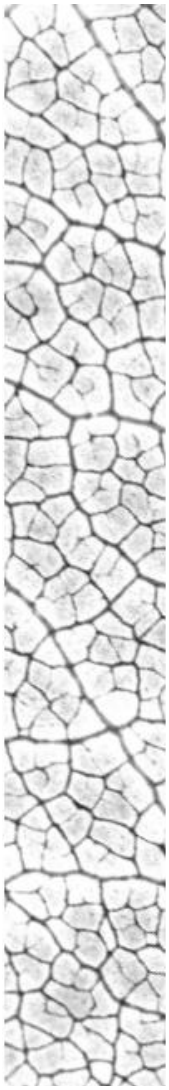

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The effect of Annona muricata and Urtica dioica on killing the proscolex of *Echinococcus granulosus*

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Abstract
This study showed the effect of Annona muricata and Urtica dioica on killing the proscolex of Echinococcus granulosus parasite. The echinococcus cysts was isolated from the livers of slaughtered sheep in Mosul city. The vitality of the cysts was studied under the microscope using a 0.1% aqueous aucine. concentrations were used (10, 20, 30, 40, 50, 60, 70, 80, 90) µg/ml from an extract of Annona muricata and Urtica dioica plants to study its influence on the vitality number of the proscolex. These proscolex were treated with the extract for different time periods ranging from (0, 15, 30, 45, 60) minutes, the study showed a significant effect of Urtica dioica plant on killing the proscolex of Echinococcus granulosus. Flavonoids had a fatal effect on killing the proscolex in the concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90) µg/ml for time periods ranging from (0, 15, 30, 45, 60) minutes. The 90 µg/ml showed a high effect on killing the proscolex. Alkaloids also showed a similar effect to flavonoids on killing the proscolex in the same concentrations and time periods, while the alkaloids were extracted from Annona muricata has a moderately effective on killing the proscolex in the same concentrations and time periods. While the cleucosides and flavonoids extracted from Urtica dioica did not show any effect in the same concentrations and time periods.

Keywords: Annona muricata, Urtica dioica, *Echinococcus granulosus*

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INTRODUCTION

The researchers turned to the use of medicinal plants that are available in the environment and people have resorted to them since ancient times in treating many diseases before the manufacture of medicines (Al-Khafaji, Al– Zubaedi , 2013). The Annona muricata plant considered one of the plants that have medicinal importance for many diseases, the plant belongs to the family Annonaceae which includes about 130 genus and more than 2,300 species.

It is considered one of the plants that spread in hot regions, especially in North and South America, as it is spread in tropical regions such as India, Malaysia and Nigeria. The plant is characterized by its green color and it is in the form of a tree whose height ranges from 5 - 8 meters and leaves are dark green and shiny and have sweet and edible fruit has a heart-like shape and yellow color (Banerjee , Das, Maji Mukherjee, S. 2018). Annona muricata contains many important compounds, including alkaloids, flavonoids, phenols, multiple proteins and oils. It also contains many elements, including K, Ca, Na, Cu, Fe and Mg, all of which are important nutrients for humans (Cardozo, et al. 2012). Annona muricata used in the treatment of diseases, especially cancerous diseases, tumors and parasitic infections, as the fruit of the plant is used in the treatment of joint diseases, nerves, diarrhea, amoebic dysentery, malaria, cystic fibrosis, diabetes, headache and insomnia. The seeds of the plant are also used in the treatment of intestinal worms and external parasites (Fatemeh, et al. 2018) While Urtica dioica plant belongs to the family Urticaceaeis it is considered one of the medicinal herbaceous plants that are used in various medical treatments, and is used in the treatment of many diseases because it is considered an inflammatory inhibitor, as it is used in the treatment of arthritis and urinary tract infections, allergies, asthma, bronchitis, gout, treatment of kidney stones, enlarged prostate, and treatment hair loss and baldness (Gemmell & Lawson 2016). The vegetative part is also used to treat internal bleeding and relieve rheumatism pain and eczema. In addition to treating psoriasis and a milk and urine supplement, in addition to its role in treating anemia (Gholami, et al. 2018). Cystic echinococcosis considered one of the important ancient diseases that are common between humans and animals (zoonotic

