Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Sciences for Women
Department of Biology

Synthesis of silver nanoparticles by some plant extracts and effects on viability of *Echinococcus granulosus*

A Thesis
Submitted to the Council of the College of Science for Women/
University of Babylon in Partial Fulfillment of the Requirements for the
Degree of Master in Sciences/ Biology

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2022 A.D **1444 A.H**

بسم الله الرحمن الرحيم

﴿ألم تروا أن الله سخر لكم ما في السماوات وما في الأرض وأسبغ عليكم نعمة ظاهرة وباطنة ومن الناس من يجادل في الله بغير علم ولا هدى ولا كتاب منير﴾

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Supervisor Certification

We certify that this thesis entitled (**Synthesis of silver nanoparticles by some plant extracts and effects on viability of *Echinococcus granulosus***) was prepared under our supervision at the Department of Biology, College of Science for Women, Babylon University, as a partial requirement of the degree of Master in Biology.

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Dedications

To My Family with Love

RIDHAB

Acknowledgements

"In the name of Allah, the Most Beneficent, the Most Merciful" Above all, I thank Allah who gives me the patience, the strength and all the causes to complete this investigation……

I am very grateful to Prof. Dr. Ahmed Khudair Al-Hamairy, and Prof. Dr. Huda Jasim Altameme, for taking the time to move forward, my supervision, outstanding assistance, and extraordinary efforts in completing this work.

I would like to express my sincere appreciation to all the workers in the slaughterhouses in the Najaf and Babil provinces, for their assistance in collecting animal samples, and the gratitude of the doctor, Dr. Surgeon Majid Al-Khafaji at Imam Sadiq Hospital, who is helping me to collect human samples.

I will not forget the efforts of Prof. Dr. Hadi Meziel Al-Rubaie from the College of Science for Women / University of Babylon, who helped me in the statistical analysis., with my deepest love and gratitude to my friends,thanks for their love and motivation to complete this investigation.

Ridhab

Summary :-

Hydatid cystic disease is a financial burden in Iraq because it reduces the productivity of sheep, goats, cows, and camels by leaving their afflicted organs unsuitable for human consumption, causing weight loss and poor health. It is one of the most prevalent zoonosis diseases between people and animals and develops in several organs, the most significant of which is the liver and lung as a hydatid cyst in numerous hosts, including humans. It causes many complications that may result in death. Until now, no safe and efficient medicines for this parasite in use, and research process is ongoing to find such treatments.

During the period from November 2021 to May 2022, 24 samples of hydatid cysts (14 human samples and 10 sheep samples) were collected and examined. The samples of humans were collected from Al-Qadisiyah province general Al-Najaf's hospitals and private laboratories, as well as hospitals of Babylon, Baghdad, and Al-Qadisiyah provinces,and stored in Kreb ringers culture media and cyst fluid (4:1) until used. For comparison, several concentrations of Albendazole and some selected plant extracts were made. The control group received one milliliter of distilled water, while the rest of the groups were provided with the concentrations of the extract and the above-mentioned treatment in three replicates. The validity rates of the protocols were determined for (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hours and their vitality was evaluated using eosin staining.

In this study, natural dyes were produced from *Hibiscus sabdarriffa* L., *Brassica oleracea* L. var. *capitata*, *Beta vulgaris* L. and *Crocus sativus* L., and the best results were obtained from the natural dye extracted from the plant extract *Crocus sativus* when used to determine the vitality of

protoscolices, as it showed that the live protoscolices had a transparent white color and the dead were distinguished by yellow, while the It is dyed green for vital protoscolices and red for unvital protoscolices when compared to standard eosin stain.

The effectiveness of the above-selected plant extracts has been tested with concentration (100, 200, and 300mg\ml) on the protoscolices, and comparing the effectiveness of plant extracts with albendazole treatments with concentration of (100, 200,300) mg\ml. An effect for *Hibiscus sabdarriffa* extract is better than other plant extracts current study as well as Albendazole. Therefore, it was chosen to synthesize silver nanoparticles from it to test the biological effect against the parasite.

Silver nanoparticles were created from the plant *H.sabdarriffa*, and their efficacy against the protoscolex of *E.granulosus* was evaluated together with treatment with albendazole *In Vitro*. The effect of different concentrations of nanoparticle *H.sabdarriffa* extracts plants (0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL) interacting on the death of the *E.granulosus* protoscolices in different time periods were tested in vitro for (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hours. Protoscolices are lost, as the viability percentage in the concentration of 0.8 mg/ml after 24 hours of treatment was 0%, compared to 85.54% in the control treatment.

Through the results of the current study, it was noted that the effect of the extract of *H.sabdarriffa* nanoparticles is better than that of albendazole treatment because the *H.sabdarriffa* nanoparticles had the ability to kill protoscolices after 48 hours at a concentration of 0.8 µg/ml, while Albendazole reached zero expiration after 120 hours and at a concentration of 300 mg/ml. Therefore, the results of boiled water extract of *H.sabdarriffa* nanoparticles revealed its efficacy against protoscolices

viability and could be used as an alternative to chemotherapy in the treatment of hydatid cyst infection.

For the molecular study, 18 samples of hydatid cysts (9 human samples and 9 sheep samples) were selected for examination to molecular identifies the most *Echinococcus granulosus* that common strains in central Euphrates provinces, as well as to study the gene sequences of the strains, draw the phylogenetic tree, and compare them to the strains recorded in NCBI-BLAST for determining the most that common strains for humans and sheep in the central Euphrates provinces. The livers and lungs were used to collect hydatid cyst samples for this study.

DNA was isolated from a germinal layer,and the amplified of the product using the technique of Conventional PCR and sequenced in gene 18S rRNA Internal Transcribed Spacer (ITS1) and (ITS2), which has a molecular weight of (750 and 1100) base pairs. The studied samples were matched with the samples installed in the gene bank. It was found that there were samples that matched the samples of the gene bank with different percentages. Human Hydatid Cysts(HC) which were chosen to draw the evolutionary tree, where the percentage of matching was 99 % with a sample which has ID: KX434757.1 named *E.granulosus* from India and which was recognized through the alignment drawn to compare it.

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List of Abbreviations

CE	Cystic Echinococcosis
HCF	Hydatid cyst fluid
HC	Hydatid cyst
Nad1	NADH dehydrogenase subunit 1
Cox1 Rpm	Cytochrome c oxidase subunit 1 Round perminte

NCBI	National Centre for Biotechnology Information
PCR	polymerase chain reaction
WHO	World Health Organization
(EDXS)	Energy Dispersive X-Ray Spectroscopy
(FTIR)	FTIR: Fourier-Transform Infrared Spectroscopic Analysis
(AFM)	Atomic Force Microscopy
DPPH	DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl
(SEM)	Scanning electronic microscopes

List of Appendix

No.	Title
1-	The Genetic Sequence Sent by the Macrogen Company in South Korean before Giving the Sequence Number for Registration on the Samples Under Study.
2	The Genetic Sequence Sent by the Macrogen Company in South Korean before Giving the Sequence Number for Registration on the Samples Under Study.
3	The Genetic Sequence Sent by the Macrogen Company in South Korean before Giving the Sequence Number for Registration on the Samples Under Study.
4	The Genetic Sequence Sent by the Macrogen Company in South Korean before Giving the Sequence Number for Registration on the Samples Under Study.
5	The Genetic Sequence Sent by the Macrogen Company in South Korean before Giving the Sequence Number for Registration on the Samples Under Study.

6	*Echinococcus granulosus* Isolate VT-SAg 5.8S Ribosomal RNAgene and Internal Transcribed Spacer2,Partial Sequence ID:KX434757.1 Length:619 Number of Matches:1
7	*Echinococcus granulosus* Isolate VT-SAg 5.8S Ribosomal RNAgene and Internal Transcribed Spacer2,Partial Sequence ID:KX434757.1 Length:619 Number of Matches:1
8	*Echinococcus granulosus* Isolate VT-SAg 5.8S Ribosomal RNAgene and Internal Transcribed Spacer2,Partial Sequence ID:KX434757.1 Length:619 Number of Matches:1

Chapter one

Introduction

1. INTRODUCTION

1-1: General Introduction

Echinococcosis or hydatidosis caused by the tapeworm *Echinococcus granulosus* larval stage are one of the most important zoonotic diseases for human and domestic animals (Hama *et al*., 2015; Ali *et al*., 2020; Khan *et al*., 2021). This disease leads to many medical, veterinary and economic problems, Iraq is regarded as one of the countries that plagued by endemic sickness of hydatidosis (Deplazes *et al*., 2017; Abdulhameed *et al*., 2019).

The pressure exerted by the cyst on the surrounding organs, which affects their growth and functions, as well as the cyst's explosion and spilling of its contents to the outside, which causes dead shock and the emergence of secondary cysts, are the causes of the clinical symptoms of infection in general (Zeibig, 2013).

Cystic Echinococcosis (CE) can infect a variety of human organs, and the treatment is extremely difficult, with surgery being required to remove the cyst from the affected organs (Sadjjadi *et al*., 2013). Various medications have been used, including mebendazole, flubendazole, praziquantel and ivermectin , Its effects are non-toxic to the human body (Hendrix and Robinson, 2006; Arziak *et al*., 2008).

As alternatives, medicinal plant extracts have been used to treat many diseases, including hydatid cysts, medicines herbals generally are used due to low cost, availability, acceptability, and assessed to be harmless than synthetic medicines (Ozioma and Chinwe, 2019), As well as those plants, produce secondary compounds for treatment like phenolic, alkaloid, and terpinate compound (Wickzkowski *et al*., 2013). Due to the appearance of various phytochemical components which have therapeutic value (Gangola *et al*., 2017) there are numerous previous studies that were conducted in Iraq such as Al-Maliki (2008), Al-Hamairy

(2010), Al-Tai (2014), Saeed (2021) and Al-Hasnawi (2022). Therefore, this study is complementary to previous studies.

Currently, the synthesis of nanoparticles from plant extracts has been used as a low-cost, environmentall friendly, non-toxic and cost-effective method for making nanoparticles of various shapes, sizes and shapes (Mohanpuria *et al*., 2008) for testing on protoscolices *In vitro*.

Molecular affinity has been used extensively to identify parasite strains, and molecular studies have determined that there are ten distinct genotypes (G1 to G10) of *E. granulosus* (Thompson, 2008) which differ in a wide variety of criteria that influence epidemiology, pathogenesis, infection (Ebrahimipour *et al.,* 2019). The genotypes of *E. granulosus* are determined and controlled molecular genetic analysis using the basis of DNA sequencing (Cao *et al.* 2020).

1-2: Aim of the Present Study

The present study aimed to applied the following objectives: -

1. Compare the efficiency of boiling water extracts of (*Crocus sativus*, *Beta vulgaris*, *Hibiscus sabdarriffa*, *Brassica oleracca*) as active material as well as using mebendazole as a comparative chemical treatment for protoscolices as control *In vitro*.
2. Using of some aqueous extracts of plants as a natural alternative to dyeing instead of eosin dye that using for the viability of protoscolices determining.
3. Investigate the influence of the best boiling water extracts of silver nanoparticles on the viability of *E.granulosus In vitro*, as well as evaluate it in a biological application.
4. Extract DNA nuclear material from the protoscolices and germinal layer of *E.granulosus* in humans and sheep , as well as investigate the genotyping sequences of *E.granulosus,* and then determine the

accession number in NCBI-BLAST and draw the phylogenetic tree of these samples.

Chapter two

Literatures Review

2. Literatures Review

2-1: Historical View

Around four centuries before Christ, Hippocratus, an ancient Greek physician, linked human hydatid Cysts to the "water-filled tumors" Hippocratus saw in post-mortem examinations of cattle (Eckert *et al*., 2001). In 1695, Hartmann discovered adult *Echinococcus granulosus* in a dog's small intestine, and in 1782, Goeze identified the larval stage hydatid cysts (Paniker and Ghosh, 2013). In a series of studies published in 1853, Carl Von Siebold revealed that cysts from sheep produced adult tapeworms in dogs, illustrating the life cycle and link between larval and adult stages (Von Siebold, 1853).

In 1886, the German scientist Leukart described the shape of the parasite and the hydatid cysts resulting from it and called it *Echinococcus granulosus* (Sabau, 2011). This term is derived from the Latin word Echinos, meaning urchin or thorny, while Kokkos means bean, i.e., granulated *Echinococcus*, and the word Granulum means small tubercles. Hydatid is a Greek word, Hydatis, meaning a drop of water (Rahmm *et al*., 2015).

In 1984, Francesco was the first to describe the parasitic nature of the disease (Khalifa *et al*., 2016; Thompson, 2017). Leuckart, a biologist, was the first to describe the adult worm's life cycle and basic descriptions (Muller *et al.*, 2007).Ecollaborated with a group of experts at the end of the nineteenth century to change the parasite's scientific nomenclature, and *Echinococcus granulosus* was established (Bhatia, 1997).

2-2: Classification of *Echinococcus granulosus*

The Parasite *E. granulosus* is classified in the following depending on (Paniker, 2013):

Kingdom: Animalia
Phylum: Platyhelminthes
Superclass: Eucestoda
Class: Cestoidea
Subclass: Cestoda
Order: Cyclophyllidea (Braun, 1900)
Family: Taeniidae (Ludwig, 1886)
Genus: *Echinococcus* (Rud, 1801)
Species:*E. granulosus* cited by (Batsch, 1786)

2-3: Species of *Echinococcus*

The recent developments in Phylogenetic systematics and Genetic evolution identify nine types of Echinococcosis:

1. *E. granulosus sensu stricto* (G1 to G3)
2. *E. equinus* (G4)
3. *E. ortleppi* (G5)
4. *E. canadensis* (G6 to G10)
5. *E. multilocularis*
6. *E. vogeli*
7. *E. oligarthrus*
8. *E. felidis*
9. *E. shiquicus*

There are more four common types of *echinococcosis* such as *E.equinus; E.orteleppi; E.multiloculaeris; E.vogeli; E.oligarthus* (Taylor *et al.*, 2016).

2-4: Parasite Description Morphology

The length of an adult *E.granulosus* is ranged from 2 to 11 mm, with two to six proglottids, the length of a terminal gravid proglottid is normally more than half the length of a mature worm, and the position of the genital hole in both mature and gravid proglottid is usually posterior to the middle, There are 26 to 40 hooks dispersed across the rostellum in two rows, with the first row hooks ranging in size from 25 to 49 mm and the second-row hooks ranging in size from 17 to 31mm (Eckert *et al*., 2001; Ekhnefer, 2012).

The body, or strobila, is segmented and made up of several proglottids. The first segment is the scolex, it is followed by the neck, and finally, the proglottid body (immature, mature, and gravid proglottid) (Almeida *et al.,* 2015). Because each proglottid receives everything it needs straight through its tegument, *E. granulosus* lacks a circulatory system, respiratory system, or digestive tract (Thompson, 2001).The gravid segment has a uterus that contains roughly 500 eggs that are released with faeces (Eckert and Deplazes, 2004).

Each proglottid has hermaphroditic reproductive organs and excretory cells called flame cells (protonephridia). The genital pore is a common aperture that connects the reproductive organs of each proglottid. The middle segment has developed testes and ovaries, while the posterior segment is pregnant with an egg-filled uterus. The gravid section is the largest and longest, with the uterus holding up eggs (Rahman *et al*., 2015). As the proglottid approaches the tail, it grows in size as Figure (2-1). Mature gravid proglottids detach from the strobila (the segment chains) and shed their outside the host (John *et al*., 2006). A mature worm lives in a definite host's (dog) small intestine (John *et al*., 2006).

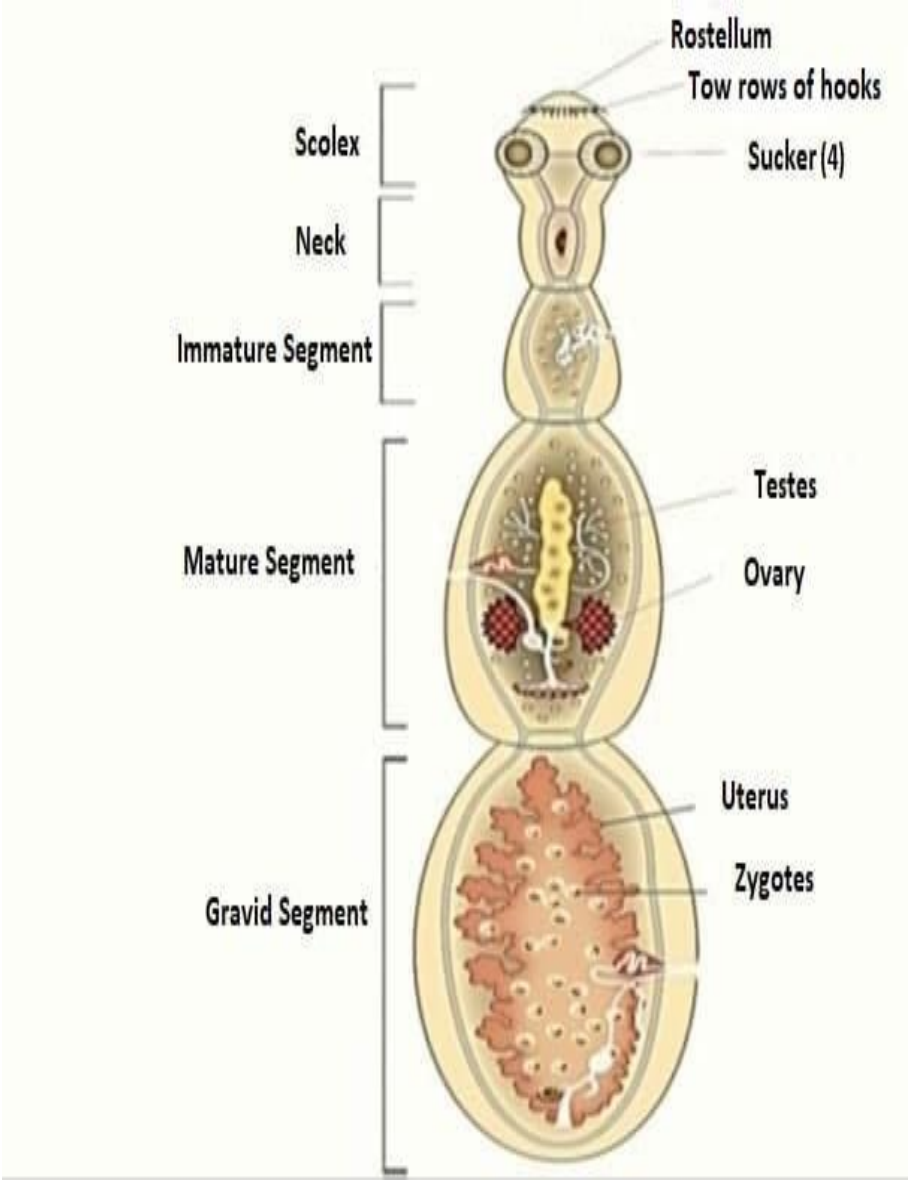

Figure 2-1: Structure of *Echinococcus granulosus* (Thompson, 2017).

2-4-1: Egg Description

The eggs have a spherical shape, with a diameter ranging between 25-35µ, containing a hexacanth embryo an oncosphere hexacanth embryo surrounded by a shell (Muller *et al*., 2007).The eggs are wrapped by three covers: a thin gelatinous shell on the exterior that has become confused with exposed faecal eggs over time, a thick yellow-brown shell with numerous microscopic pores that gives the egg a striated appearance, and

an egg cell membrane that lines the inside of the egg. *E.granulosus* eggs have a morphology that is similar to that of other Taeniidae species (Eckert *et al*., 2001).

The eggs are completely immune to climatic conditions and can remain infectious for months or perhaps a year in a wet environment at temperatures between 4 and 15 Cº, but heating to 60-80 Cº kills the eggs in less than five minutes (Al-Saqi, 2001). The clearest of which gives the egg its shape dark striped, and these eggs are highly resistant to unfavorable conditions and for a long time (Muller *et al*., 2007).

2-4-2: Metacestode

The larval stage is known as hydatid, which proliferates asexually in numerous animals including humans. Cystic echinococcosis is the name of the infection at this stage (Gottstein *et al*.2014). The metacestode is developing as a bladder which coated with a cellular layer that comes from intermediate host activities as fibrous tissue around the bladder of the metacestode (Hodžić *et al*., 2018), the inner germinal layer capable to produce protoscolices (Eckert *et al*., 2001; Halajian *et al.,* 2017).

2-5: Life cycle

McManus *et al*. (2012) and Zhang *et al*. (2018) state that *E.granulosus* is transmitted by two mammalians hosts: a carnivore predator (canine family) as the final host , its herbivores prey (most herbivorous) as intermediate host; infections in humans occurs accidentally through ingested the infective egg across contamination food or water resources.

The eggs are transmitted to the intermediate host through contaminated food and water or upon direct children, they should avoid contact with

diseased dogs, as the eggs stick for the dogs' the hair on the anus (Yang *et al*., 2006; Thatte and Thatte, 2016).

