± 0.0
	40	100 ± 0.0
	60	100 ± 0.0
	5	24.3 ± 1.15
300	7	57.3 ± 4.3
	12	87.6 ± 3.15
	20	100 ± 0.0

	40	100 ± 0.0
	60	100 ± 0.0
	5	5.6 ± 0.5
150	7	22.6 ± 1.15
	12	30.3 ± 2.88
	20	51.6 ± 4.51
	40	73.3 ± 4.51
	60	89.6 ± 4.51
	5	0.0 ± 0.0
Normal saline + Tween 20	7	1.3 ± 0.57
	12	4.3 ± 0.57
	20	6.6 ± 1.15
	40	7.6 ± 0.57
	60	8.3 ± 1.15
Ag-nitrate	5	42.3 ± 2.88
	7	100 ± 0.0
	12	100 ± 0.0
	20	100 ± 0.0
	40	100 ± 0.0
	60	100 ± 0.0

4. DISCUSSION

Nowadays, processed drugs are a good alternative to chemicals. One of the important causes of this substitution is less side effects of herbal medicines than chemical drugs [11]. *C. spinosa* is a plant with many medicinal properties that are briefly as follows: liver healer, a strong antibiotic, and anti-rheumatism. It is also used in the treatment of epilepsy, diabetes, stroke prevention and eradication of kidney infection. *C. spinosa* has anti-inflammatory, antimicrobial, anti-gastric worm and taenia, and anti-leishmaniasis parasite [13, 14].

Hydatid cyst disease is caused by the larval stage of the *Echinococcus* worm and the adult worm is the small intestine of the Canidea. This cyst can develop in different organs and the symptoms of the disease depend on which organ is infected [1]. The only definitive way to treat this disease is surgery, but medications can also reduce the size of the cyst [4]. Nowadays, drugs such as hypertonic saline and Ag-nitrate are used to prevent protoscoleces leaks during surgery, which have serious side effects such as necrosis [4, 5].

In this study, we evaluated the scoliocidal effect of the extract of *C. spinosa* on protoscoleces of hydatid cyst. The results demonstrate that *C. spinosa* extract eliminates 100% of protoscoleces at a concentration of 600 mg/ml at all exposure times. Also, the concentration of 300 mg/ml of this extract during 10, 20, and 30 minutes exposure to protoscoleces and the concentration of 150 mg/ml after 30 minutes, eliminates 100% of protoscoleces. These findings indicate that the protoscoleces activity of *C.spinosa* extract is as good as that of scoliocidal agents such as 20% hypersaline (within 15 minutes), 20% nitrate sulfate (within 10 minutes), 0.5%-1% cetrimide (within 10 minutes), 3% hydrogen peroxide (within 15 minutes), 95% ethyl alcohol (within 15 minutes).

An appropriate protoscolicidal agent must have certain properties such as ability to at low doses, the highest effect in the shortest time, the ability to maintain the effect of the cyst dilute liquid , high availability, low toxicity and the possibility of rapid preparation as mentioned in previous studies [12, 21]. Many studies have pointed to sevral activity of *C. spinosa* such as anti-inflammatory ,anti-hyperglycemic ,anti-obesity ,anti-hepatotoxic and antioxidant. Another of these activity is the antimicrobial activity of this plant [13, 14]. In 2011, Boga et al. showed that *C. spinosa* root can inhibit of *Deinococcus radiophilus* growth [22]. In 2013, Muhaidat et al. found that butanolic and aqueous methanolic extract of *C. spinosa* have antibacterial activity against *Staphylococcus epidermis* [23]. It has also been reported that t butanol extract of *C. spinosa* has

antifungal activity against *C. albicans* and *A. flavus* [24]. In 2009, Lam et al.showed that *C. spinosa* fresh seeds from fruits can have anti-fungal activity against *Valsa mali* and inhibition of HIV-1 reverse transcriptase activities [25] .

In a study by Gull T et al. (2015) in Pakistan, the effect of aqueous-methanol, ethanol and acetone extracts of bark, stem, fruit, flower, and root of *C. spinosa* on its antimicrobial potential compared with amoxicillin and ciprofloxacin (control) were evaluated. The effect of these extracts on the growth of four bacteria of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pasteurella multocida* was evaluated by "disk diffusion" method and minimum inhibitory concentration (MIC) [26]. In addition, another study conducted by Arena et al. (2008) emphasizes the therapeutic role of *C. spinosa* extract on *Herpes simplex* virus type 2 (HSV-2) [27]. In 2014, Kiani et al. investigated the inhibitory effect of aqueous extract of *C. spinosa* flower on *Meloidogyne incognita* nematode *in vitro* [28]. The concentration of the extract was examined at four levels of 1, 2, 6 and 10% on mortality of second instar larvae and at three levels of 0.1, 0.5 and 1% on hatching in 4 replicates for each treatment, while in control treatment, about 97% of hatching occurred, and *C. spinosa* extract reduced egg hatching by 96.5% [28].