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disease), It is also a chronic parasitic disease, as the echinococcus cysts often remains throughout the life of the host (Marquardt Demarec & Grieve 2012). The disease is caused by development of echinococcus cysts in different parts of the human body and other intermediate hosts (Moghadamtousi, et al. 2015; Odok, et al, 2018) After swallowing taenia eggs that belongs to the genus Echinococcus graneulosis , Taeniidae family, Cyclophyllidea rank, Cestoda class and flat worms section (Moro,et al. 2017) Studies have indicated that the most susceptible organs to infection with echinococcus cysts are the liver at 50-70% and the lung 20-30% and the rest of the organs in which the infection occurs in less than 10% of the body, such as the spleen, brain, kidney, bone and heart (Patel, Patel, 2016) The echinococcus cysts are circular in shape and filled with fluid and proscolex, surrounded by several layers, including a generating layer, a cellular layer and a specific thick layer as it is surrounded from the outside by a layer of host tissue (Pedro, & Peter, 2019) .Echinococcus granulosus is a small taenia worm with a length of (3.2-9.2) mm (Rausch, (2017)." composed of a pear-shaped head with a length of (0.3) mm, equipped with a apical Rostellum surrounded by two rows of hook, its number ranging from (28-40), the head hold four muscular suckers sepals in shape, after the head there is a short neck then the proglottids, The first is immature proglottid, followed by mature proglottid, then gravid proglottids. The gravid proglottids contains about (200-800) egg (Moro,et al. 2017) The eggs are spherical with a diameter of (32-38) micrometers surrounded by a thick sheath like the rest of the tapeworm eggs belonging to the family of Taenia (Salih, Arif 2014). The Echinococcus granulosus worm needs two hosts to complete its life cycle, the first one called the final host in which the parasite reaches sexual puberty in the small intestine of Carnivorous from the Caniidae family that is infected as a result of ingestion of live heads resulting from asexual reproduction of the larval stage, the second host It is known as an intermediate host of herbivorous animals such as sheep, livestock and pigs, as well as humans when eating vegetables contaminated with worm eggs (Smith Hussain & Allen 2014).

MATERIAL AND METHODS

1. Hydatid Cysts

Hydatid cysts were obtained from the butcher's shops in Mosul, which were isolated from the livers of slaughtered sheep. The vitality of proscolex was examined in the laboratory under a microscope and using aqueous eosin dye at a concentration of 0.1%.

2. Collection of protoscolices

(5) method was used to obtain the proscolex. The surface of the Hydatid cysts was sterilized with ethyl alcohol at a concentration of 70%, then the proscolex were withdrawn by medical syringes with a capacity of (10-20) cm^3, with needles (21g), and the collection was performed under sterile conditions. The proscolex were washed with a phosphate buffer saline (PBS) with a pH (pH7.2).

The proscolex were collected in sterile clean test tubes and separated by centrifugation three times quickly (3000 r / min) for 15 minutes at one time. After completion of the deposition process the floating part was removed and then a few sterile PBS were added to the precipitate and the Protoscoleces Viability was estimated.

Estimation of Protoscoleces Viability

The viability of protoscoleces was estimated by mixing 20 µl of the of the suspended protoscoleces solution using a micropipette with a similar volume of 0.1% aqueous eosin dye and shaking the solution well. Drop was taken and examined directly under the microscope. The ratio of the number of protoscoleces viability was calculated that appeared in a bright green color to the number of dead protoscoleces stained in red as an average of three replicates (Moro et al. 2017).

The effect of plant extract of Annona muricata and Urtica dioica on the number of protoscoleces

Concentrations were used (10, 20, 30, 40, 50, 60, 70, 80, 90) µg/ml from an extract of Annona muricata and Urtica dioica plants to study its influence on the vitality number of the proscolex. These proscolex were treated with the extract for different time periods ranging from (0, 15, 30, 45, 60) minutes.