When the final host eats the infected members the parasite will reach the small intestine of the intermediate host, where the primary parasite will be found.Protoscolices grow to adults worms with 4-7 week, each worm produce thousands of eggs each day to start the cycle again (Higuita *et al*., 2016). Inside the small intestine of the intermediate host, the swallowed egg hatches, producing hexacanth embryos (Mandal,2012). The adult or gravid proglottide separates from the worm's body and may rupture and the eggs will come out with the faeces (Muller *et al*., 2007).

The protoscolices attach to the diseased organs when a carnivore eats them, small intestine mucosa and develop into several adult worms that shed eggs after 6-8 weeks.Humans are referred to as dead end hosts because human was death end hosts the life cycle short, (Kamiyaet *et al*., 2007; Mandal, 2012).

While the cycle is completed in sheep and other herbivores by feeding the definitive host from dogs and other members of the Canidae family on the infected guts by protoscolices, a mature worm that can begin a new life cycle (Moro *et al*., 1999). In addition to the liver and lungs, other organs can be affected, but to a lesser extent, including the spleen, kidneys, brain, bones and heart, after which a primary rupture of the cyst occurs to form a new cyst in new organs, this condition is called secondary cystic echinococcosis or secondary hydatid cysts (Eckert *et al*., 2001).

The cysts have a wall made from both host tissue (pericyst) and origins of larvae (endocyst), the cysts are fluid-filled and grow slowly (about one centimeter in diameter each year) (Al-Qura'n, 2008).

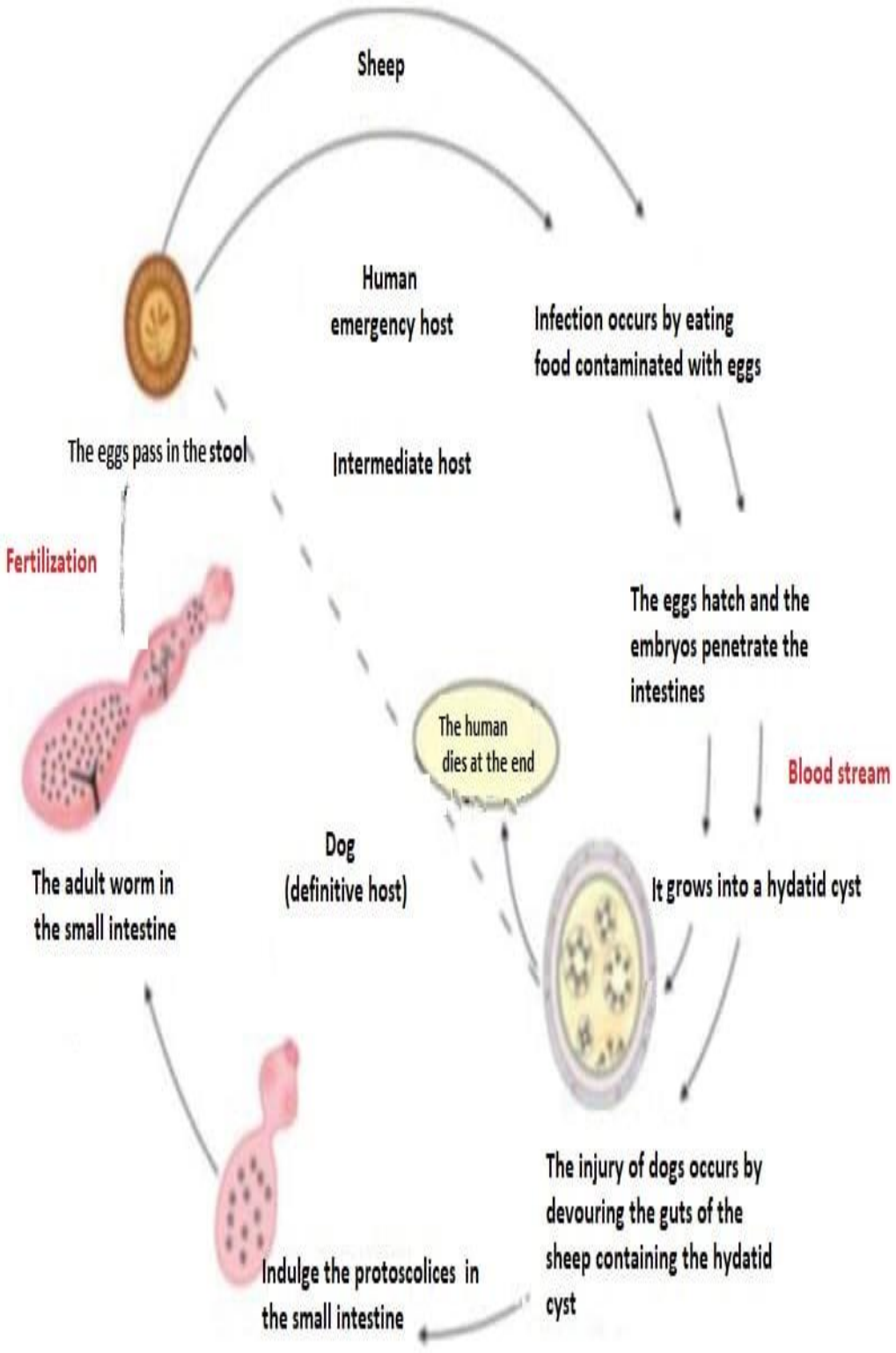

Figure 2-2: -The Life cycle of *E. granulosus* (Sankar and Jaypee, 2014).

2-6: Structure of Hydatid Cyst

2-6-1: The Outer Pericyst Layer

Golzari and Sokouti(2014) state that the layer was created by cells' of host adaptation of protective thick fibrous tissue a response of infection, any breakdown of the outer layer causes the hydatid cyst to degenerate or explode. Layer of pericyst diameters depending on the hosting organ in which the hydatid is found, and depending on the host organ in which the hydatid is found, but they are typically a few millimeters in diameter and play role in sheltering the parasite from the immunological reaction of the host.

2-6-2: Laminated or Medial Layer

When observed under the electron microscope, it is a white layer consisting of microfibrils and granules rich in amino acids and carbohydrates (Muller, 2007). It is secreted by the parasite and reaches a thickness of one millimeter. It plays the key involvement in parasite defense against immunological response or the reaction of the hosts tissues against it and providing the appropriate environments for it was continued in growth, In addition it has a role in lowering the side effects of medications to treat the disease and role in regulating the entry and exit of nutrients from and to the hydatid cyst, in addition to this, this layer acts to support the generative germ layer (Gottstein and Beldi, 2017).

2-6-3: Germinal or Inner Layer

The nuclei-containing cellular active layer is linked to the lamellar layer via the germinal layer's prints (Arora, 2017). This layer, which has a thickness of 22-25 μm, is responsible for the parasite's asexual reproduction. The hydatid cyst fluid and brood capsule, in which the protoscolices originate, are produced by this layer (Loker and Hofkin,

2015). It helps it to grow by regulating the permeability of the wall and controlling the osmotic pressure of the hydatid cyst wall (Roberts and Janovy, 2009).

2-6-4: Daughter Vesicles Brood Capsules

They are cysts that form inside the hydatid cyst and are identical to the mother's cyst in composition. They can be formed in one of three ways, either from the germ layer, the primary primates, or the brood pouches compared to the brood capsule or secondary gradually from generative layers was float in the fluid of the mother cyst (Mehlhorn, 2008).

2-6-5: Hydatid sand

When the brood capsule splits from the germinal layer and the brood capsule and protoscolices become loose in the hydatid fluid, and hydatid sand is generated. It has both evaginated and invaginated protoscolices, the latter of which is more dangerous (Marquardt, 2000). The brood capsules, which range in size from(250 to 500) mm, develop internally from the germinative layer and asexually bud to create several protoscolices, some brood capsules will be free inside the cyst fluid with their scolices, and these are known as hydatid sand because of their appearance (Lynne *et al*., 2000).

2-6-6: The Fluid of Hydatid Cyst

It is a yellowish or colourless fluid that fills the cavity of the cyst with its specific weight (1.009-1.005) and has a pH of roughly (6.7-7.2), providing nutrients for the growth of Protoscolices, and has harmful effects on the host (Aziz *et al*.,2011; Juyi *et al*., 2013). Also, Albumin, Creatinine, Lecithin, Urea, minor amounts of Glucose, Sodium Chloride,

Phosphates, Sodium sulfate, Sodium succinate, and Calcium, as well as trace elements like Iron, Copper, Zinc, Cadmium, Nickel, Chromium, Magnesium, and Manganese (Erin, 2007; Ekhnefer, 2012).

The parasite's metabolic functions, such as ammonia, bilirubin, and creatine, are also present in hydatid cyst fluid. In addition to some inorganic materials like iron, magnesium, chlorine, cadmium, sodium, nickel, chromium, calcium, copper, and enzymes like acidophosphatase, Oxidase, Protease, Lipase, and others, the quantity and quality of these materials varies depending on the parasite's source and cyst site (Sastry and Bhat, 2014). The components of the fluid differ according to the organ in which it is located, it was found that the hydatid cyst fluid in the liver contains bile compounds (Smyth, 1964) .

The hydatid cyst fluid is the main source of parasite antigens that are used in serological tests to diagnose the disease (Izadi and Ajami, 2006). The size and shape of the fluid of the hydatid cyst are determined by the location and the organ in which it grows (Marquardt *et al*., 2000).

2-7: Types of hydatid cysts: -

The general structure of the hydatid cyst takes the form of a bladder. There are four types described by Smyth (1964) which are

2-7-1: Unilocular (Univesicular):-It is characterized by the presence of simply one bladder or a number of fully separate bladders, each with its own curdle (Nakao, 2013).

2-7-2: Multilocularis: - Each space is made up of a series of small voids or cavities that are separated from one another or filled with connective tissue.It is a jelly-like matrix that is usually sterile but may include protoscolices on occasion (Chin, 2001).The germinal and laminated

layers are underdeveloped, and the fibrous capsule is absent (Samad and Mohsen, 2014).

2-7-3: Alveolar: - It is characterized by a malignant type of growth made up of a series of proliferating vesicles embedded in a dense fibrous stroma. In the vesicles, the hydatid fluid is replaced by a jelly-like mass in older cysts, and there are few protoscolices (Barnouti, 1985).

2-7-4: Osseous Cyst: - bony bag does not take a form spherically to the presence of tissue bone as it extends to the bone length and a shape is an irregular containing little liquid so be small and solid attacks the bone marrow, leading to bone necrosis and break (Song *et al*., 2007).

2-8: Protoscolices

Protoscolices are protoscolices for each proboscis and sucker but are self-contained and originate from the internal surfaces of the germinal layer of the brood capsule (Schantz *et al*., 2006; Thompson, 2017). A single hydatid cyst can produce millions of protoscolices according to Cox (2004). The protoscolices go through the following stages in their evolution to mature (Galindo *et al*., 2002).

At the first phase, the cellular buds that produce protoscolices are formed by the affinity of cells in the germinal layer. The buds grow longer and the number of cells in the base of the buds decreases. Trenches form as elongated buds that divide the anterior, which will become the future scolex and the caudal which will eventually become the body. Hooks will be the first structures to emerge from the growing protoscolices' apical area. The front of the forming protoscolices which

subsequently evolve into suckers may be seen with circular projections and reduced in the more advanced stages.

Then, at the center of the circle created by the hooks, a conical structure representing the Proboscis emerges, the body region grows larger, and the neck space between the scolex and the body clears. The protoscolices are linked to the germinal layer by a thin Stalk throughout their development, after full differentiation, the protoscolices are detached for the germinal layer and it float freely in the fluid for hydatid cyst.

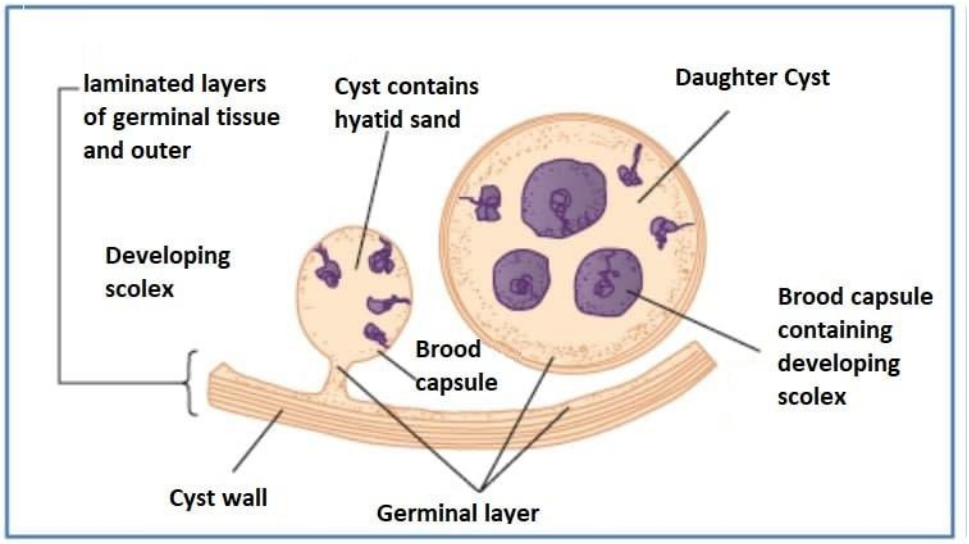

Figure (2 - 3): Structure of Hydatid Cyst (Gockel-Blessing, 2013)

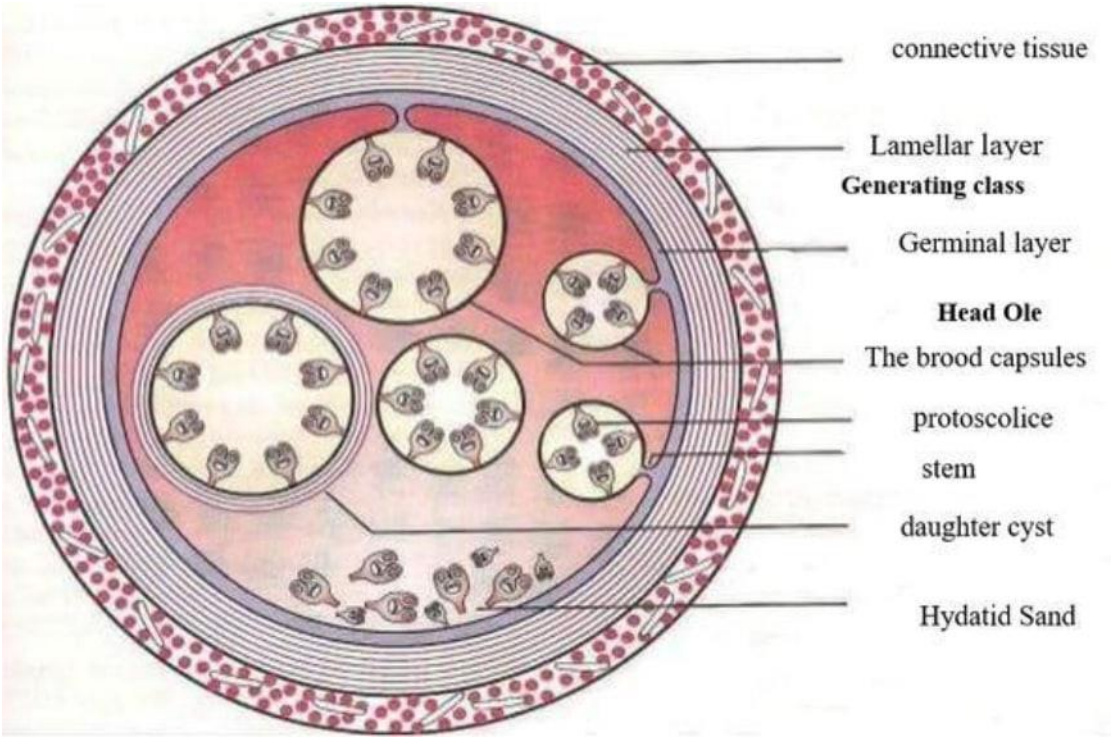

Figure (2-4): Structure of Hydatid Cyst **(Thompson and McManus, 2002).**

2-9: Pathogenicity and Epidemiology

Cystic Echinococcosis (CE) is spread largely due to the presence of a significant number of stray dogs. They were infrequently vaccinated, had easy access to contaminated offal at slaughterhouses, and received inadequate or unsuitable anthelmintic therapy (Khan, 2020).

Humans can be infected after inadvertently consuming eggs from domestic dogs, which are transmitted to humans through polluted water, vegetables, and fruits. This can happen if human eats raw veggies or other foods that have been contaminated with dog faces. While searching for pet dogs, fingers infected with eggs may take them to the mouth (Thompson, 2017).Karyakarte and Damle (2008) explain that Childhood

is the most prevalent time for infection because they are more likely to have intimate contact with pet dogs.

In Iraq, Hydatidosis is caused by the helminths *E.granulosus* and *E.multicularis*, which are hyperendemic. It is one of the most dangerous helminth infections with a substantial socio-economic impact because it affects both humans and livestock, and the cost has been estimated to be millions of dollars (Saida, 2011). According to the findings of a prior study, CE infected 60% of the patients, with the livers being the mostly afflicted organ (57.8%), the lung (26.4%) (Shafiei *et al*., 2018).

Galeh *et al*. (2018) and Wen *et al*. (2019) have found that the incidence rates of CE in endemic locations in Central Asia, South America the and Mediterranean countries have been reported to range from (1-200) per 100,000 people, with a low fatality rate (2-4%).

The epidemiologic studies and surveillance of control programs, determine that the infection rate in dogs is valuable for assessing the dynamics of transmission and the risk of infection. In the past, infection in dogs was determined by looking for worms in intestinal washes after death or after arecoline purgation (WHO, 1981; Mehmood *et al*., 2022).

Certain endemic locations, preventative activities such as hygiene education, control and surveillance in slaughterhouses, farms and dog-sheep-human interactions were planned, several methods for preventing hydatid cysts include disposing of all dogs that may have eaten uncooked sheep or internal organs, setting out of dead and infected animals, control of stray dogs and personal hygiene hand washing after use or playing with dogs. The control was essentially dependent on a community area or county (Craig *et al*., 2017).

2-10: Clinical Signs of Hydatidosis

Hydatid disease is characterized by asymptomatic cysts that are developed slowly and adversely in the liver, lungs, spleen, brain, bone marrow, and occasionally other organs (Budke *et al*., 2013; Hajizadeh *et al*., 2013; Casulli, 2020).

Clinical indications are determined by the parasite's location and size (David and Petri,2006). In humans, diseases are mostly caused by pressure effects induced by growing cysts (obstruction) and hypersensitivity to *Echinococcus* antigen (Paniker, 2007). Hemoptysis, Dyspnea, and Hematuria can also occur in the kidney (Paniker, 2013).

If the cyst ruptures and there are accompanying symptoms such as pruritus, urticarial rash, and severe anaphylactic shock, hypersensitivity or anaphylactic reactions to worm antigens can ensue (Harvey and Fisher, 2007). Bronchopneumonia, hepatic disorders leading to a scitis; jaundice; heart failure, the protoscolices infiltrate from the cyst and transport to the other part of the body and grow their secondary hydatid cyst (Paniker, 2013).

The invasion of the metacestode stage in the liver can cause parenchymal atrophy and cirrhosis. When alveolar echinococcosis spreads to the liver, it forms clusters of tiny gelatinous cysts that resemble malignant neoplasia (Taylor *et al*., 2007). Infection in the definitive hosts is frequently asymptomatic and subclinical (Zajac and Conboy, 2006). The liver is inflamed and sensitive when palpated with abscesses, as well as stomach nausea, vomiting, and pain, like a rise hepatic blood pressure and the lower vena cava cavity. The bile ducts have also developed fibrosis, and the hydatid cyst exerts a lot of tension on the diaphragm (Brunetti, 2015).

2-11: Diagnosis

Echinococcosis cysts are normally asymptomatic until they grow large enough to cause damage to nearby tissues and organs. The clinical signs are similar to those of a mass lesion (Eckert *et al*., 2001; Hansh *et al*., 2016).

Ultrasonography, CT scanning, and magnetic resonance imaging (MR imaging) are all common imaging procedures. Ultrasonography is the most popular imaging method for detecting hydatid lesions and determining the number and size of hydatid cysts in practically all anatomical locations (Brunetti *et al*., 2018).

Many serological procedures are employed in diagnosis, including the Complement Fixation Test, Indirect Hemagglutination Test, Latex Agglutination Test, Western Blot, and others. The presence of Protoscolices suggests that the cyst is active (Pawowski *et al*., 2001). Even during the postmortem, the majority of cases are observed and confirmed. Imaging studies, cyst fluid examinations, and serologic tests are used to make a diagnosis immunodiagnostic test (Beers, 2006).

In the identification of Hydatidosis, contemporary techniques polymerase chain reaction, for example (PCR) with high sensitivity and sequencing of DNA specificity are used (Ghosh, 2013).

A biopsy can be utilized to diagnose *Echinococcus*, although ultrasonographic controlled tiny needle penetration for cyst fluid aspiration can result in cyst collapse or leaking (Sbihi *et al*., 2001).