In this study, in line with the previous study [15, 29], the findings referred to primary phytochemical screening of the *C. spinosa* methanolic extract demonstrated the presence of tannins, flavonoids, terpenoids, glycosides and alkaloids in this plant. Considering antimicrobial mechanisms of this compounds, in 2001 , Puupponen-pimiä et al. shown that polyphenolic compounds can inhibit bacterial growth by destroying the outer membrane.[30] In the other study by Tasdemir et al in 2006 showed that flavonoid compounds can have anti-leishmanial and anti-trypanosomal effects [31]. In 1998, Helander et al. found that phenolic compounds inhibit the growth of bacteria such as *Salmonella* and *E.coli* by destroying the outer membrane [32]. Therefore, based on the antimicrobial effect of polyphenolic and flavonoid compounds, it can be said that the antiparasitic activity of this plant is due to these compounds.

CONCLUSION

The obtained results *in vitro* and *ex vivo* exhibited that potent protoscolicidal effects of C. spinosa extract particularly at the concentrations of 600 and 300 mg/ml which entirely eliminates the parasite after 5-20 min exposure. However, more and supplementary works are required to verify these findings through assessing in animal models and clinical subjects.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests

FUNDING

Not applicable

REFERENCES

[1] Agudelo Higuita NI, Brunetti E, McCloskey C. J Clin Microbiol. Cystic Echinococcosis 2016; 54(3): 518-23.

[2] Mahmoudi S, Mamishi S, Banar M, Pourakbari B, Keshavarz H. Epidemiology of echinococcosis in Iran: a systematic review and meta-analysis. BMC Infect Dis 2019; 19(1): 929. http://dx.doi.org/10.1186/s12879-019-4458-5 PMID: 31684882

[3] Fasihi Harandi M, Budke CM, Rostami S. The monetary burden of cystic echinococcosis in Iran. PLoS Negl Trop Dis 2012; 6(11)e1915 http://dx.doi.org/10.1371/journal.pntd.0001915 PMID: 23209857

[4] Brunetti E, Kern P, Vuitton DA. Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 2010; 114(1): 1-16. http://dx.doi.org/10.1016/j.actatropica.2009.11.001 PMID: 19931502

[5] Wen H, Vuitton L, Tuxun T, *et al.* Echinococcosis: Advances in the 21st Century. Clin Microbiol Rev 2019; 13;32(2): e00075-18.

[6] Stojković M, Weber TF, Junghanss T. Clinical management of cystic echinococcosis: state of the art and perspectives. Curr Opin Infect Dis 2018; 31(5): 383-92. http://dx.doi.org/10.1097/QCO.0000000000000485 PMID: 30124496

[7] Junghanss T, da Silva AM, Horton J, Chiodini PL, Brunetti E. Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. Am J Trop Med Hyg 2008; 79(3): 301-11. http://dx.doi.org/10.4269/ajtmh.2008.79.301 PMID: 18784219

[8] Ormeci N. PAIR vs Örmeci technique for the treatment of hydatid cyst. Turk J Gastroenterol 2014; 25(4): 358-64. http://dx.doi.org/10.5152/tjg.2014.13018 PMID: 25254515

[9] Rajabi MA. Fatal reactions and methaemoglobinaemia after silver nitrate irrigation of hydatid cyst. Surg Pract 2009; 13: 2-7. http://dx.doi.org/10.1111/j.1744-1633.2008.00427.x

[10] Sahin M, Eryilmaz R, Bulbuloglu E. The effect of scolicidal agents on liver and biliary tree (experimental study). J Invest Surg 2004; 17(6): 323-6. http://dx.doi.org/10.1080/08941930490524363 PMID: 15764499

[11] Gunjan M, Naing TW, Saini RS, Ahmad A, Naidu JR, Kumar I. Marketing trends & future prospects of herbal medicine in the treatment of various disease. World J Pharm Res 2015; 4: 132-55. [Google Scholar].

[12] Kohansal MH, Nourian A, Rahimi MT, Daryani A, Spotin A, Ahmadpour E. Natural products applied against hydatid cyst protoscolices: A review of past to present. Acta Trop 2017; 176: 385-94. http://dx.doi.org/10.1016/j.actatropica.2017.09.013 PMID: 28935552