RESULTS

In this study, the effectiveness of Annona muricata plant extract on killing the proscolex of Echinococcus granulosus. Echinococcosis is one of the dangerous diseases that affect humans and transmitted to humans through food and drink contaminated with eggs of the worm (Gholami, et al. 2018). This study also showed the effectiveness of Urtica dioica plant extract on killing the proscolex of Echinococcus granulosus. The study showed a significant effect of Urtica dioica plant on killing the proscolex of Echinococcus granulosus. Flavonoids had a fatal effect on killing the proscolex in the concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90) µg/ml for time periods ranging from (0, 15, 30, 45, 60) minutes. The 90 µg/ml showed a high effect on killing the proscolex. Alkaloids also showed a similar effect to flavonoids on killing the proscolex in the same concentrations and time periods. The effectiveness of Urtica dioica in killing the proscolex due to it contain many important chemical compounds, including alkaloid, phenols, glycosides, tannins, resins, saponin flavonoids formic acid, and histamine, as they are considered as anti-inflammatory compounds (Smyth & Barett 2017). While the alkaloids were extracted from Annona muricata has a moderately effective on killing

Table 1. Effect of flavonoids extracted from Urtica dioica on the viability proscolex of Echinococcus granulosus

Time/minute	Average number of live proscolex after the time in minutes					
Mm/ml	0	15	30	45	60	Control
10	85	75	70	66	50	
20	82	73	68	55	48	
30	80	70	65	50	45	
40	70	68	56	49	43	
50	66	65	60	44	39	97%
60	60	55	50	41	35	
70	50	45	40	34	30	
80	40	36	30	22	20	
90	33	21	15	9	0	

Table 2. Effect of Glycosides extracted from Urtica dioica on the viability proscolex of Echinococcus granulosus

Time/minute	Average number of live proscolex after the time in minutes					
Mm/ml	0	15	30	45	60	Control
10	85	84	84	83	82	
20	82	80	78	75	73	
30	80	78	77	75	75	
40	75	73	73	70	70	
50	75	73	73	73	70	97%
60	73	72	70	70	69	
70	70	65	63	62	60	
80	70	65	63	63	62	
90	70	65	63	63	62	

Table 3. The effect of alkaloids extracted from Urtica dioica on the viability of the proscolex of Echinococcus granulosus

Time/minute	Average number of live proscolex after the time in minutes					
Mm/ml	0	15	30	45	60	Control
10	88	80	73	68	60	
20	83	76	70	65	55	
30	77	63	56	53	45	
40	70	64	60	50	41	
50	66	60	55	47	39	97%
60	58	50	45	40	33	
70	50	43	37	30	27	
80	45	40	35	28	20	
90	36	30	21	13	3	

Table 4. The effect of alkaloids extracted from Annona muricata on the viability of the proscolex of Echinococcus granulosus

Time/minute Mm/ml	Average number of live proscolex after the time in minutes					
control	0	15	30	45	60	
10	90	86	80	77	70	
30	80	80	76	70	68	
40	77	73	67	64	60	
50	68	63	60	55	51	97%
60	60	57	54	50	44	
70	56	50	48	43	39	
80	53	46	40	35	27	
90	50	41	36	30	26	

Table 5. Effect of Glycosides extracted from Annona muricata on the viability proscolex of Echinococcus granulosus

Time/minute	Average number of live proscolex after the time in minutes					
Mm/ml	0	15	30	45	60	Control
10	85	75	70	66	50	
20	86	85	83	78	75	
30	84	83	80	78	45	
40	82	85	83	78	75	
50	81	85	80	78	75	97%
60	85	83	79	75	75	
70	86	85	80	78	75	
80	85	83	79	78	75	
90	85	83	79	75	75	

Table 6. Effect of flavonoids extracted from Annona muricata on the viability proscolex of Echinococcus granulosus

Time/minute	Average number of live proscolex after the time in minutes					
Mm/ml	0	15	30	45	60	Control
10	86	85	77	76	74	
20	85	83	78	75	74	
30	88	88	87	84	80	
40	85	83	78	75	74	
50	86	85	83	78	75	97%
60	85	83	78	75	74	
70	86	85	83	78	75	
80	86	85	83	78	75	
90	88	88	87	84	80	

the proscolex in the same concentrations and time periods, while the cleucosides and flavonoids extracted from Urtica dioica did not show any effect in the same concentrations and time periods. Studies have indicated the importance of alkaloids in treating many diseases (Patel, 2016) this agree with our study.

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