The presence of adult *Echinococcus* spp. cestodes in the feces or small intestine, or the identification of particular coproantigens or copro DNA, are required for the diagnosis of *Echinococcosis* in dogs and other carnivores (OIE, 2008).

2-12: Treatment

2-12-1: Surgical Treatment

Where cysts are accessible, surgical excision is the best treatment option in humans, but recurrence following surgery is common (Paniker, 2007). Iraq (where the current study was conducted) is regarded as one of the countries that is plagued by the endemic sickness, in most circumstances, the most effective treatment for that disease is surgery, which can be challenging in some cases when cysts have spread to many organs or established in dangerous places (Norouzi *et al*., 2020).

Unless the larval mass as a whole can be removed, surgery is recommended if it is possible, which is determined by the size, location, and symptoms of the lesion, Albendazole has been shown to slow the progression of inoperable lesions. In a few cases, liver transplantation has saved their lives (Paniker, 2007).

2-12-2: Chemotherapy

As a result of the failure of some surgical removals in some cysts located in places that are difficult for the surgeon to deal with or reach, some researchers resorted to finding other treatment alternatives for this disease, including the use of the treatments below (Moro and Cantey, 2018).

Methyl-5-(6)- bropylthiobenzimidazole –2- carbamate, is one of the preferred treatments in the treatment of hydatid cyst disease, which works to break down the generative layer, shrink it and decay, as well as the disappearance of the daughter cysts inside the cyst (Yarsan *et al*., 2003).

The use of the treatment for people with this disease showed improvement when continued use and the disappearance of the cysts, leaving the affected tissue naturally, but it may cause simple poisoning in the liver and the lack of white blood cells, and give a dose of 10 mg/kg

daily divided into a treatment arrangement for 28 days, or the order is repeated several times with rest periods of not less than 14 days (Adas *et al*., 2009).

Albendazole, an oral anthelmintic with a broad spectrum of action, is the treatment of choice for hydatid illness (Adas *et al*., 2009). Albendazole 400 mg orally daily for (1-6) months (7.5 mg/kg) cures 30%–90% of individuals with *E.granulosus* and can be used to inhibit growth in inoperable cases, if cyst contents spill, albendazole is frequently given before surgery to prevent metastatic infections (Beers *et al*., 2006).

Albendazole was used to treat a patient with hydatid cysts in the lung, heart, liver, and spleen. After therapy, a cyst in the heart was entirely removed, and the size of the liver cyst decreased. However, the number and size of lung hydatid cysts did not be improved (Eser *et al*., 2013).

Pensel *et al.* (2014) investigated the effects of albendazole and poloxamer 188 on protoscolices survivability. Both medications, when used together, slowed the progression of hydatid cysts *in vitro*. Treatments with mebendazole, albendazole, and praziquantel are effective (Taylor *et al*., 2007).

Ivermectin and other treatments can be used successfully in the treatment of dermatophytosis in cattle generated by immunopotentiation due to an increase in total white blood cells (lymphocytes) after ivermectin injection, according to a study by (Ghassan and Amjad, 2015).

In vitro combined therapy with flubendazole and ivermectin had a significant effect on *Echinococcus granulosus* protoscolices and metacestodes (Elissonodo *et al*., 2009).

2-13: Medicinal Plants

Medicinal plant extracts have been used to treat many diseases, including hydatid cysts, medicines herbals generally are used due to their low cost, availability, acceptability, and assessed to be more harmless than synthetic medicines (Ozioma and Chinwe, 2019), Moreover those plants produce secondary compounds for treatment like phenolic, alkaloid, and terpene compounds (Wickzkowski *et al.*, 2012).

Because some plants contain important components that induce specific reactions that aid in the treatment illness of humans, they are known as medicinal plants (Kumar *et al*., 2015). These plants are employed because their chemicals have antibacterial activity, and these compounds are produced by the plant's secondary metabolism (Bakht *et al*., 2011).

Fruit and vegetable purees have therapeutic capabilities not only because of their sensory qualities, but also because of physiologically active substances including vitamins, minerals, and antioxidants that are beneficial to one's health (Vasudha and Mishra, 2013).

Raoof *et al*. (2009) studied the impact of *Dendrosicyos socotrana* and *Jatropha unicostate* plant leaves alcohol extracts, finding that the *D.socotrana* plant killed protoscolices in vitro for fifteen days at a dosage of 5000 kg/ml. According to the study, the alcoholic extract of the *J.unicostata* plant, as well as the dose, were shown to have a clear effect in lowering the number of secondary hydatid cysts and their weights, and this extract had a comparable effect on the pharmaceutical albendazole. The second extract of the *D.succotrana* plant had a weak impact on lowering the quantity and weight of subsequent hydatid cysts.

In the experiment conducted on rats infected with live primary protoscolices and treated after six months with the extract of Propolis at a

concentration of 50, 100 and 150 mg/ kg of body weight, it was found that this substance at a concentration of 150 mg/kg led to a reduction in the weights and numbers of secondary hydatid cyst compared to untreated control (Kismet *et al.*, 2008) .

The protoscolices were collected from hydatid cysts from sheep livers infected, the suspension of these protoscolices was added to the different concentrations of the boiled aqueous extract of *Aloe vera* at concentrations (2.5, 5, 10) mg/ml and cold aqueous extracts of *Aloe vera* at concentrations (2.5, 5, 10) mg/ml, for different periods of time (0, 1, 24, 48, 72, 96, 120) hours, and the viability of these protoscolices was measured using eosin aqueous dye (0.1%). The results showed that the percentage of viability of the protoscolices was 92.73%, and the percentage of viability of the protoscolices ranged 68, and the results showed that the boiled aqueous extract of *Aloe vera* at concentration 10 mg/ml more succeded to killing protoscolices initial, after five days the viability of the protoscolices was 60.9-68%, it eliminated the viability of the protoscolices after one day from the start of the experiment (Al-Khalidi and Al-Hamairy, 2016).

2-13-1: *Hibiscus sabdariffa* L.

The classification of *Hibiscus sabdariffa* L. is done cited by

Acevedo-Rodríguez and Strong (2007) as follows:

Kingdom: - Plantae – Plantes, Planta, Vegetal, plants
Subkingdom: - Viridiplantae – green plants
Infrakingdom: - Streptophyta – land plants
Super division: - Embryophyta
Division: - Tracheophyta – vascular plants, tracheophytes
Subdivision: - Spermatophytina – spermatophytes, seed plants,
Class: - Magnoliopsida

Superorder: - Rosanae
Order: - Malvales
Family: - Malvaceae – mallows, mauves
Genus: - *Hibiscus* L. – *rosemallow*
Species: - *Hibiscus sabdariffa* L. – *roselle*

Hibiscus sabdariffa (Roselle) is a 2–2.5m tall annual or perennial herb or woody-based subshrub with a woody base belonging to the Malvaceae family. The leaves are 8–15 cm long and deeply 3-5 palmately lobed on the smooth, and cylindrical red stems alternately (Mohamed *et al*., 2007).

The flowers are 8–10 cm in diameter, white to pale yellow with a dark red mark at the base of each petal, and have a strong meaty calyx at the base, 1–2 cm broad, growing to 3–3.5 cm, as the fruit matures, fleshy and bright red. It takes around six months for it to reach full maturity, Roselle is planted at the start of the rainy season, around mid-April, and harvested for the calyces of the fruits around three weeks before flowering begins (Naim and Ahmed, 2010).

Ali-Bradeldin *et al*. (2005) stated that roselle is a rich source of vitamins, minerals, and other active components such as phytosterols, organic acids, and polyphenols, many of which have antibacterial properties.

The commercially significant portion of the plant is the calyx (sepals) that surrounds the fruit (capsules). Chen *et al*. (2013) the whole plant can be consumed as a beverage, or the dried calyces can be soaked in water to make a colorful cold drink, or they can be boiled in water and consumed hot. It also has medicinal benefits.

Olaleye (2007) investigated the phytochemical components and cytotoxicity of the aqueous methanolic extract of Roselle (*Hibiscus sabdariffa*) and discovered cardiac glycosides, flavonoids, saponins, and alkaloids in the extract.

Fullerton *et al*.(2011) stated that the findings support that used of these plants in traditional medicine for diseases such as cancer, bilious conditions, abscesses, and coughs, also point to the possibility of isolating anticancer and antibacterial agent while Fullerton tested antimicrobial activity of *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* isolates from food, veterinary, and clinical sample .

In India, the traditional Chinese medical system, the medicinal plants are gaining appeal a natural antibacterial agent. In folk medicine, the Roselle and Linne (Malvaceae) have been used as diuretic, a mild laxative, and treatments for heart and nerves disease (Puro *et al*., 2014). These plants are highly rich in vital minerals and nutrients such as alkaloids, tannins, saponins, glycosides, phenols, and flavonoids in their extract (Okereke *et al*., 2015).

Elowni *et al*. (2020) looked into the possibility of using an aqueous extract of plant calyces to test the viability of PSCs using plant pigment uptake/exclusion as a criterion because the extract can be employed as an objectively quantified low-cost assay for assessing the viability of PSCs from hydatid cysts comparable to that of eosin.

2-13-2: *Beta vulgaris* L.

Beta vulgaris is classified according to (ITIS, 2022) as:

Kingdom: Plantae – Plantes, Planta, Vegetal, plant
Subkingdom: Viridiplantae – green plants
Infrakingdom: Streptophyta – land plants

Super division: Embryophyta
Division: Tracheophyta – vascular plants, tracheophytes
Subdivision: Spermatophytina – spermatophytes, seed plants
Class: Magnoliopsida
Superorder: Caryophyllanae
Order: Caryophyllales
Family: Amaranthaceae-pigweed, amaranthes
Genus: *Beta* L. – beet
Species: *Beta vulgaris* L.

(Beta vulgaris ssp. *vulgaris* L.) red beetroot is a herbaceous biennial (the flowering in second year for growth) or, occasionally, perennial plants that grow to a height of 120 cm (200 cm in second year), However, most cultivate variants are tow year. Cultivated varieties have dark red, white, or yellow roots that are moderately to strongly colored severely swollening and fleshy, but the wild subspecies' roots are brown, fibrous, and occasionally swollen and woody (Chhikara *et al*., 2019).

The Plants in the *Beta* genus are thought to have originated in North Africa and spread over Europe, Asia, and the Americas via the Mediterranean Sea route (Neelwarne and Halagur, 2013).

Beetroot is distinguished by the presence of a functional component called betalain pigment, which can minimize the detrimental effects of free radicals produced during the oxidation process in cells (Niari *et al*., 2012). Betacyanins have an anti-inflammatory action as well as the ability to inhibit radical scavenging activities, in cervical, ovarian, and bladder cancer cells (Pedreno and Escribano, 2000). hepatoprotective (Olumese and Oboh, 2018), inorganic nitrate (Clifford *et al*., 2015), cardiovascular health protector other benefits mentioned by (Kale *et al*., 2018), and suppression of lipid peroxidation and chemopreventative effects (Babarykin *et al*., 2019).

The bioactive components in red beet are abundant, it high concentration of physiologically actives chemicals, betalain, inorganic nitrates, and others polyphenol, and folate since the tuberous root contains nutrients and vitamins (Sing and Sing Hathan, 2014; Babarykin *et al.*, 2019). Sugar beets (*Beta vulgaris saccharifera*), fodder beets (*Beta vulgaris crassa*), leaf beets (*Beta vulgaris cicla*), and garden beets (*Beta vulgaris rubra*) are among the cultivated beets (Lewellen *et al*., 2009).

2-13-3: *Brassica oleracea* L.

A classify of *Brassica oleracea* L. according to (Dias *et al*., 1993) as:

Kingdom: Planata
Division: Magnoliophyta
Class: Magnoliopsida
Order: Brassicales
Family: Brassicaceae or Cruciferae
Genus: *Brassica*
Species: olearacea L.

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of most significant the crop plant of Brassica *oleracea* L. species, that belong to the Cruciferae family which has a long history in Iraq (Faltusová *et al*., 2011). It is a blooming herbaceous, biennial, dicotyledonous plant with a distinctive compacts crown of leaves, white heads cabbages (*B. oleracea* var. *capitata* sub. var. *alba*) and red heads cabbages (*B. oleracea* var. *capitata* sub. var. *rubra*) are two kinds of the cabbage (Faltusová *et al*., 2011).

Red cabbages *(Brassica oleracea*) is a type of the cabbage that originated in Southwestern Europe and the Mediterranean region. It is currently grown all over the world (Arapitsas and Turner, 2008). It is a herbaceous flowering plant with pressed leaves in the shape of a head,

The origin of the word (cabbage) is derived from the French word caboche, and the meaning of the head for the name of the genus *Brassica*, is derived from the Latin word Brassicaceae, which includes about (300) genera and about (3000) species of plants spread all over the world (Sharma *et al*.,2022), which also includes broccoli, cauliflower, and kale. The size, shape, and color of the leaves, as well as the texture of the head, differ significantly amongst cultivated cabbage kinds (Singh *et al*., 2006).

Sarikamiş *et al*. (2009) state that cabbage eating has a positive impact on human health, and it is linked to the secondary metabolites termed glucosinolates it is recognized to have anticarcinogenic characteristics for eighteen cabbage cultivars, discovered variations phytochemicals rich in antioxidants (ascorbic acid, lutein, -carotene, tocopherol, and phenolics) (Singh *et al*., 2006).

Cabbage is often used in traditional medicine to treat symptoms related to gastrointestinal diseases (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) and small cuts and wounds and mastitis due to its antioxidant, anti-inflammatory, and antibacterial characteristics (Samec *et al*., 2011; Ramirez *et al*., 2020). Popular Cabbage contains simple phenols, Polyphenols, Phenolic acid, Flavonoids, Hydroxycinnamic acid, and Carotenoids (Jahangir *et al*., 2009; Wickzkowski *et al*., 2013).

Also, it contains Carotenoids, Hydroxycinnamic, and Antioxidants are found naturally in this product. There are also plenty of vitamins including tocopherol (E) and ascorbic acid (Vitamin C). These antioxidant compounds protect the human body from oxidative damage caused by free radicals, particularly active forms of oxygen, and so help to prevent chronic diseases like cardiovascular disease and cancer, and this food is high in fiber, minerals, and glucosinolates (Jahangir and colleagues, 2009).

According to Wu and Prior (2005), red cabbage has a distinct anthocyanin pattern with a high concentration of anthocyanins (cyanidin glycosides). Numerous studies have confirmed that anthocyanins have pro-health functions, acting as an antioxidant, anticancer, antiulcer, antitumor, antimutagenic, cardioprotective, vision improvement, antidiabetic, antineurode generative, antibacterial, and ocular (Canter *et al*., 2004; Bell, 2006).

2-13-4: *Crocus sativus* L.

The classification of *C.sativus* is as follows: (Saxena, 2002)

Division: Spermatophyta
Sub-division: Angiospermae
Class: Monocotyledonae
Sub-class: Liliidae
Order: Liliales
Family: Iridaceae
Genus: *Crocus*
Species: *sativus* L.

Saffron (*Crocus sativus* L.), the dried stigmas, is one of 85 known spices in the world belonging to the Iridaceae family which is the most expensive. It's also at the top of the list of the most valuable agricultural commodities. Saffron is also known as Red Saffron for its use in many products, for which reason it is unsurprising that it is called red gold (Husaini *et al*., 2009). Because of their perfume, color, and bitter taste, saffron flowers, which have only three stigmas each, are used as a food additive (Singh *et al*., 2010).

Crocus sativus is a purple or lilac tuber plant that looks like grass. Flowers of various colors, a flower stalk is a long, thin stalk that grows from a bulb. The flower itself is huge and gorgeous, with a white, slender tube. Leaves are radial and linear, with a dark green upper surface and a

pale green lower surface, sometimes still wrapped in a membranous sheath fresh for almost the entire winter (Molina *et al.*, 2003). The stigma, which is where the saffron comes from has six violet tepals, three yellow stamens, and a single pistil on this flower. with three red filaments (Gresta, 2007).

Crocus sativus is a flowering plant that produces saffron. The word "saffron" comes from the Arabic word zafaran, which means “yellow" (Rios *et al*., 1996). Saffron is used as an oregano spice, a food colour, perfume, dye, and ink. The origin of saffron is said to have originated in Iran, India, and China, it has also been successfully grown in other areas, such as Europe. (Caballero-Ortega *et al*., 2007).

In ancient Egyptian medicine, saffron was used to heal kidney and stomach problems, as well as to aid in the delivery of newborns (Lev, 2002). It is mentioned in both The Bible and ancient literature (Bachrach, 2012).

Saffron extract and tincture are used as a food flavoring, digestive, appetite suppressant, sedative, diaphoretic, expectorant, and tonic, as well as for the treatment of liver and gallbladder disorders, convulsions, spasms, toothache, inflammation of the nasal and throat mucosa, bloating, insomnia, cognitive disorders, acute postpartum haemorrhage and pain, cough, asthma, nausea, urinary tract infections (Kianbakh and Ghazavi, 2005). In addition to main metabolites, like, minerals, carbohydrates, vitamins and fats, Crocin (mono-glycosyl polytene esters), crocetin, and crocus sativus L include four primary bioactive components (a natural carotenoid dicarboxylic acid precursor of crocin),Picrocrocin (a monoterpene glycoside precursor for safranal as well as a byproduct of xeaxanthin breakdown) and safranal are two compounds that are related. (Melnyk *et al*., 2010). Crocins, which are extremely water-soluble, are widely utilized as a natural food colorant and antioxidant, quenching free

radicals and protecting cells and tissues from oxidation (Melnyk *et al*, 2010).

2-14: Nanobiotechnology:

Nanobiotechnology is a new growing subject of nanoscience that combines nanotechnology with biotechnology to generate pioneer materials for use in health, medicine, the environment, economics, science and technology, and other fields, resulting in a major shift in technological development (Palmqvist, 2017).

Biswas and Dey, (2015) stated that the study, manufacture and synthesis of materials with a diameter of less than one millimeter is known as nanotechnology. The term "nano" is derived from a Greek word that means "dwarf" or "extremely small (Rai *et al.*, 2008). The chemical and physical properties from nanomaterials can become substantially distinct from others of the same type substance in bigger bulk for, Nanoparticles are atom clusters that range in size from 1 to 100 nanometers.

Gazit and Mitraki(2013)explained that the health effects of nanotechnology , people's wealth and life will be at least equal to the combined effects of microelectronics, "Medical imaging, computer-aided engineering, and man-made polymers" are some of the topics covered. Nanobiotechnology refers to the use of nanotechnology techniques to generate and improve biotechnological processes and products.

Silver is thought to be a benign and beneficial antimicrobial metal due to its non-toxicity to animal cells. Silver nanoparticles, or AgNPs, are the metal nanoparticles of interest because of their shape and size, which are influenced by optical, magnetic, catalytic (Hassan *et al*., 2019) and

electrical properties (Zhang *et al*., 2016). Antimicrobials, cosmetics, biosensors, composite fibers, superconductors, and electronic components are all examples of AgNP applications (Thamilselvi and Radha, 2017). Nanomaterials have been used in a variety of fields, including semiconductors. (Tahir *et al*., 2020), biomaterials (Kang *et al*., 2020), medical treatment (Devi, 2019), treatment delivery (Anoop *et al*.2020), and cancer treatment (Korkmaz *et al*., 2020).

2-14-1: Synthesis of the Silver Nanoparticles

In general, the silver nanoparticles were made using many techniques: Chemical, Biological, and Physical methods. Vanaraj *et al.* (2017) stated that physical and chemical synthesis processes have the drawbacks of being very expensive, the energy-intensive, and high toxic for environment, as well as being unsuitable for biological applications due to the usage of poisonous and hazardous compounds that are responsible for a variety of biological dangers. It also reduces the need for high-temperature, high-pressure, high-energy, and harmful chemicals.

Zuas *et al*. (2014) state that biosynthesis processes have the advantages of being cost-effective and environmentally friendly, capping and stabilizing are found in plant or microbial cultures and are hence harmless.

Silver nanoparticles can be generated by biological processes (biosynthesis) using microbial or extract methods. from plants. Synthesis of silver nanoparticles by (microorganisms, bacteria, fungi, plants) (Gazit and Mitraki, 2013).

Currently, the synthesis of nanoparticles from plant extracts has been used as a low-cost, environmentally friendly, non-toxic and cost-effective method for making nanoparticles of various shapes, sizes and shapes for

testing hydatid cysts (Mohanpuria *et al*., 2008), such as the study of Salih *et al.* (2020) when they used three distinct plant extracts taken from *Piper nigrum*, *Ziziphus Spina-Christi*, and *Eucalyptus globulus* leaves to speed up AgNP biosynthesis. In addition, the scolicidal activity against *Echinococcus granulosus* is being investigated.

2-14-2: Characterization of Silver Nanoparticles

2-14-2-1: Color Change

The color reaction mixture change is documented through visual observation. The changing color is pointed to the synthesis of silver nanoparticles (Korbekandi *et al.*, 2013; Benakashani *et al*., 2016).