[13] Ao M, Gao Y, Yu L. Advances in studies on constituents and their pharmacological activities of Capparis spinosa. Chin Tradit Herbal Drugs 2007; 38: 463-7.
[14] Zhang H, Ma ZF. Phytochemical and Pharmacological Properties of Capparis spinosa as a Medicinal Plant. Nutrients 2018; 24;10(2): E116. http://dx.doi.org/10.3390/nu10020116
[15] Nabavi SF, Maggi F, Daglia M, Habtemariam S, Rastrelli L, Nabavi SM. Pharmacological Effects of Capparis spinosa L. Phytother Res 2016; 30(11): 1733-44. http://dx.doi.org/10.1002/ptr.5684 PMID: 27406313
[16] Jahanbakhsh S, Azadpour M, Tavakoli Kareshk A, Keyhani A, Mahmoudvand H. *Zataria multiflora* Bioss: lethal effects of methanolic extract against protoscoleces of *Echinococcus granulosus.* J Parasit Dis 2016; 40(4): 1289-92. http://dx.doi.org/10.1007/s12639-015-0670-4 PMID: 27876932
[17] Taheri F, Sepehri G, Sheibani V, Sharififar F. Amelioration of Prenatal Lead-Induced Learning and Memory Impairments by Methanolic Extract of Zataria Multiflora in Male Rats. Basic Clin Neurosci 2019; 10(2): 175-84. PMID: 31031904
[18] Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers 2002; pp. 42-4.
[19] Moazeni M, Borji H, Saboor Darbandi M, Saharkhiz MJ. In vitro and in vivo antihydatid activity of a nano emulsion of Zataria multiflora essential oil. Res Vet Sci 2017; 114: 308-12. http://dx.doi.org/10.1016/j.rvsc.2017.06.003 PMID: 28646741
[20] Niazi M, Saki M, Sepahvand M, Jahanbakhsh S, Khatami M, Beyranvand M. *In vitro* and *ex vivo* scolicidal effects of *Olea europaea* L. to inactivate the protoscolecs during hydatid cyst surgery. Ann Med Surg (Lond) 2019; 42: 7-10. http://dx.doi.org/10.1016/j.amsu.2019.04.006 PMID: 31193397
[21] Fabbri J, Maggiore MA, Pensel PE, Denegri GM, Elissondo MC. In vitro efficacy study of Cinnamomum zeylanicum essential oil and cinnamaldehyde against the larval stage of Echinococcus granulosus. Exp Parasitol 2020; 1;214: 107904. http://dx.doi.org/10.1016/j.exppara.2020.107904
[22] Boga A, Forlani L, Calienni R, *et al.* On the antibacterial activity of roots of Capparis spinosa L 2011; 25(4): 417-21.
[23] Muhaidat R, Al-Qudah AM, Al-Shayeb A, Jacob HJ, Al-Jaber H, Hussein E, *et al.* Chemical profile and antibacterial activity of crude fractions and essential oils of Capparis ovata Desf. And Capparis spinosa L. (Capparaceae). Int J Integr Biol 2013; 14: 39-47.
[24] Mahasneh AM. Screening of some indigenous Qatari medicinal plants for antimicrobial activity. Phytother Res 2002; 16(8): 751-3. http://dx.doi.org/10.1002/ptr.1037 PMID: 12458480
[25] Lam SK, Ng TB. A protein with antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities from caper (Capparis spinosa) seeds. Phytomedicine 2009; 16(5): 444-50. http://dx.doi.org/10.1016/j.phymed.2008.09.006 PMID: 19019643
[26] Gull T, Sultana B, Ahmad I Bhatti, Amer Jamil. Antibacterial Potential of Capparis spinosa and Capparis decidua Extracts. International Journal of Agriculture and Biology 2015; 17(4): 727-33. http://dx.doi.org/10.17957/IJAB/14.0007
[27] Arena A, Bisignano G, Pavone B, *et al.* Antiviral and immunomodulatory effect of a lyophilized extract of Capparis spinosa L. buds. In: Phytotherapy research. 2008; 22: pp. (3)313-7.
[28] Kiani Gh Abdollahi M. Inhibitory effect of aqueous extracts of Capparis spinosa flower and Ficus carica leaf on Meloidogyne incognita, under laboratory condition. Research in Plant Pathology/Vol 3/No 1/Autumn 2014.
[29] Vahid H, Rakhshandeh H, Ghorbani A. Antidiabetic properties of Capparis spinosa L. and its components. Biomed Pharmacother 2017; 92: 293-302. http://dx.doi.org/10.1016/j.biopha.2017.05.082 PMID: 28551550
[30] Puupponen-Pimiä R, Nohynek L, Meier C, *et al.* Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol 2001; 90(4): 494-507. http://dx.doi.org/10.1046/j.1365-2672.2001.01271.x PMID: 11309059
[31] Tasdemir D, Kaiser M, Brun R, *et al.* Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. Antimicrob Agents Chemother 2006; 50(4): 1352-64. http://dx.doi.org/10.1128/AAC.50.4.1352-1364.2006 PMID: 16569852
[32] Helander LM, Alakomi H-L, Latva-Kala K, *et al.* Characterization of the action of selected essential oil componentson Gram-negative bacteria. J Agric Food Chem 1998; 46: 3590-5. http://dx.doi.org/10.1021/jf980154m