2-14-2-2: UV-Visible Spectral Analysis

The UV-Visible and characterization absorption spectra of AgNPs are key attributes, and it is proved to be a good technique for characterization of AgNP formation and growth. The disparity in spectra can be explained by the quantity of particles in the solution and their size distribution (Zhou and Wang, 2012).

2-14-2-3: Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) gives topographical and elemental information on NPs at relevant magnifications and with a virtually infinite depth of field, the elemental composition of NPs, grain size, surface roughness, porosity, size distributions, homogeneity, intermetallic distribution, and diffusion can all be evaluated using SEM (Palmqvist, 2017).

2-14-2-4: Energy-dispersive X-ray spectroscopy (EDXS)

Surface analysis and elemental characterization of a sample are investigated using energy dispersive spectroscopy. The examination of the released X-rays of various energy from the sample when an electron beam contacts its constituents is the basic premise. The amount and composition of metal nanoparticles may be easily determined by looking at the sample's surface (Rades *et al*., 2014).

2-14-2-5: Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR is one of the techniquesthat is used in nanomaterials characterization. FTIR determines heterogeneous nature and surface activity by collecting data from absorption and reflection spectra at certain lambdas. FTIR technique is used to explore the vibrational structures of materials (Chen *et al*., 2015). Nanoparticle sizes and crystalline structure can also be determined using FTIR (Kumar and Kumbhat, 2016).

2-14-2-6:X-Ray Diffraction (XRD)

X-ray diffraction is a widely used and extremely effective non-destructive characterization technique for studying the physical characteristics, crystallographic structure, and chemical composition of materials, as well as defect structure, grain size, and strain (Sharma *et al*., 2012). Aside from amorphous materials like polymers, it's also used to figure out atomic configurations and thickness of thin films (Sharma *et al.*, 2012; Castillo-Michel *et al.*, 2017).

2-15: Polymerase Chain Reaction (PCR)

In vitro, the polymerase chain reaction (PCR) is a method used in molecular biology and genetics to amplify and multiply one or a few copies of DNA to make thousands to millions of copies of DNA sequences (Newton and Graham, 1997).

In medical and biological research laboratories, it has a variety of important applications, including the detection of genetic mutations and in the fields of forensic medicine and crime investigation, the identification of a specific gene not only for humans but also for bacteria and viruses, the detection of genes that play a role in some illnesses, such as cancer, and the diagnosis of several diseases and opportunistic infections (Joshi and Deshpand, 2010; Garibyan and Avashia, 2013; Mehmood *et al*., 2020).

Ten genotypes, G1-G10, have been found around the world based on nucleotide sequence analysis of the mitochondrial cytochrome oxidizes subunit 1 (Cox1), dehydrogenase subunit 1 (Nad1), and internal stranscribed spacer 1 (ITS1) genes. Sánchez *et al.* (2010) related these genotypes to a variety of intermediate hosts, including sheep, pigs, cattle, horses, camels, and goats. Various genetic diversity investigations discovered that *E. granulosus* had 10 strains, which were located in various parts of the world (Hammad *et al*., 2018). Cao *et al*. (2020) evaluated *Echinococcus* genotypes in patients with hydatidosis in Qinghai Province utilizing cytochrome c oxidase subunit partial sequencing one (Cox1) with NADH dehydrogenase - 1 (Nad1) genes.

The goal of the work of Hama *et al*., (2014) was to use polymerase chain reaction (PCR) and a specific sheep strain (G1) primer to identify sheep strains from intermediary hosts, including humans, and to detect

genetic diversity by sequencing, However, the species status of genotypes G6, G7, G8, and G10 of *E.granulosus* is unknown (Romig *et al*., 2017). Human cases with the G9 genotype, first discovered in 1997, are now regarded to have the G7 genotype (Cucher *et al*., 2016). Camels are also important in the epidemiology of *E.intermedius* (G6), a disease that can be transferred to humans (Thompson, 2008). The sheep strain of *E.granulosus* (G1 genotype) is the most common and prone to infect humans (WHO, 2001).

The most prevalent strain is sheep strain (G1), which is mainly responsible for human Echinococcosis; nevertheless, various other genotypes like G2, G3, G4, G5, and G6 have been discovered in humans. The first step in efficient CE management and limiting infection is molecular identification and strain determination (Hama *et al*., 2013).

Amplification of the ribosomal DNA internal transcribed spacer ITS (ITS-1 and ITS-2) regions, followed by genomic DNA extraction from hydatid cysts, were also used to identify the parasite morphologically and molecularly. Camels and cattle may play a role in the zoonotic parasite's transmission cycle, according to morphological and genetic studies (Gareh *et al*., 2021).

Abdul Kadhim (2020) used PCR to molecularly identify common strains of *Echinococcus granulosus*, study the gene sequences of the strains and draw the phylogenetic tree and then compare them to strains identified using NCBI-BLAST to discover the most prevalent strains in humans provinces of the Euphrates , using 14 samples from *Echinococcus granulosus* hydatid cyst from livers and lungs gene Internal transcribed spacer 1 of gene 18S rRNA DNA sequencing (ITS1) with molecular weights of 579 bp was targeted after the product was amplified by PCR.

Seven samples were found to be positive, while the remaining seven were found to be negative.

PCR test primers were used to determine the sex of the aquatic cyst worm and its genotypes in the germ of hydatid cysts samples of animal or human origin based on Internal transcribed spacer 1 of gene 18S rRNA DNA sequencing (ITS1), which had been amplified using the PCR technique (Nikmanesh *et al*., 2014).

A biologically developed pattern of strain variation is used to classify them. The mitochondrial DNA (mtDNA) genes coding for cytochrome c oxidase I (cox1) and small subunit ribosomal RNA (ssRNA) were directly sequenced in 47 hydatid cyst specimens collected from slaughterhouses from patients, as well as domestic the intermediate hosts such as sheep, cattle, goats and the buffalo to detect the molecular analysis in *E. granulosus*, strains circulating in two northern Iraqi cities (Kirkuk and Sulaimania). The sheep strain (G1) had a high prevalence, the buffalo strain (G3) had an isolated discovery, and cox1 and rrnS had seven and three distinct micro variants, respectively (Hammad *et al*., 2018).

In the Misan regions in southern Iraq, Al-Quzweeni (2019) undertook phenotypic and molecular research on *E. granulosus*. The first molecular diagnosis of *E. granulosus* infections in humans and other farm animals was performed in Misan province utilizing the technique of multi-polymerase chain interaction of the Cox1 and Nad1 genes.

The findings of another study on people from Iraq's Kurdistan region to determine the features, location, cyst stage, and species/genotypes of *Echinococcus granulosus* after surgical removal of 64 echinococcal cysts from 62 patients revealed that the liver was the most common anatomical

site of CE. Molecular studies of the mitochondrial NAD5 gene revealed that 59 of the 62 samples were G1 or G3 genotypes (Issa *et al*., 2022).

Chapter three

Materials

&

Methods

3-1: Materials

3-1-1: Equipment and Tools

Table 3-1: The Devices or Equipment used in the current study with the name of the manufacture and origin country

No.	**Equipment Name**	**Company and origin**
1.	Autoclave	Binder (USA)
2.	Camera	Xiaomi (China)
3.	Centrifuge	Fanem (Saupaulo- Brazil)
4.	Deep Freezer	Liebherr (Germany)
5.	Digital camera	Nikon (Japan)
6.	Electric blender	Silver crest (Germany)
7.	Electric oven	Memmert (Germany)
8.	Electronic balance sensitive	Metter (Switzerland)
9.	Exispin centrifuge	Bioneer (Korea)
10.	Fridge	Kiriazi (Egypt)
11.	Gel electrophoresis	Lab net (USA)
12.	High-Speed Cold centrifuge	Eppendorf (Germany)
13.	Incubator	Mammert (Germany)
14.	Light microscope	Snitch Xsz-N107 (Malaysia
15.	Thermo cycler PCR	Bio Rad (USA)
16.	UV Transilluminator	ATTA (south Korea)
17.	Vortex	Bioneer **(**south Korea**)**
18.	Water Bath	Jona lab (south Korea)

3-1-2: Tools and instruments

Table 3-2: Laboratory tools used in the current study with the name of the manufacturer and origin country

No.	Tools name	Company and origin
1.	Anatomy sets	Samco stainless (England)
2.	Beaker	Lab (Germany)
3.	Centrifuge tubes	Afco-Dispo (Jordan)
4.	Cotton	Aslanli (Turkey)
5.	Masks	Broche (Malaysia)
6.	Eppendorf tube	Pioneer (South Korea)
7.	Micropipettes (different sizes)	CYAN (Belgium)
8.	Gloves	Broche (Turkey)
9.	Class Cylinder Graduated	Lab (Germany)
10.	Slides	C.S.M.D (China)
11.	Slide covers	C.S.M.D (China)
12.	Tube Plain	Afco-Dispo (Jordan)
13.	Washing bottle	Co-Ltd (China)
14.	Syringes	C.S.M.D.CO (China)

3-1-3: Chemicals

Table 3-3: Laboratory tools used in the current study with the name of the manufacturer and origin country

No.	Material name	Company and origin
1.	Absolute Ethanol (99%)	BDH (England)
2.	Agarose	BioBasic (Canada)
3.	Ethidium Bromide	BioBasic (Canada)
4.	TBE buffer	BioBasic (Canada)
5.	Free nuclease water	BioBasic (Canada)
6.	DNA Marker ladder	BioBasic (Canada)

3-1-4: The kits that used in the present study

Table 3-4: The Kit that Used in the present study with the name of the manufacturer and country of origin

DNA Extraction Kit \Favrogen	Company and origin
DNA Marker Ladder	BioBasic (Canada)
RBC Lysis Buffer	South Korea
buffer FATG	
FABG Buffer	
W1 Buffer	
Wash Buffer(concentrate)	
Elution Buffer	
FABG mini-column	
Collection Tube	

Table 3-5: Components of Master Mix that was used in this study.

Maxime PCR PreMix	Company and origin
Taq DNA polymerase	Intron (south Korea)
dNTPs (dATP, dCTP, dGTP, dTTP)	
MgCl2, KCl, Tris-HCl pH 9.0	
Tracking dye	

3-1-5: The Primers

PCR primers were used in the current study for detecting and genotyping of *E.granulosus* based on Gareh (2021) (ITS1-BD1Forward 4S reverse, ITS2−3S- forward A28 - reverse) genes that amplification by using the PCR technique. provided by (South Korea) in the following table:

Table 3-6: Primers that were used in this study.

Primers	Sequence		Amplicon
ITS1-BD1 Forward 4S (reverse)	F	5′GTCGTAACAAGGTTTCCGTA-3′	1,100 bp
	R	5′TCTAGATGCGTTCGAATGTCGATG-3′	
ITS2−3S- forward A28 – reverse	F	5′GGTACCGGTGGATCACTCGGCTCG3′	750 bp
	R	5′GGGATCCTGGTTAGTTTCTTTTCCTC CGC-3′	

3-2: Preparation of chemical solution

3-2-1: Kreb Ringers Solution

This solution is considered one of the finest for maintaining protoscolices alive outside the hydatid cysts (Al-Rubaiey,1999).

Table 3-7: Materials used in preparing kreb ringer solution depend on Routunno *et al*. (1974).

No.	Materials	Weight
1.	KCL	0.157 gm
2.	NaCL2	0.481 gm
3.	CaCL2	0.0137 gm
4.	NaHCO3	0.28 gm

5.	NaH2PO4	0.097 gm
6.	NaHPO4	0.490 gm
7.	MgSO4	0.072 gm

The ingredients' chemical compounds were progressively 100 mL pure water, dissolved and the volume was increased to one liter. The solution was then autoclaved for 15 minutes at 121°C and 15 pounds per inch2 pressure. It was then maintained in the refrigerator at 4°C, using a sterile kreb ringer distillation solution with a 4:1 hydatid cysts fluid ratio as a culture media to maintain protoscolices and then measuring their viability.

3-2-2: Water Eosin Stain (0.1%)

According to Smyth and Barret (1980), this dye is made by dissolving 0.1 gm of dye powder in 10 ml of distilled water and storing it in a refrigerator at 4 C°. The viability of the protoscolices was determined using this dye. Because the dye does not pass through alive protoscolices' with their membranes whereas the dead protoscolices take the dye because their membrane lacks the permeability for any materials, the protoscolices that are stained green are alive in accordance with the viability calculation formula.

Viability (%) = The rate of live protoscolices/ the total number of protoscolices (alive + dead) ×100 (Smyth, 1985).

3-2-3: Preparation of Phosphate Buffer Saline (PBS) pH=7.2

Phosphate Buffer Saline (PBS) was prepared according to Dacie and Lewis (1991) as shown in Table (3-8).

Table 3-8: The Materials Used in Preparing Phosphate Buffer Saline: -

No.	Materials	Weight
1.	Potassium Hydrogen Phosphate	0.2 gm
2.	Sodium Chloride	0.8 gm
3.	Potassium Chloride	0.2 gm
4.	Disodium Hydrogen Phosphate	1.15 gm
5.	Distilled water	80 ml

3-2-4: Preparation of Formalin (% 10) depend on (Suvarna *et al*., 2013) to Prepare the Formalin.

Table 3-9: Component of Preparation of Formalin

No.	Materials	Weight
1.	Formalin (%40-37)	100 ml
2.	Sodium Phosphate dibasic	6.5gm.
3.	Distilled water	900 ml
4.	Sodium Phosphate monobasic	4 gm

3-3-1-: Collection samples of hydatid cysts from the human:

The samples of human were collected of Al-Qadisiyah province, Al-Najaf's general and private, as well as hospitals of Babylon and Baghdad as shown in (Figure 3-1).

The hydatid cyst samples were taken from infected humans preserved in formalin solution who had previously been diagnosed by ultrasound (sonar) or CT scan and had undergone surgical procedures. Those

samples were promptly relocated to the College of Sciences for Women's advanced parasitology laboratory / University of Babylon by the designated container (Barzanji *et al*., 2019; Issa *et al*., 2022).

3-3-2: Collection Samples of a Hydatid Cyst from Animals:

Samples of hydatid cysts were collected from infected organs of sheep slaughtered at slaughterhouses in Babylon and Al-Najaf provinces. The samples were packed in sealed and labelled polyethylene bags and sent to the laboratory for advanced parasitology in the College of Sciences for Women's (Figure 3-2).

The protoscolices were collected and stored in a conservative medium Kreb Ringers solution for the current experiment. The viability of the protoscolices were assessed using the eosin stain (1%) approach (Smyth, 1985).

3-3-3: Preparation of Protoscolices

To avoid contamination with host tissue, the cysts were rinsed many times with sterile phosphate buffer saline and extensively cleaned with 70% alcohol. The cysts were then opened longitudinally by using forceps and scissors, and the cyst fluid contents were drained aseptically via sterile disposable syringes 10 milliliters into sterile tubes. The protoscolices were then pelleted by centrifugation at 3000 rpm for 10 minutes at room temperature.

Only two milliliters of fluid with precipitate remained in the bottom of the test tube, after which one drop was taken with a pasture pipette and mixed with one drop of eosin (0.1%) stain and then placed on a slide covered by a cover slip with examined by the microscope (40x) to check for the protoscolices. Live protoscolices were then transferred into a sterile tube and stored at (4C°) until they were used (Hussein, 2020).

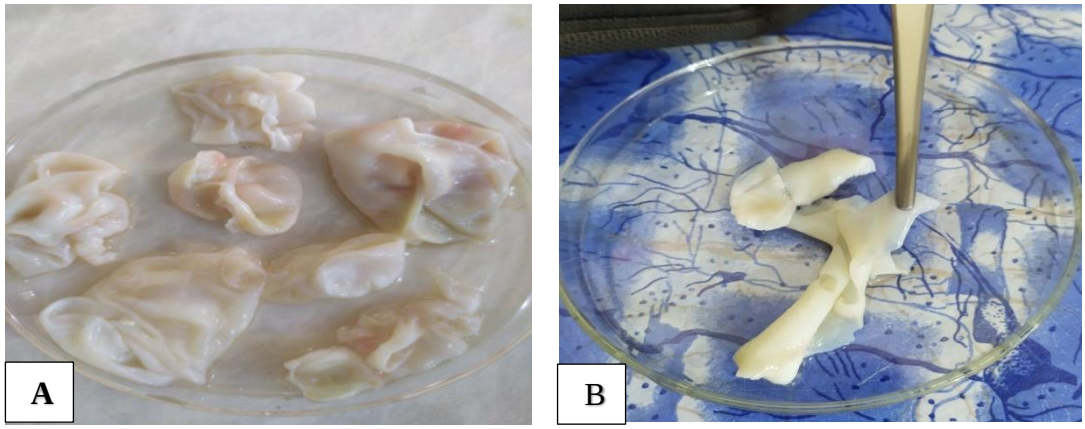

Figure 3-1: The germinal layer isolated from the hydatid cyst A- of liver sheep, B- of humans.

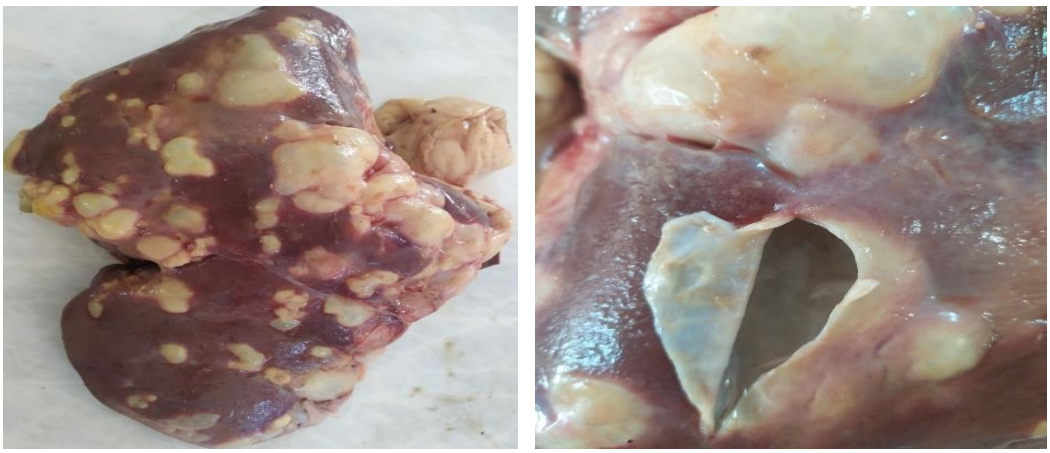

Figure 3-2: Infected Sheep Liver Hydatid Cyst

3-4: Estimating the Viability of Protoscolices

The viability of protoscolices were estimated using a micropipette to extract 10 milliliters from sediment in centrfuge tube containing clear protoscolices suspension, then place one drop (10 micromiters) on a glass slide with the similar volume of the eosin dye (0.1%), mixed well, examined under optical microscopy. The percentage of live protoscolices that appear bright green was calculated, as the percentage of protoscolices that were dyed in red color for the purpose of determining the protoscolices percentage as shown in (Figure 3-3), using magnification power of 100 and 400. (Smyth and Barrett,1980**;** Al-Aloosi *et al*., 2018).

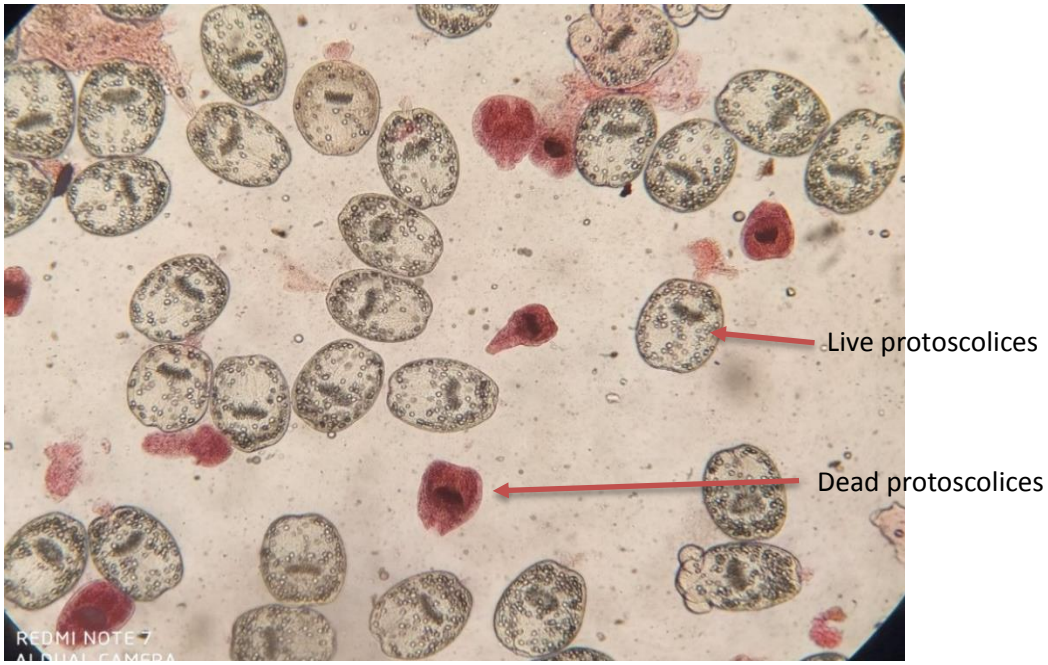

Figure 3-3: Microscopic appearance demonstrates the live protoscolices (Green) and the dead protoscolices (Red Color) stained with (0.1%) Eosin Stain (10X).

3-5: Plants are used in the study

3-5-1: Collection of Plant samples:

Plant samples were collected from different markets in Babylon provine, as shown in table (3-10), The plant samples were cleaned well by washing them with water to get rid of dust and left at room temperature to dry from the water completely. After making sure that it was dry and not infected with fungi, parts used in the current study were cut into very small pieces and placed in sterile special containers for the purpose of preparing the plant extract (Ahad *et al*., 2011).

Table 3-10: Plant species and their parts used in the current study

Plant species	Part used
Brassica oleracea var. capitata	Leaves
Crocus sativus	flower buds
Beta vulgaris	Roots
Hibiscus sabdarriffa L.	Calyces

Figure 3-4: Plants used in the current study (A- *Brassica oleraceae,* B - *Beta vulgaris,* C- *Crocus sativus,* D- *Hibiscus sabdarriffa).*

3-5-2: Preparation of the extracts of boiled water for plants

The hot boiled water extract was prepared for the above plant parts according to Harborne's maceration method (1998).

1- Macerate 40 g of plant material in a beaker with 500 ml hot boiled water, agitated for 30 minutes
2- After leaving for 24 hours to decompose the active ingredients more effectively, cover them tightly to prevent any foreign materials from entering.
3- Filter the solutions through two layers of gauze and centrifuge them for 10 minutes at 3000 rpm.
4- Take the leachate and leave the residue, then place the solution in (40-45) C° an electric oven to acquire a dry material from the extract.
5- Keep refrigerated in glass containers until ready to use (Figure 3-5). The process was repeated numerous times to obtain a significant amount of the extract to conduct experiments.
6- Prepare the stock solution concentration equivalent to 300 mg/ml after 7.5 gm of the crude plant was dissolved in 25 ml of distilled water. The concentrations (200,100) mg/ml were produced from this solution using the formula N1V1 = N2V2 (Al-Nakeeb, 2004 **;** Awwad *et al*., 2014).

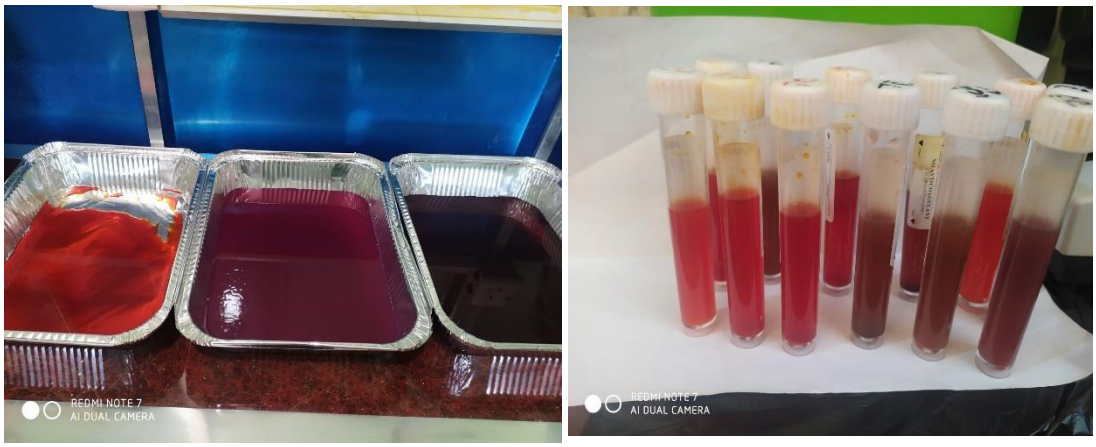

Figure 3-5: Stages of Preparation of the plant Extracts

3-6: Preparation dyes of the plants

One gram of each powder from the plant were dissolved in 20 ml of distilled water, spinning the flask occasionally to ensure that any grains stuck to the walls of the flask entered the solution, the solution was filtered with Whatman filter papers No.1 until it became clear, then stored in the refrigerator until needed (Kamal, 2018).

3-7: The effect of Boiled Water Extracts of Plants as Natural Products on the Viability for Protoscolices of Hydatid Cysts *In vitro.*

At the beginning of the experiment, the protoscolices were collected and their number, viability, and other factors were calculated, transfer (0.7-1) ml of protoscolices stock (Kreb Ringers solution + hydatid cysts fluid) to each of the test tubes in a 4:1 ratio, where the average number of protoscolices per tube was around (2000 protoscolices), For each extract separately, the tubes contained suspended protoscolices at concentrations of (300, 200, 100) mg/ml for each extract separately, the control was putting in distilled water for one militer, all the concentrations of the above were used three replications for all the extracts for each treatment , After that, the rate of protoscolices' viability were determined for the period of (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hour for treatment that bevidence to penetration of eosin dye (0.1%), A dead protoscolices identified by their color red, whereas a live protoscolices identified by their color bright green, and stored the treated tube at 25C° in the laboratory temperature (Al-Hamairy, 2010).

3-7-1: The Effect of Albendazole as a Comparison Treatment on the Viability of Protoscolices *In vitro: -*

The effect of albendazole medicines on protoscolices' viability was done *in vitro*. It is available at local pharmacies in Babylon province and

can be purchased there. The albendazole as an emulsion and a treatment of 10ml at a concentration of 400 mg as a stock solution, and prepared the concentrations 300 mg/ml, the concentrations 200 mg/ml and the concentrations 100 mg/ml from the stock solution using the formula (N1V1=N2V2), as mentioned above, to treat the viability of protoscolices (Al-Nakeeb, 2004; Ravis *et al*., 2018**).**

3-8: Preparation of Silver Nanoparticles

Silver nanoparticles were made by combining 5mL of aqueous extract of *H.sabdarriffa* with silver nitrate solution (weight 0.017g silver nitrate, dissolved in 100 mL deionized water) and put in an incubator shaker for 24 hours. The brown tint suggested the production of silver nanoparticles, and after that began to turn dark brown. The change in color shows the creation of AgNPs. The solution was held at room temperature for 24 hours to ensure that the nanoparticles were utterly stable. The particles were separated after this time by centrifugation at 1500 round perminte (rpm) for 15 minutes, followed by washing with deionized water, freezing the powdered, and using it for additional analyses (Choudhary *et al*., 2015).

Fill a dish with the solution and let it dry in the dark before collecting the powder, the experiment was carried out under settings, including silver ion concentration and to find the best conditions for nanoparticle production.

3-8-1: Characterization of Green Synthesis of Silver Nanoparticles.

3-8-1-1: Color Change

The color reaction mixture change was documented through visual observation. The transition from light brown to dark brown pointed to the

synthesis of silver nanoparticles (Korbekandi *et al.*, 2013; Benakashani *et al*., 2016).

3-8-1-2: UV-Visible Spectral Analysis.

Silver nanoparticles were created by reducing silver ions (Ag^{+}). It was identified spectrometrically at different wavelengths using a double beam UV-Vis spectrophotometer (PD-303UV) at different wavelengths (300-800 nm), Deionized water is used as a reference in the X-axis wavelength graph and the Y-axis absorbance graph, the procedure of making silver nanoparticles has been undertaken several times in order to fine-tune various parameters (Ahmad *et al*., 2013; Benakashani *et al*., 2016).

3-8-2-1: X- Ray Diffraction (XRD)

X-ray diffraction is a technique used primarily for the analytic determination of the crystallite size of the solution silver nanoparticles (Robertson, 1979; Li *et al.,* 2016). The sample was examined in Al-Ameen Center for Research and Advanced Biotechnology, Al-Najaf province .

3-8-2-2: Scanning Electron Microscope

Scanning electron microscope (SEM) has also performed characterization, It was a kind of electron microscope this images a sample (AgNP) by scanning it with an electron beam in a raster scan pattern, The interaction of the electrons with the atoms that make the sample produces signals that carry the information about the topography and composition of a sample the surface for the samples (Sadeghi and Gholamhoseinpoor, 2015; Palmqvist, 2017).

3-9: Study the Effect of *H.sabdarriffa* AgNPs on Protoscolices' Viability for *Echinococcus granulosus In vitro*

3-9-1: Preparation of *H. sabdarriffa* AgNPs concentrations:

Sterilized distilled water was used to make concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL from a stock solution of AgNPs (5mg/mL).

3-9-2: Effect of *H.sabdarriffa* AgNPs on Protoscolices' Viability for *Echinococcus granulosus In vitro*:

After collecting the sample in the same way as the previous one, concentrations (0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL) were tested, in addition to the control, on the primate, three replicates, after that the viability of protoscolices determined for duration (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hours, Penetration of the eosin stain (0.1%) is evidence of the death of primates, and the live is green (Al-Hamairy, 2010).

3-10: Haemolysis assays

According to the method of Laloy *et al* (2014), about 15 ml blood of one healthy donor, haemolysis tests were performed:

15 µL of nanoparticles (in Tyrode as a negative control) or 285 liters of whole blood (in Triton X-100 as a positive control) are combined with 285 liters of whole blood, the suspension is incubated at room temperature for one, four, and twenty-four hours on a shaking plate. The suspension is centrifuged for five minutes at 10000g after the incubation time.

In this experiment, four concentrations of *H.sabdarriffa* AgNPs extract were used (512, 256, 128, 64) µg\ml. A microplate scanning spectrophotometer XMark (Biorad, USA) reads the supernatant in a 96-

well plate at 550 nm. The following formula was used to compte the percent haemolysis:

$$\mathbf{H\ (\%) = (OD_{550}nm\ sample - OD_{550}nm\ tyrode) / (OD_{550}nm\ Triton\ X\text{-}100\ 1\% - OD_{550}nm\ tyrode) *100}.$$

The relevant interference was subtracted from each term of the equation. Except for the fact that there are no RBCs in the solution, the interference conforms to the identical conditions. The lysis rates in the negative and positive controls were (100%, 0%) respectively (Laloy *et al.*,2014).

3-11: Antioxidant Activity Evaluation Using the DPPH Assay

To assess the free of radical-scavenging action of the chemical compounds, DPPH radical cation technique was adapted (Pellegrini *et al*., 1999). DPPH was the DPPH reagent (8 mg) diluted in 100 mL MeOH to 80 µg/mL solution concentration. In a 96-well microplate, 100µl of DPPH reagent were combined with 100µl of sample then incubated for 30 minutes at ambient temperature to test scavenging activity. In this experiment, four concentrations of *H.sabdarriffa* AgNPs were used (512, 256, 128, 64) µg\ml.

After the incubation, the absorbance was measured at 514 nm using an ELISA reader (TECAN, Gröding, Austria) with methanol (100%) as control. The following formula was used to compute the DPPH scavenging effect:

$$\textbf{Radical scavenging (\%)} = \left\{\frac{\mathbf{A_{control} - A_{sample}}}{\mathbf{A_{control}}}\right\} \times \mathbf{100}$$

Extrapolation from the IC50 DPPH value (the concentration of sample required to inhibit 50 % of DPPH radical) determined by regression analysis. IC50 value was used to determine the antioxidant effectiveness. (Lee *et al*., 2015).

3-12: Molecular Study

3-12-1: Prepare the Germinal Layer for Extraction

Small pieces of the hydatid cyst germinal layers were extracted, scraped and well crushed to get the proper amount for DNA extraction, and then handled in the same way as DNA extracts from hydatid cyst fluid or protoscolices suspension.

3-12-2: PCR Technique

PCR technique was used to detect and genotype *E.granulosus* hydatid cyst. The method outlined by Gareh *et al*. (2021) based on (ITS1-BD1Forward 4S reverse, ITS2−3S- forward A28–reverse) genes in isolates, was used to carry out this method.

3-12-3: The Genomic DNA Extraction:

The DNA Extraction Kit was used to extract genomic DNA from samples of hydatid cyst fluid and germinal layers Kit Geneaid\Taiwan, following the manufacturer's instructions:

1. 200µl of frozen hydatid cyst fluid was transferred for a 1.5 mL sterile microcentrifuge tube and centrifuged for 20 seconds at 60000 rpm.
2. The supernatants were discarded and added 150µl of RBC Lysis Buffer and mixed by vortex for five seconds.
3. Each tube was then filled with 200 l of FABG buffer, which was properly mixed by vortexing, after which all tubes were incubated in a 70°C water bath for (10) minutes, or until the sample was clear, and inverted every three minutes during the incubation period.
4. To the lysate, 200 l absolute ethanol was added and vortexed forcefully. The entire mixture was placed in a 2ml collecting tube with the DNA filter column (including any precipitate) was

transferred for it. After that, it was centrifuged at (14000-18000) rpm for one minute. In a fresh 2 ml collection tube, the column was put, and the flow-through 2 ml collection tube was discarded.

5. The DNA filter column was centrifuged for 10 minutes after adding 400 W1 buffer for 30 seconds at (14000-18000) rpm. The column was placed back in the 2 ml collecting tube after the flow-through was discarded.
6. Each column received 600µl of Wash Buffer (ethanol). Then it was centrifuged for 30 seconds at (14000-18000) rpm. The column was placed back in the 2 ml collecting tube after flow-through discarded.
7. All of the tubes were centrifuged for three minutes at (14000-18000) rpm to dry the column matrix.
8. A clean 1.5ml microcentrifuge tube was used to transfer the dried DNA filter column, along with 100l of warmed elution buffer or TE was added to center of the column matrix's.
9. To ensure that the elution buffer was absorbed by a matrix, the tubes were let to stand for at least 10 minutes. The purified DNA was then centrifuged for one minute at (14000-18000) rpm to elute it.
10. Transfer the dried and DNA-containing DNA filters to sterile and new sterilized tubes, adding 100µl Preheated Elution (total volume could be 200µl), and storing the DNA fragment at (-4) or (-20) C^{o}.

3-12-4: The preparation of PCR Master Mix

The PCR master mix was made with the (Maxime PCR PreMix Kit) and followed the company's instructions as shown in the table (3-11) :

Table (3-11): The Polymerase Chain Reaction Mixture by Using (PCR Maxime PreMix Kit)

PCR mixture	Volume (µl)
Master mix	12.5
Foreword primer (10 p/mol)	2
Reverse primer (10 p/mol)	2
DNA (50 ng/µl of DNA template)	5
Deionized distilled water.	3.5
Total volume	25

Following that, the PCR master mix components listed in Table (3-9) are placed in a normal PCR PreMix Kit, which includes all additional PCR reaction components such as primers and probes (dNTPs, Taq DNA polymerase, KCl, MgCl2, Tris-HCl pH: 9.0, tracking dye and stabilizer). The PCR tubes were then placed in an Exispin vortex centrifuge to three minutes at 3000rpm. The samples were then deposited in a PCR thermocycler (BioRad. USA).

3-12-5: The Conditions PCR Thermocycler

The following table shows the PCR thermocycler conditions using a typical PCR thermocycler system:

Table 3-12: Conditions of PCR Technique

Phase	Tm (C°)	Time	No. of cycles
Initial Denaturation	95 C°	5 min.	1 cycle
Denaturation	95 C°	1min.	40 cycle
Annealing	60 C°	30 second	
Extension	72 C°	3min	
Final extension	72 C°	10min	1 cycle

3-12-6: PCR Product Analysis

The following steps were used to examine the PCR results (ITS1, ITS2) of mitochondrial genes on an agarose gel electrophoresis (Figure 3-6):

1. A gel of agarose containing 1.5% was created by dissolving 1X TBE in the water bath at 100 C° to 15 minutes and then chilling to 50 c°.
2. The dye ethidium bromide was then added to the solution of agarose gel in a volume of 4µl.
3. After the comb was properly positioned, the agarose gel solution was placed in a tray then after allowing 15 minutes for the comb to solidify at room temperature, it was carefully removed from the tray and 8 µl. of PCR product and 5µl of (100bp-1500 bp Ladder) were added to each combs well.
4. 4. In the electrophoresis chamber, the gel tray was put and 1X TBE buffer was added to it. Then, for 1 hour, an electric current of 100 volts and 75 AM was applied.
5. The UV transilluminator was used to visualize the PCR products.

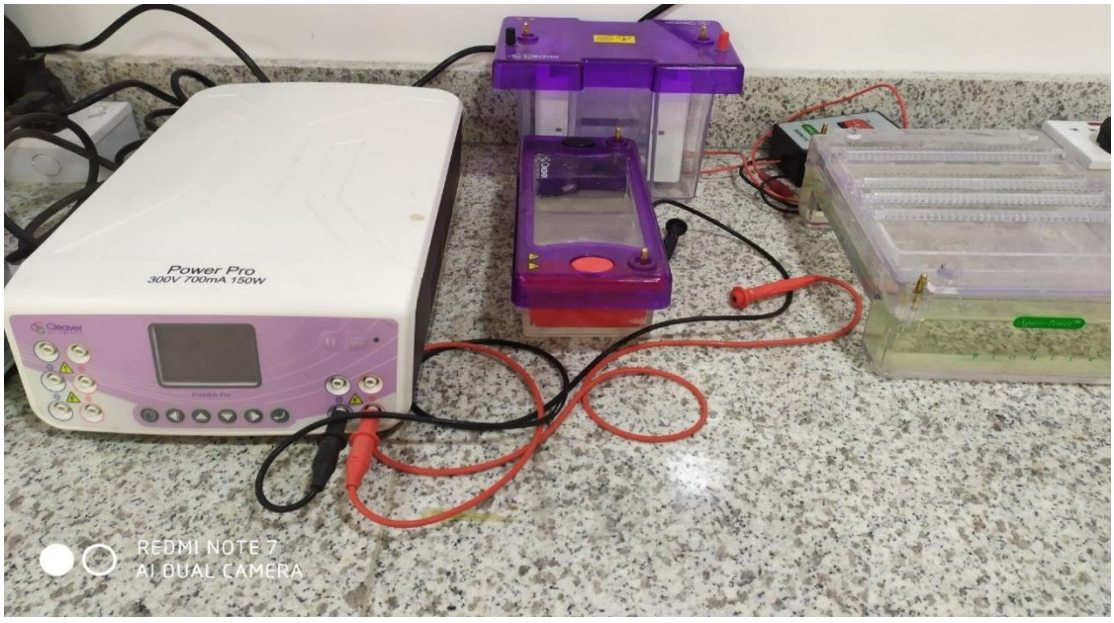

Figure 3-6: Agarose Gel Electrophoresis Apparatus

3-12-7: DNA Sequencing Method:

The products of DNA replication were sent by PCR technique to the Korean company Macrogene to sequence the nitrogenous bases of the studied sample and the results were compared with the BLAST program and BioEdit program with the genes registered in the NCBI Gene Bank, After obtaining the sequence of the genetic bases of the DNA of the products of molecular polymerization if they were analyzed and compared with the data obtained from the NCBI Gene Bank, the studied type was registered within the gene bank,The sequence of local, *E. granulosus* no. 2 strain was pair alignment with references strains deposited in gene bank , the tree was draw based on NCBI data references online.

3-13: The Statistical Analysis

The study was done as factorial experiments with a perfectly randomized design, and significant differences were examined using the least significant difference (L.S.D.) at level (0.05) for demonstrating significant results (Al-Rawii and Khalfalla, 2000).

Chapter Four

Results

&

Discussion

4: Results and Discussion

4-1: Estimating the viability of protoscolices

Table (4-1) shows the average number of protoscolices of the *E.granulosus* parasite in 6µl for five replications which were calculated by using the fixed volume method for different time periods. As the average number of protoscolices per hour was zero time (468) protoscolices, while per 216 hours was (zero) protoscolices. The results of the statistical analysis indicated significant in the differences obtained results.

Table 4-1: Average Number of Protoscolices for the *E. granulosus* at 30µl at the Different Time Periods (hour).

Time/ hour	The average number of protoscolices/30 µl
0	468
24	436
48	389
72	358
96	330
120	323
144	314
168	299
192	253
216	0
The value of L.S.D. at level 0.05 about the effect of time period on the viability of protoscolices =6.54	

4-2: Calculating the Percentage of the Viability of the Protoscolices:

Table (4-2) shows that the average number of total protoscolices (viable and dead) was (501) protoscolices, while the average number of a live protoscolices were (468) protoscolices, while the rate of dead protoscolices was (33) protoscolices, so the percentage of protoscolices viability was (89.06) %.

Table 4-2: Percentage of the Viability of Protoscolices for the *E.granulosus* in 50 µl.

Viability of the protoscolices percentage	Average of the total number
Average total number of protoscolices	501
Average total number of a live protoscolices	468
Average total number of dead protoscolices	33
Percentage	93.41 %

There are several differences in the viability of the samples, as it reached 67% on the seventh day and zero on the ninth-day according to the current study, although Al-Tai (2014) found that the viability of protoscolices which were (61-64%) after 12 days, Al-Hasnawi (2020) stated that the viability of the samples protoscolices was 60.95% after seven days of conservation and zero after nine days.

In the conservative culture, the average number of suspended protoscolices was computed (Kreb Ringer's solution + fluid HCF) in a ratio of (4:1), which was used. The findings revealed that this conservative culture is the most effective at preserving protoscolices for an extended period of time (Al-Hamiay, 2010) but differed from him in the percentage of (60.1%) after seven days.

This discrepancy in the results can be related to the environmental conditions in which the samples were brought, such as high or low temperature which has an impact on the protoscolices' survival. The reason for the protoscolices' decreased viability is that they have become accustomed to the temperature, pH, and ratio of organic and inorganic

chemicals, as well as other nutrients such as sugars, salts, and so on (Hasnawi *et al*., 2021).

The nutrients gradually decrease when these viable protoscolices are taken and placed in the culture medium HC: KRP at a ratio of (4:1), resulting in a lack of viability and activity until death, as shown in tables (4-1) and (4-2).

4-3: Different Natural Stains Used to Stain and Detect Protoscolices: New stains, including natural stains,were used for the first time in this work to detect protoscolices of *E. granulosus*, and they were compared to the classic eosin stain (0.01%), which is approved for staining protoscolices. The results showed that the protoscolices of the dead specimens viewed under the microscope were clear, with a red color because the eosin stain penetrated through the wall, while the live specimens were green in color as shown in Figure (4-1).

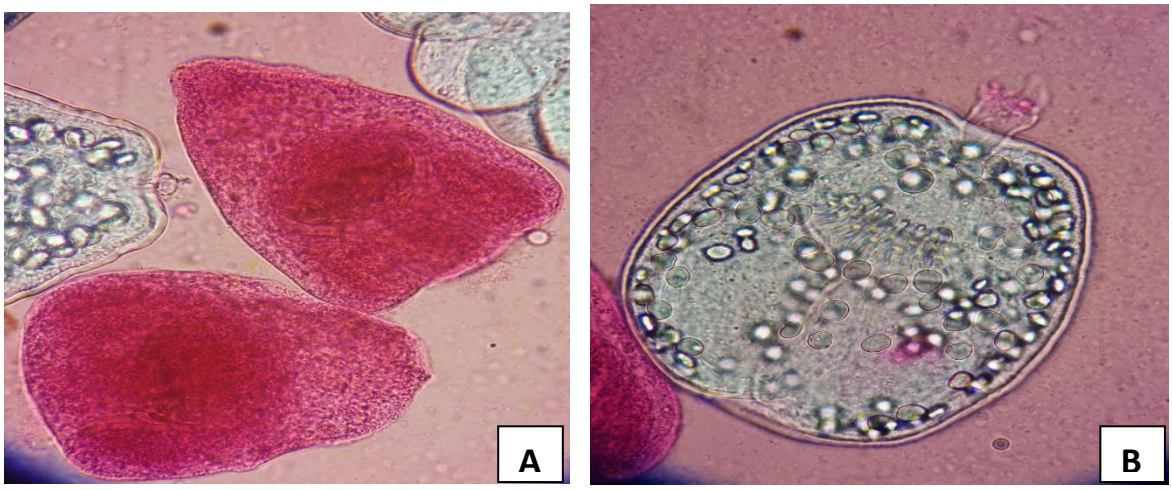

Figure 4-1: Protoscolices Stained with Eosin, (A) Dead Protoscolic with Red Color and (B) Living Protoscolices with Green Color. (40x)

4-3-1: Natural stains

Table 4-3: Protoscolices (viable and dead) Color that Revealed with Numerous Different Natural Stain Extract.

No.	Extract kind	viable protoscolices color	dead protoscolices color
1	*Crocus sativus*	Transparent white	Yellow
2	*Beta vulgaris*	greenish lead	Black
3	*Hibiscus sabdarriffa*	Brownish	dark nutty
4	*Brassica oleracca*	yellowish white	light brown

4-3-1-1: *Crocus sativus* Stain

The dead protoscolices looked yellow in an aqueous extract of *Crocus sativus*, while the live protoscolices appeared transparent white in an aqueous extract of *C. sativus*, as shown in figure (4-2) and table (4-3).

4-3-1-2: *Beta vulgaris* Stain

The aqueous extract stain appeared on the live protoscolices with greenish lead color while the dead protoscolices black appeared as in figure (4-3) and table (4-3).

4-3-1-3- *Hibiscus sabdarriffa* Stain

The aqueous extract stain appeared as dead protoscolices with dark nutty color while the live protoscolices appeared brownish as in figure (4-4) and table (4-3).

4-3-1-4-*Brassica oleracca* Stain:

The aqueous extract stain appeared the dead protoscolices with light brown color while the live protoscolices appeared yellowish white as shown in figure (4-5) and table (4-3).

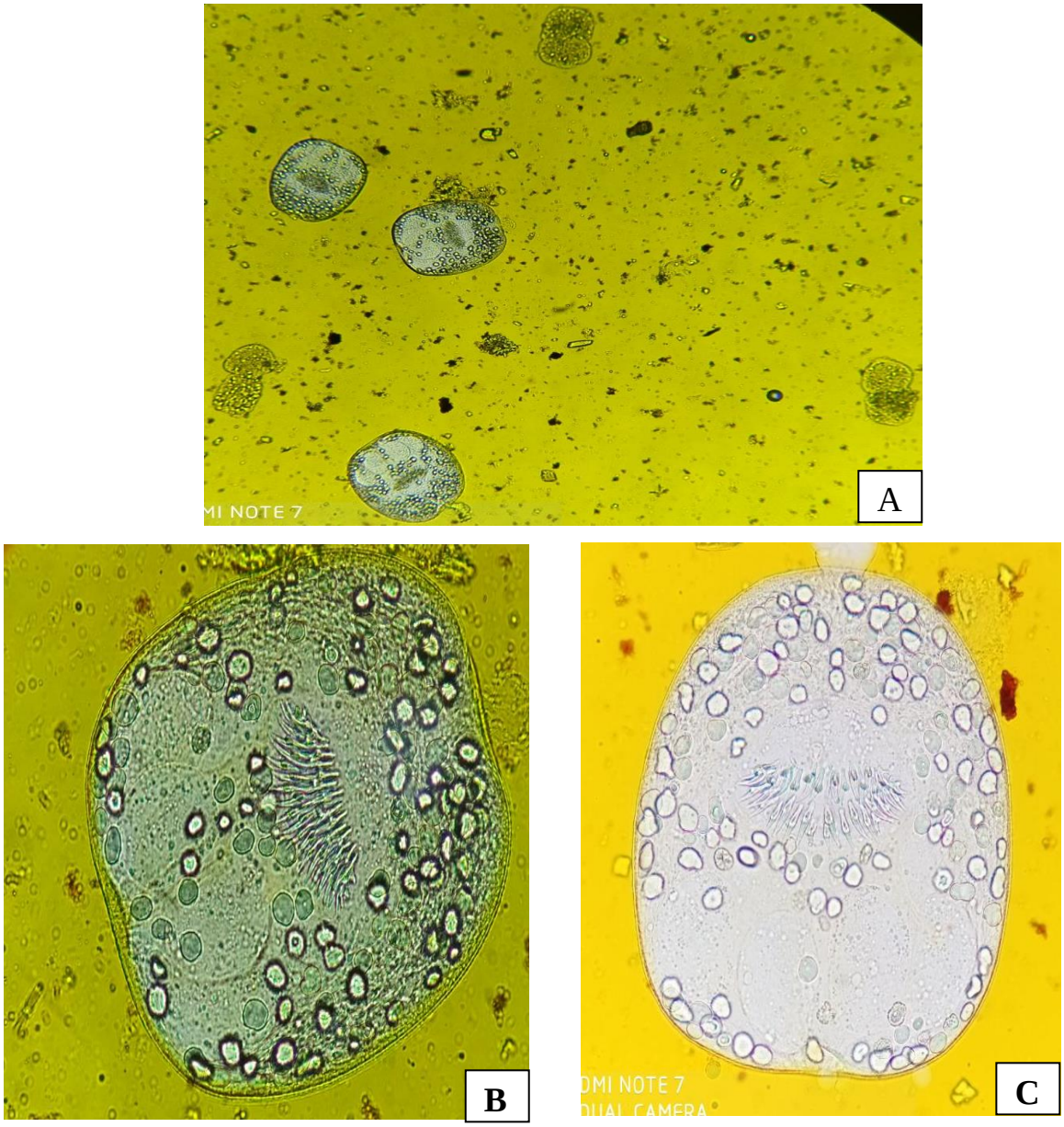

Figure 4-2: (A) Protoscolices Stained with *Crocus sativus* Appeared Live as Clear White and Dead as Yellow (10 X). (B) Live Protoscolices Stained in Zero Hour (40 X). (C) Live Protoscolices Stained in After an Hour (40X).

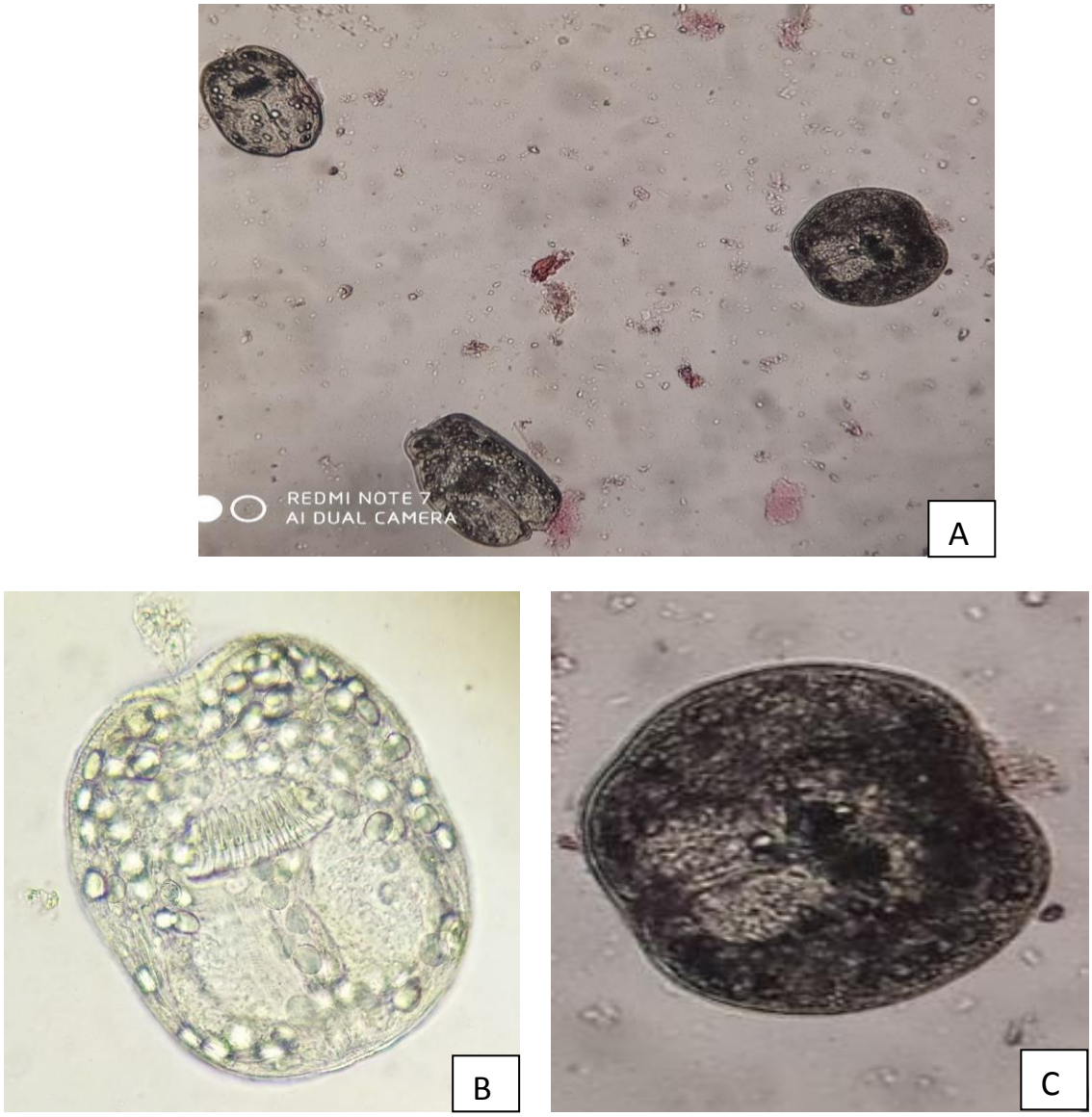

Figure 4-3: (A) Protoscolices Stained with Beta *vulgaris* Appeared live as Greenish Lead and Dead as Black (10X). (B) Live Protoscolices Stained Greenish Lead (40X). (C) Dead Protoscolices Stained Black (40 X).

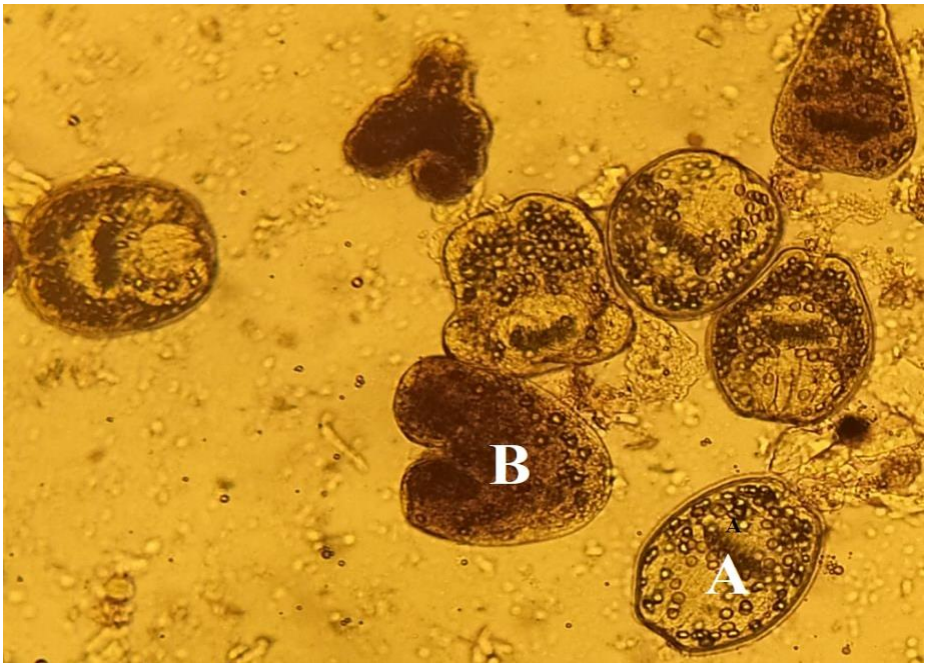

Figure 4-4: Microscopic Examination of Protoscolices Stained with (A) the Live Protoscolices Appeared Brownish Revealed that and (B) the Dead Protoscolices Appeared Dark Nutty (40X).

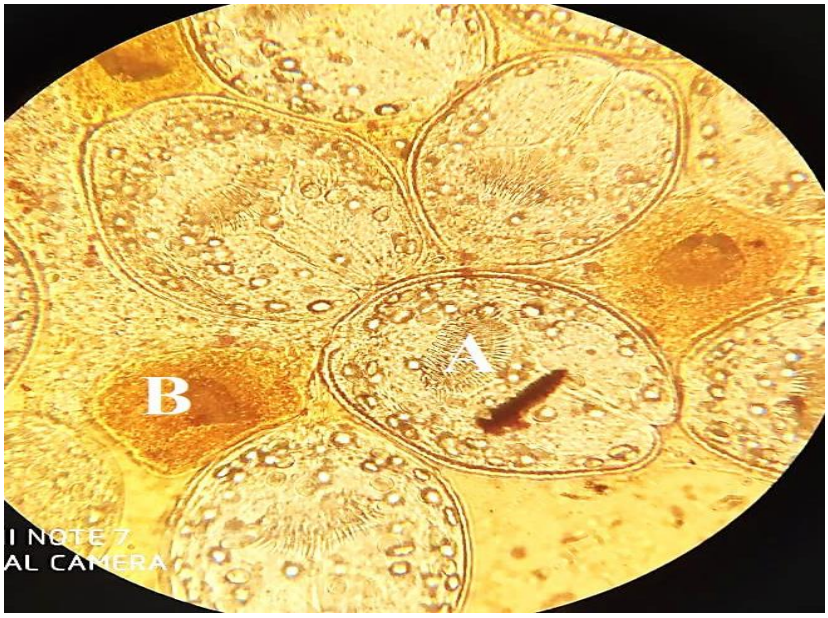

Figure 4-5: Microscopic Examination of Protoscolices Stained with (A) the Live Protoscolicse Appeared Yellowish White and (B) the Dead Protoscolicse Appeared Light Brown (40X).

The dead protoscolices of *Crocus sativus* appeared as yellow, and the live protoscolices as a transparent white when stained with industrial stain when compared to eosin-stained as a reference sample.

The stained protoscolices developed a variable of pigmentation with the distinction between dead and live protoscolices, as an eosin stain 0.01% (standard stain), was colored the red stain of the dead protoscolices and the viable shows green, whilst the yellow stain appeared the dead yellow and the living white. According to the observation, the color stained on the protoscolices suggested that these extractions were able to enter the cell membrane of the dead protoscolices (Saeed, 2021).

Also, saffron components are responsible for the bitter flavor and scent. Crocin pigments are responsible for the golden yellow-orange hue of saffron as well as anthocyanins, flavonoids, vitamins (particularly riboflavin and thiamine), amino acids, proteins, carbohydrates, mineral matter, gums, and other chemical components. Crocetin, one of the ingredients of the saffron extract is primarily responsible for its pharmacological effects (Marhaba and Haniloo, 2018).

Aqueous extracts of *H.sabdariffa* appeared utilized as natural dyes, and also in health aspects as natural red colorants (Abdallah, 2015). Anthocyanins which are flavonoids and are water-soluble natural pigments showed a dark crimson color and they have acquired parasites' structural features and organs a rich color. (Marhaba and Haniloo, 2018).

Both extracts were effective stains that were successfully used for staining and diagnosing hydatid cyst protoscolices of *Echinococcus granulosus*, as opposed to conventional stain eosin, this study is compatible with Al-Azizz's study (2010) on the aqueous extract for natural stain extracted from Kujarat flowers were used for detection of the viability of protoscolices in hydatid cysts, The live protoscolices took

on a brownish show in the Kujarat stain, while the dead protoscolices took on a pink color.

The study and identification of microorganisms with the staining process is a crucial necessity. Enzymes, temperature, oxygen, and pH all play a role in pigment stability (Sarkar *et al*., 2015). It has been used to stain helminths, intestinal nematode ova, buccal cavity smears of *Ancylostoma* and tissues as well as employed as a fluorescent dye in tissue staining (Udonkang *et al*., 2018).

Beetroot is a vegetable, but its juice and extracts are also used in traditional medicine, as a food colorant, and as a cosmetic ingredient, this plant is abundant in antioxidants and anti-inflammatory compounds, and it may be useful in the treatment of a variety of disorders (Ceclu and Nistor, 2020). Some researchers claim that phenolic compounds, dietary fibers, fructo-oligosaccharides, non-starch carbohydrates, and other chemicals found in fruits/vegetables and beetroot extracts can be digested by probiotic bacteria and used as a prebiotic source (Soccol *et al*. 2010; Malik *et al*., 2019). Antioxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant, and carminative are only a few of the medical properties of this plant (Jasmitha *et al*., 2018). Aqueous preparations of beetroot color stained most basic tissue structures because they included slightly acidic betalain pigments (Udonkang *et al*., 2018).

Another study used the aqueous extracts of red beet, China rose, henna and acid carmine stains were used to stain the adult worms of *Fasciola gigantca*. The stained flukes showed varying degrees of pigmentation with well-defined external and internal structure, oral and ventral sucker, muscular pharynx, oesophagus, intestinal ceca and cirrus sac took a rose to light cherry color in aqueous extract of china rose, orange to the brown in aqueous extract of sugar beet, beige to pale brown in aqueous extract

of henna while they took deep red color in carmine stain with the best quality achieved by china rose aqueous extract (Aly, 2020).

The most well-known synthetic dyes used to stain *Leishmania* parasites for diagnostic purposes are Giemsa stain and Leishman stain, Because of the harm caused by the use of synthetic dyes, such as vast amounts of pollution and harmful toxic side effects, much research was undertaken with the goal of acquiring alternative colors for parasite staining from natural sources, It is inexpensive and simple to obtain, The alcoholic extracts of Damask rose (*Rosa damascena*) and China rose (*Hibiscus rosa sinensis*), as well as the aqueous extract of Kujarat blossoms (*H.sabdariffa*) were made and employed in staining the *Leishmania donovani* promastigotes, and the results were compared to Giemsa stain. The three natural floral extracts were effective in staining *Leishmania donovani* promastigote (Kamal., 2018).

The aqueous extract *Brassica oleracca* appeared the dead protoscolices with light brown color while the live protoscolices appeared colorless.

Anthocyanins are almost ubiquitous in the plant kingdom and are responsible for most of the red, blue and purple colors of flowers and fruits. These water-soluble pigments are also widely used in the food industry to color soft drinks, jams and dairy products, *in vitro*, flavonoids contained in red cabbage, notably acylated anthocyanins, can act as scavengers of reactive oxygen species and electrophiles, as well as chelators of metal ions (Radziejewska-Kubzdela and Bieganska-Marecik, 2015).

4-4: Effect of Different Concentration of Albendazole Treatments on Percentage Viability of Protoscolices for *E.granulosus In vitro.*

The effect of the albendazole concentration factor on protoscolices viability is depicted in figure (4-6). Compared to 89.06% in the control treatment, the percentage of viability was 21.33% at the highest concentration (300 mg/ml). The viability of protoscolices was shown to decrease as medication concentrations were increased, indicating a reversal connection. On the fifth day, it reached zero with a concentration of 300 mg/ml and It reached zero on the seventh day with a concentration of 200 mg/ml, the statistical analysis revealed considerable disparities in the obtained results.

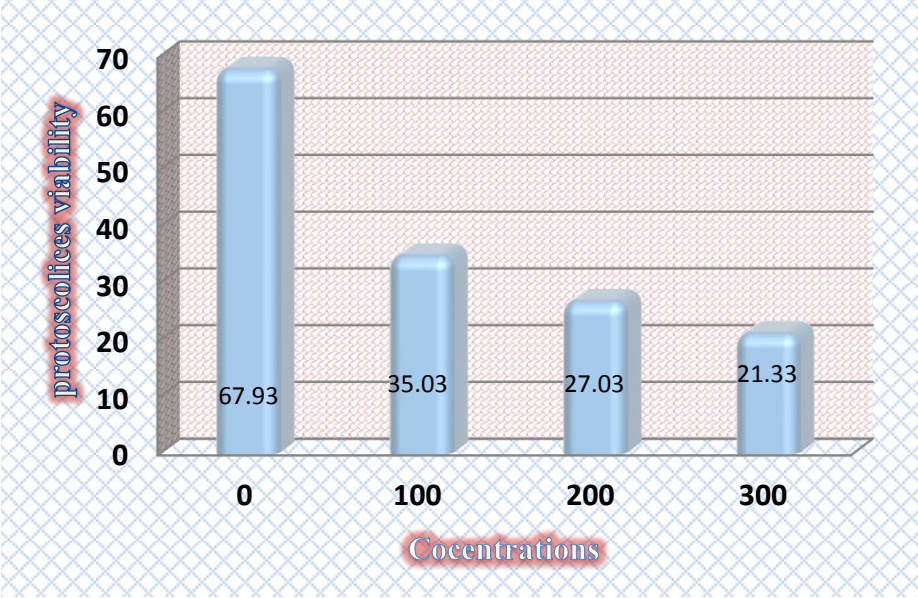

Figure 4-6: Effect of Albendazole Concentration Factor on the Number of Protoscolices *In vitro*. (L.S.D value least significant differences at level 0.05 =2.042).

Table (4-4) shows the effect of the time period on the protoscolices viability for (100,200,300) mg/ml concentration (96, 72, 48) hr. to (33.93, 25.14, 58.62) % getting to zero at (192, 168, 120) hr. sequentially.

Table 4-4: Effect of the Concentrations for Albendazole on the Viability of the Protoscolices for *E.granulosus In vitro* .

Time (hour)	0	24	48	72	96	120	144	168	192	216
concentration mg/ml	No. of protoscolices									
Control (0)	93.41	78.31	62.75	52.25	42.85	40.44	37.41	32.7	17.51	0
100	88.89	80.42	74.65	62.34	33.93	12.44	10.03	0.08	0	0
200	89.99	81.03	66.53	25.14	22.93	17.55	0.11	0	0	0
300	90.45	74.74	58.62	2.6	0.16	0	0	0	0	0
L.S.D value for interference at probability level 0.05 =5.07										

The effect of the time period factor on the percentage of viability of the protoscolices for *E.granulosus* parasite is shown in Figure (4-7). The viability ratio was 4.378 % in the time period 192 hours after treatment, compared to zero 89.06 % in the control group after hours of treatment.

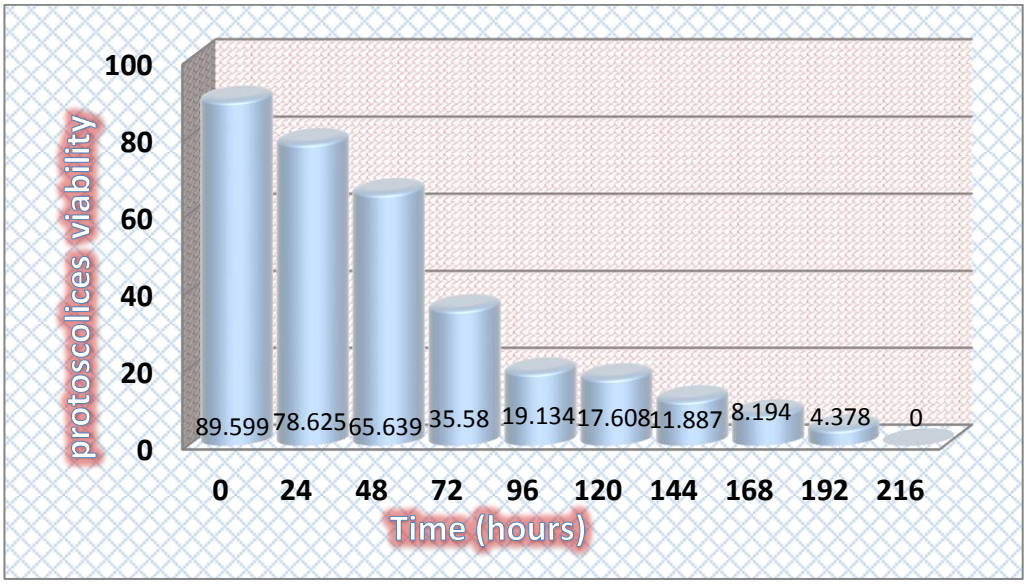

Figure (4-7): Effect of Time Duration on the Number of Protoscolices Treated with Albendazole *in vitro* (L.S.D value least significant differences at level 0.05 =7.012).

From Figures (4-6) and (4-7) a significant difference appeared between the concentration ,where the 300 mg/ml albendazole treatment concentration was most effective for protoscolices viability, decreasing from 90% to zero at 120 hr. From the beginning of the study, the viability declined to zero when accompanied by a 200 mg/ml concentration at 168 hr., although the 100 mg/ml viability after 192 hr.

The increasing in time period had a substantial impact on the protoscolices' viability, which is consistent with the findings of Al-Tai (2014) who discovered that as the time of the experiment increases, the influence on protoscolices' survivability reduces. This might be owing to the fact that as the time period lengthens, the active compounds in the protoscolices membranes penetrate deeper, shattering them and weakening the larval stage of the parasite and finally its death.

The reason for the decrease of the viability of protoscolices for the presence of compounds active, Phenolic dissolved in water.The active Phenolic compounds have a role in weakening the protoscolices membranes of the parasite (Abdullha, 2013).

In the previous studies and the results of the current study, it is noted that the effect on the viability of protoscolices decreases with increasing treatment concentration since increased treatment concentration contributes to an increase in the penetration of the active materials into the membranes of protoscolices and then the fracturing of the membrane and the shattering of these protoscolices and then the weakness finally death (Al-Hamiary, 2010).

In addition, all patients should undergo benzimidazole chemotherapy, primarily Albendazole (ABZ) and Mebendazole (MBZ) because benzimidazoles are parasitostatic, they must be used for a long time (Kern *et al*., 2017). By altering the glucose absorption mechanism at the membrane of the hydatid cyst, benzimidazole chemicals reduce glycogen

levels, cellular autolysis is caused by degenerative alterations in the endoplasmic reticulum and mitochondria of germinal cells, as well as an increase in the number and activity of lysosomes, Albendazole is commonly used as a first-line treatment for hydatid disease of the liver, with promising outcomes (Khuroo *et al*., 1993; Balik *et al*., 1999).

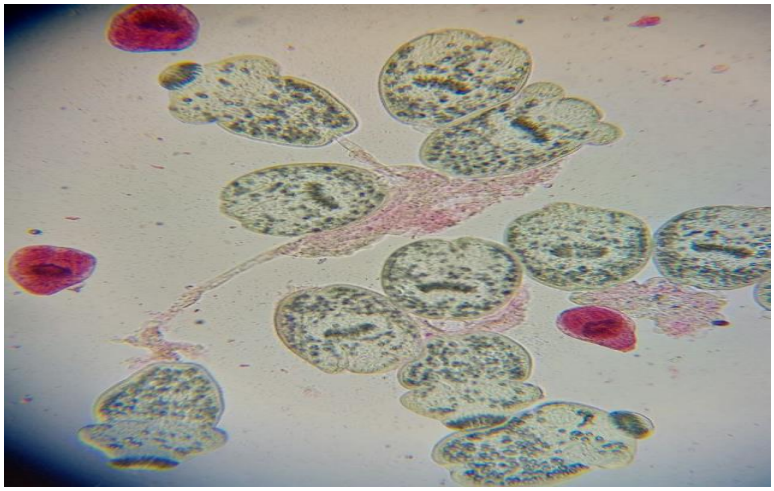

Figure 4-8: Effect of Albendazole Treatment Concentration 300 mg/ml on the Viability of Protoscolices *In vitro*.

4-5: The Effect of the Plant Type Extract on the Viability of the Protoscolices of the *E.granulosus*

Figure (4-9) shows the effect of the plant type extract on the viability of the protoscolices of the *E.granulosus*, The extract of *Hibiscus sabdarriffa* was more effective in the decay of the protoscolices compared to the extract of the *Brassica oleraacca*, *Beta vulgaris* and *Crocus sativus* extracts. As the percentage of viability (20.9%) in *Hibiscus sabdarriffa* extract compared with (34.3 and 38%) for *Brassica oleraacca* and *Beta vulgaris* extracts, while *Crocus sativus* extracts were less effective (43.2%),and differences in the obtained findings statistical analysis were statistically significant.

Boiled water extract was more effective, possibly because it inhibited enzymes, allowing the active component to be more efficient resulting in

a drop in the viability of *E.granulosus* protoscolices, Phenolic was one of the active molecules dissolved in water from these active chemicals (Harborne, 1984). The current study agrees with Hussain *et al* study (2014) in the presence of significant differences between the four plant extracts in the effect on the viability of the protoscolices of *E.granulosus*.

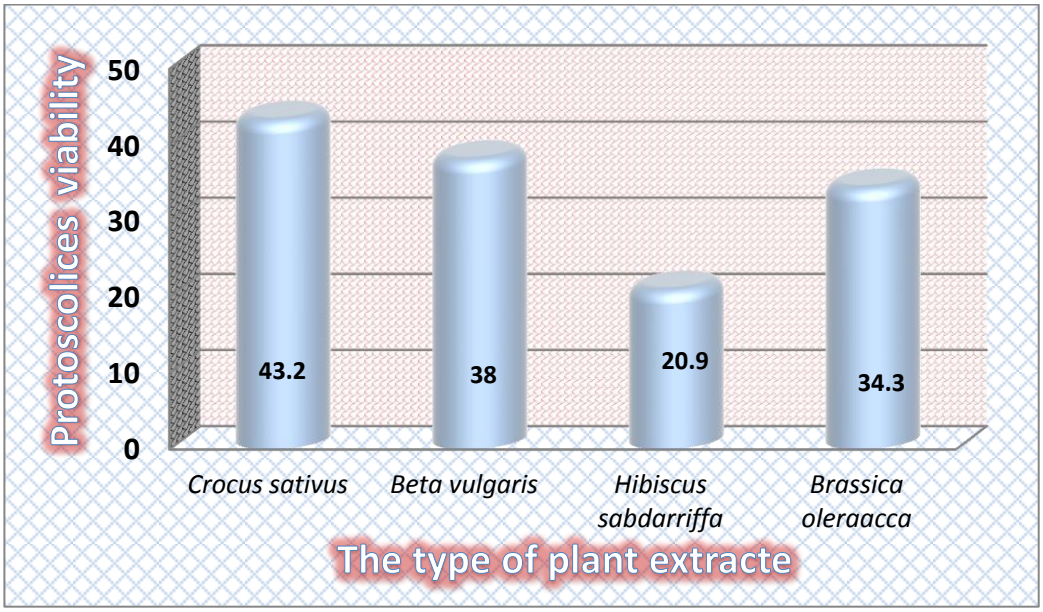

Figure 4-9: Effect of Type of Plant Extracts on the Number of Cysts of Protoscolices of Hydatid Cyst (L.S.D value least significant differences at level 0.05 = 9.531).

Medicinal plants with reliable therapeutic properties were important as natural herbal and medicinal systems of modern discovery, Plants may serve as a director medicinal source of agents, and these bioactive materials act as raw products, producing more complex semisynthetic chemical compounds, isolated medicinal plant compounds can contribute to new medicines being discovered (Bhalshing and Maheshwar, 1998; Ovais *et al*., 2018).

Saffron stigmas are the portions of the plant that have been employed in medicine. They have a lovely bitterness to them that is slightly

warming. They have a lot of extractive matter and a little bit of volatile oil in them. The powdered stigmas were one of the medications used by Charaka to impair them (Kalesi *et al.*, 2004).

The current study findings revealed that high concentrations of plant extracts had a substantial impact on protoscolices reduction when compared to smaller amounts (Figure 4-10). The results of the study show that there are consistent with the findings of the Al-Omari (2012) study which discovered that higher concentration had a significant impact on the decreased number of protoscolices, which discovered that higher concentration has a considerable impact on protoscolices loss, and which concurs with Al-Mhaisen (2020) According to results, increasing the concentration has a demonstrable effect on protoscolices viability.

The presence of active materials in plant extracts, such as alkaloids and phenolic compounds, has an effect on the tegument layer of the cell membrane, causing cell death and allowing the eosin dye to enter, resulting in protoscolices death. As these compounds transfer into the cytoplasm of protoscolices, interfere with biological processes, have deleterious effect on the protoscolices, and eventually kill them, they may impact tubulin or the phosphorescent proteins present in the cell wall and work to dissolve it (Delorenzi *et al.*, 2001).

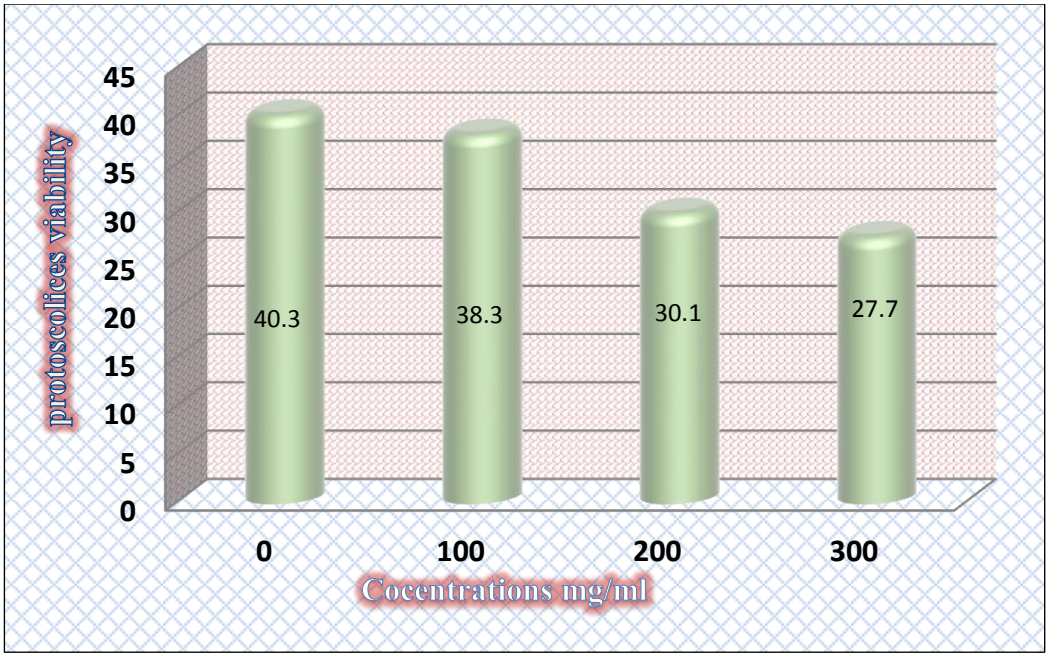

Figure 4-10: Effect of the Plant Extracts Concentration Factor on the Number of *E.granulosus* Protoscolices. (L.S.D value least significance differences at level 0.05 = 2.401).

Figure (4-11) shows the influence of time duration on the viability of *E.granulosus* protoscolices, revealing an inverse association between decreased viability and increasing time period, After 192 hours of treatment, the viability ratio were 0.8%, compared to 84.9%, after zero hours of therapy, According to the statistical analysis,there is significance differences was indicated.

The present study's results were consistent with those of a previous study of Al-Musawi (2012), who discovered that as time passes, the influence on the viability of protoscolices grows. The reason for this is because as the renewal, active compounds such as alkaloids, phenols, terpenoids, and others enter the membranes of Protoscolices, breaking them down and weakening the parasite and then death.

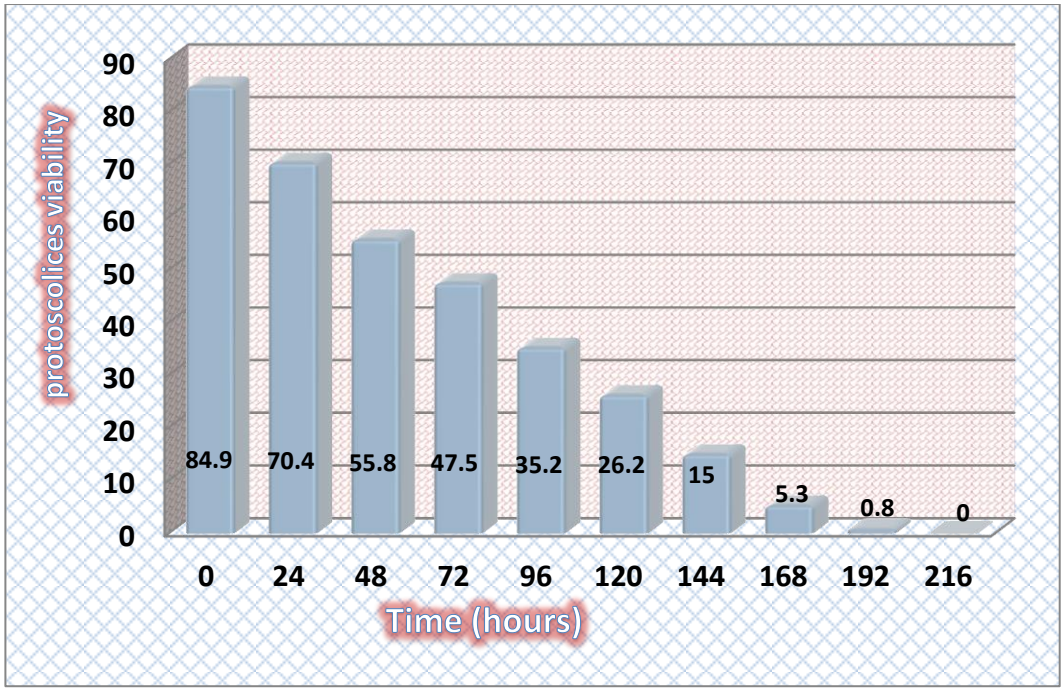

Figure 4-11: Effect of Time Duration on the Total Number of Protoscolices of *E.granulosus* Treated with Plant Extracts. (L.S.D value least significance differences at level 0.05 = 7.042).

The results in table (4-5) show the effect of different concentrations of boiling water extract plants interacting (*C.sativus*, *B.vulgaris*, *H.sabdarriffa* and *B.oleraacca*) on the demise of the *E.granulosus* protoscolices at different time periods were tested *In vitro*. Protoscolices died, as the viability percentage in concentration 300mg/ml after 24 hours of treatment was 0%, compared to 84.9% in the control treatment. Significant differences were discovered as a result.

Table (4-5) reveals that the boiled water extract for *H.sabdarriffa* is more effective than the other plant extracts employed in the present study. The inhibitory mechanism mediated by alkaloids has been explained as interfering with a chain of protein metabolism events required for the microscopic organism's vitality, due to its ability to break down the protoscolices cell wall, proteins, and lipids, leading to the parasite's death, this could be due to the presence of glucoraphanin which

has the ability to increase mitochondrial antioxidant fatty acids, as well as lipid and steroid synthesis (Zhou *et al*., 2014).

Through the present results, it was found that the *H.sabdarriffa* was more effective against the viability of protoscolices and therefore it was chosen for the manufacture of *H.sabdarriffa* nanoparticle.

Table 4-5: Effect of interfering plant extracts concentrations on the number of *E.granulosus* cysts.

Extracts	*Crocus sativus*				*Beta vulgaris*				*Hibiscus sabdarriffa*				*Brassica oleraacca*			
Concentrations Mg / ml / Time (hour)	Control	100	200	300	Control	100	200	300	Control	100	200	300	Control	100	200	300
0	80.9	81.45	79.11	83.65	80.9	80.15	81.69	78.98	92.26	93.13	93.96	94.7	83.72	83.87	84.75	85.01
24	71.81	75.79	71.49	80.79	71.81	75.91	64.6	74.9	75	55.22	45.51	40.87	80.44	82.31	80.43	79.23
48	67.22	70.11	69.55	74.85	65.36	77.09	65.36	77.89	60.66	0	0	0	70.64	76.68	74.87	42.37
72	64.62	65.67	65.41	71.41	51.72	75.64	72.17	67.53	51.36	0	0	0	65.38	66	20.76	21.95
96	47.62	91.55	61.52	69	42.56	66.86	56.91	24.41	47.52	0	0	0	51.11	51.73	4.6	0.667
120	34.39	54.91	58.47	36.61	25.3	60.81	28.02	0	41.35	0	0	0	32.39	46.67	0	0
144	29.29	28.91	25.13	24.62	16.52	33.11	0	0	32.06	0	0	0	23.66	26.73	0	0
168	24.42	27.5	0	0	3	0	0	0	13.64	0	0	0	12.35	11.95	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0	8	4.137	0	0
216	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4-6: Formation and Characterization of Silver Nanoparticles from *H.sabdarriffa* Extract:

4-6-1: The Color Change:

In principle, the silver nanoparticle formation was well known by changing the colour while adding silver nitrate solution to *Hibiscus sabdarriffa* extract as show in Figure (4-12), The color started to change after 24 hours, the light brown was changed into dark brown, This change in color indicates the formation of AgNPs, and the result is corresponding with (Benakashani *et al*., 2016). In which the synthesis of silver particles was observed during change the color of the mixture from light brown to dark brown.

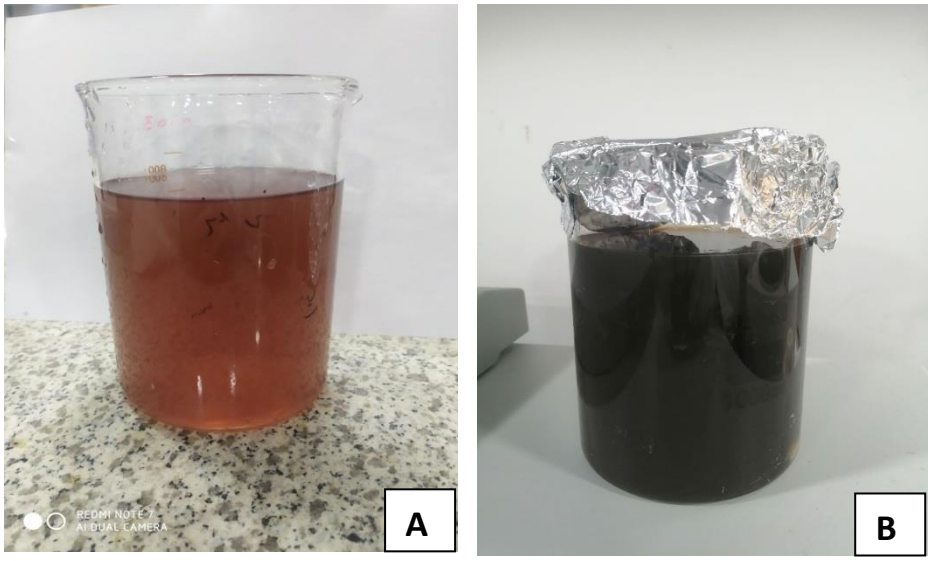

Figure 4-12: (A) *Hibiscus sabdarriffa* Extract with Color Light Brown, (B) Dark Brown Color Indicate the Formation of Silver Nanoparticles.

4-6-2: UV-Visible Spectral Analysis

The UV-Visible spectra and the characterization absorption spectra were important properties of AgNPs, and they proved to be a successful tool for the characterization of the synthesis of AgNPs. The difference in spectra may be attributed to the number of particles and the size distribution in the solution (Zhou and Wang, 2012).

In the present study, the *H.sabdarriffa* AgNPs were confirmed by obtaining a spectrum in the visible range of 300nm-800nm using UV- a visible spectrophotometer as shown in Figure (4-13). From this analysis, the absorbance peak was found at around 420nm, which was specific for Ag nanoparticles, the results corroborate those of previous studies such as Ashokkumar *et al.* (2015) who showed that the absorption peak is about 400nm, which is specific to AgNPs.

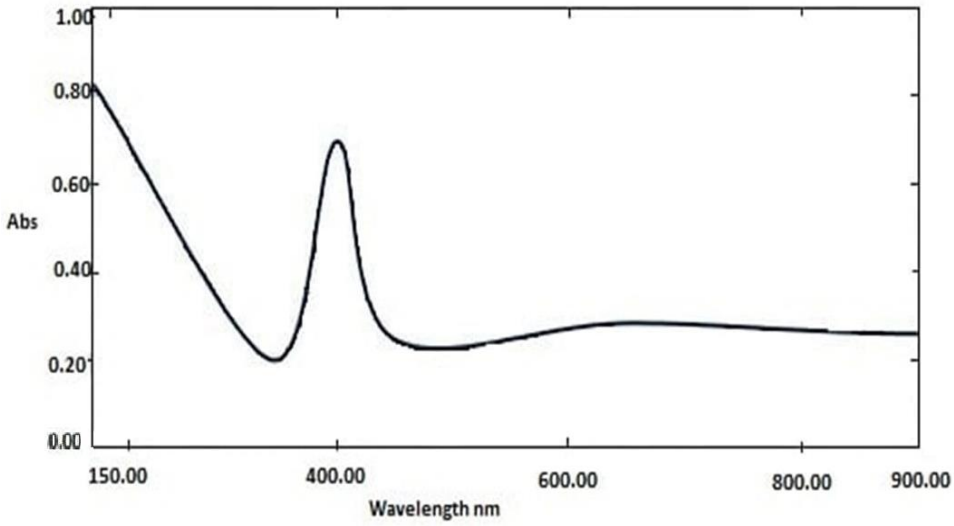

Figure 4-13: UV-Visible Spectra of *Hibiscus sabdarriffa* AgNP Synthesis.

4-7: Physical Characterization of Biogenic AgNPs

Physical characteristics of AgNPs are represented by Scanning Electron Microscopy analysis (SEM), Energy Dispersive X-Ray Spectroscopy **(**EDX), Atomic Force Microscopy (AFM), and X-ray diffraction (XRD) analysis.

4-7-1: Scanning Electron Microscopy (SEM) analysis

Scanning Electron Microscopy analysis results revealed that exhibited characteristics manufactured by *H.sabdarriffa* AgNPs, the most predominant shape found to be spherical. Their spherical sizes range from

(38.71to 86.69) nm as shown in figure (4-14).Scanning electron microscope was used to determine the shape and size of biogenic AgNPs, results displayed well-dispersed, spherical AgNPs (Palmqvist, 2017).

It is found that the physical characteristics are highly affected and determined by the composition of the reaction environment.

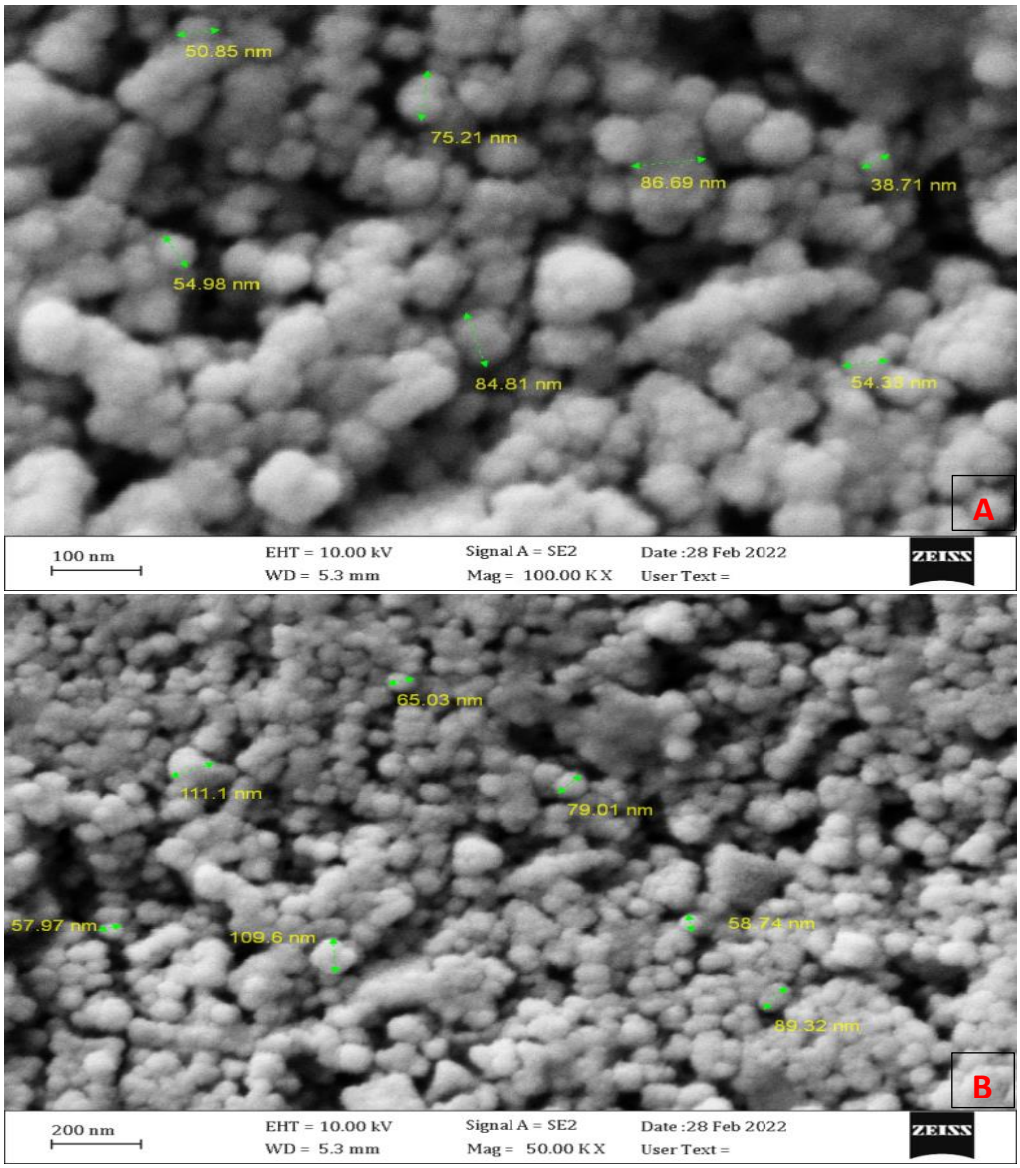

Figure 4-14: SEM Micrograph of Silver Nanoparticle of *Hibiscus sabdarriffa* Manufactured, (A) at 100nm and (B) at 200 nm

4-7-2: Energy Dispersive X-Ray Spectroscopy (EDXS)

Energy Dispersive-X-ray Spectroscopy (EDXS) have been used in the quantitative assessment of AgNPs by detecting the peaks of optical absorption by silver metal. The process of silver ions reduction into elemental silver is improved by the presence of elemental silver through recording of the spot profile mode of the EDXS spectrum. Several signals were detected, the strongest one reflected by silver, while the medium signal was reflected by chloride, and the other weaker signals were carbon, reflections from other atoms.

The percentage of elemental constituent's weight of the AgNPs shown by *H.sabdarriffa* was 80.3% silver and 12.7% of chloride and 7.1% of carbon as shown in (Figure 4-15), Optical absorption peak of AgNPs shown by *H.sabdarriffa* been detected at 4keV which is a typical absorbance of metallic AgNPs. Indicate that the AgNPs are capped by biomolecules through oxygen atoms. Other small peaks were observed attributed to the biomolecules present in the cell-free medium during synthesis (Rades *et al*., 2014).

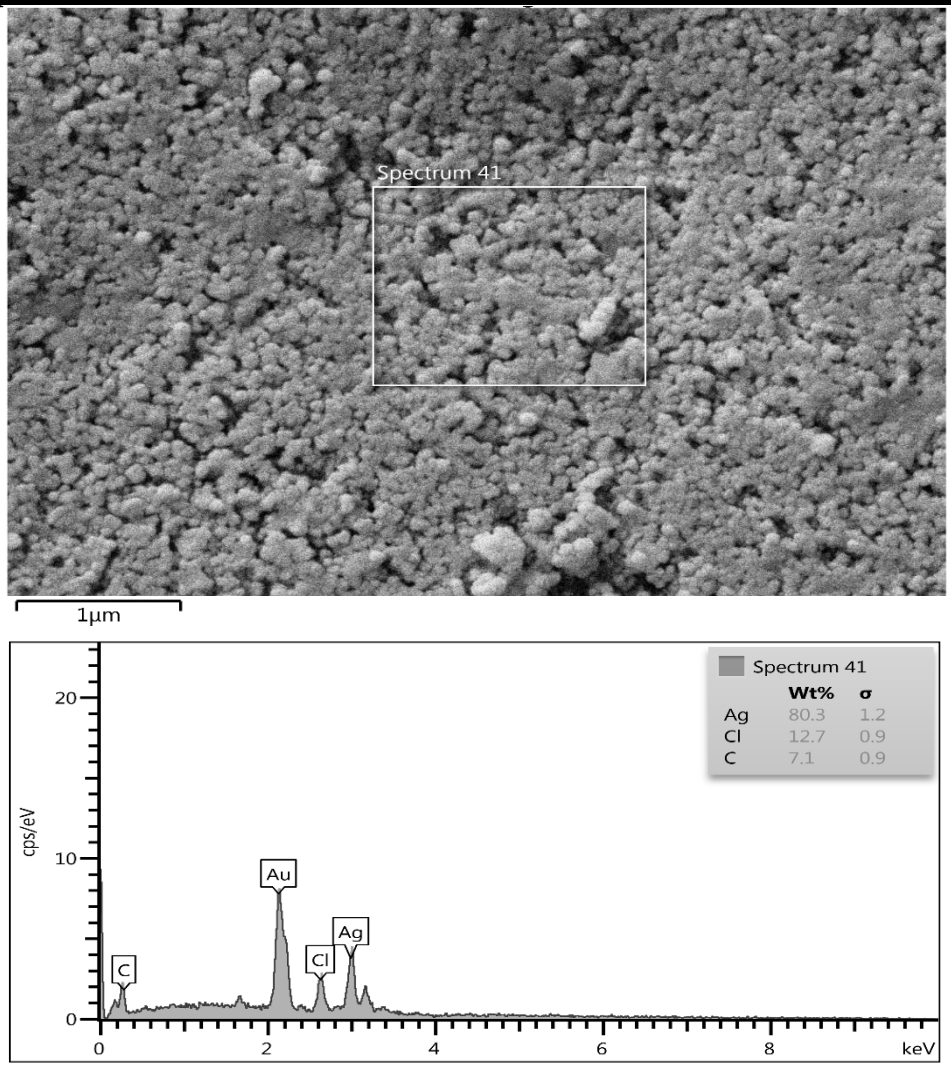

Figure 4-15: EDXS Point Analysis Spectrum Showing Availability and Abundance of AgNPs.

4-7-3: Fourier-Transform infrared spectroscopic analysis (FTIR)

The present results in Table (4-6) and Figure (4-16) show the FTIR spectrum that has been appeared in the region (3452.36 CM^{-1}) where it contains three starting regions (OH) and the (bonds) in it are wide as well (NH) in which the peak appears, It appears in the area (NH3) watching (Forked) and the semi-fork appears. The condition (NH) appears because the pick is sharp and strong at the same time, which means that the absorbance is high in this area.

In the area (2923.65 CM^{-1}), this case appears (CH) and is usually called (Aliphatic) for Alkanes, as this pike is weak, that is, high permeability and low absorption because in the spectrum of (FTIR) there is a strong, medium and weak pike, and what the current study records in the figure (4-16) the appearance of two peak in the very important region of the (IR) spectrum, which extends from (1650-1850CM^{-1}) in which (C=O) appeared, which is within the (carbonyl) group, where we notice the peak being distinctive, sharp and strong as a result of the high absorbance in this region where the first peak has a value of (1628.02 CM^{-1}) and the second of medium intensity has a value of (1712.54 CM-1) As for what is noticed in the extended region (500 -1500 CM^{-1}), which is called the fingerprint area, which contains the materials that have been worked on, and is often not explained except in some cases when a state of substitution occurs that occurs in some molecules in organic compounds here only appeared peak of the material taken as shown in the Figure (4-16).

FTIR was used to identify the extract's beneficial groups. The bands 3448.84 cm^{-1} due to phenolic, 2924.18 cm^{-1} due to C-H, 1734.06 cm^{-1} due to C=O 1654.98, 1610.61 due to C=C, 1516.10, 1454.38, 1342.50 cm^{-1} due to C-H, 1147.68, 1033.88, 1232.55 due to C-O were seen in the ethanolic extract, whereas the same result was seen in the nanoparticle extract but absence the bands at 1512.24, 1654.98, 1734.06 due to the silver ions were redacted (Berthomieu and Hienerwadel, 2009; Cisse *et al*. 2009).

Table 4-6: The Peak Values, Functional Group and Bond Type of *H.sabdarriffa* nanoparticles.

No	Peak values	Functional group	Group
1	3452.35	Alcohol and phenols	O –H
2	2923.55	Alkanes	C –H
3	1712.54	Carboxylic acid	C =O
4	1628.02	Alkenes	C =C
5	1385.54	(Methyl or-CH3)	C –H
6	1257.95	Ether, epoxide	C –O
7	1070.13	Alcohol and phenols	C –O
8	10 12.83	Alcohol and phenols	C-O
9	607.07	Halogen compound (Iodo-compound)	C –I
10	507.61	Halogen compound (Chloro-compound)	C –Cl

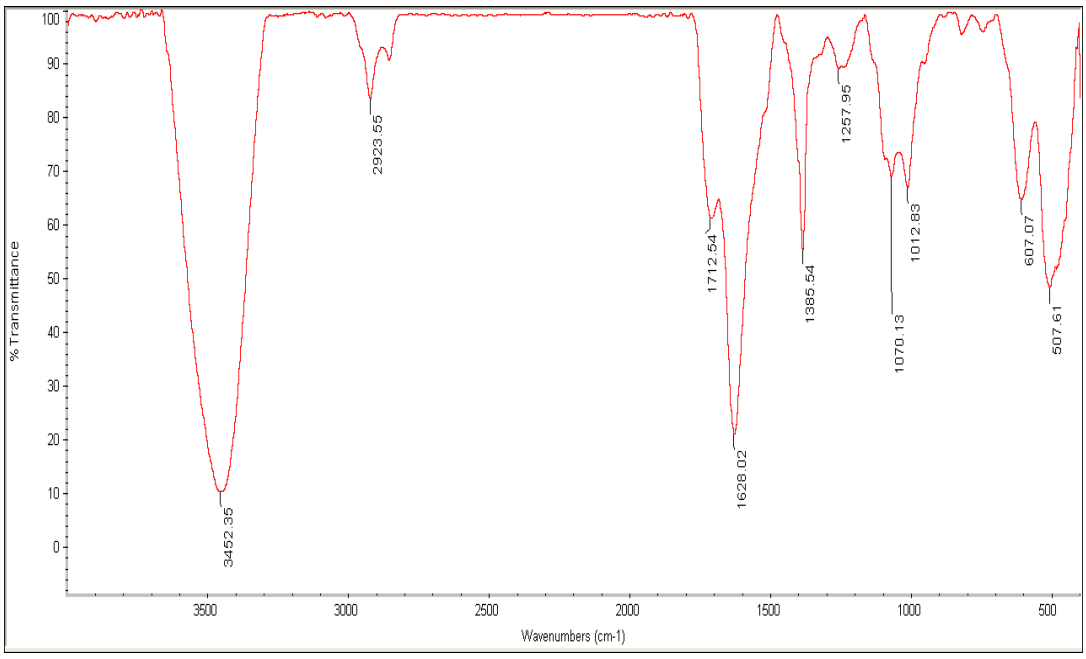

Figure 4-16: FTIR Spectrum of Synthesized *H.sabdarriffa* Nanoparticles.

4-7-4: Atomic Force Microscopy (AFM)

Atomic force microscope photos of AgNPs shown by *H.sabdarriffa* have been used to describe the morphology, the average diameter and the roughness of AgNPs. Both etching time and current density have been used to control the shape and size of the final structure. Analysis of AFM imaging of the biogenic AgNPs shown revealed that the average diameter was 63.65 nm as shows in figure (4-17).

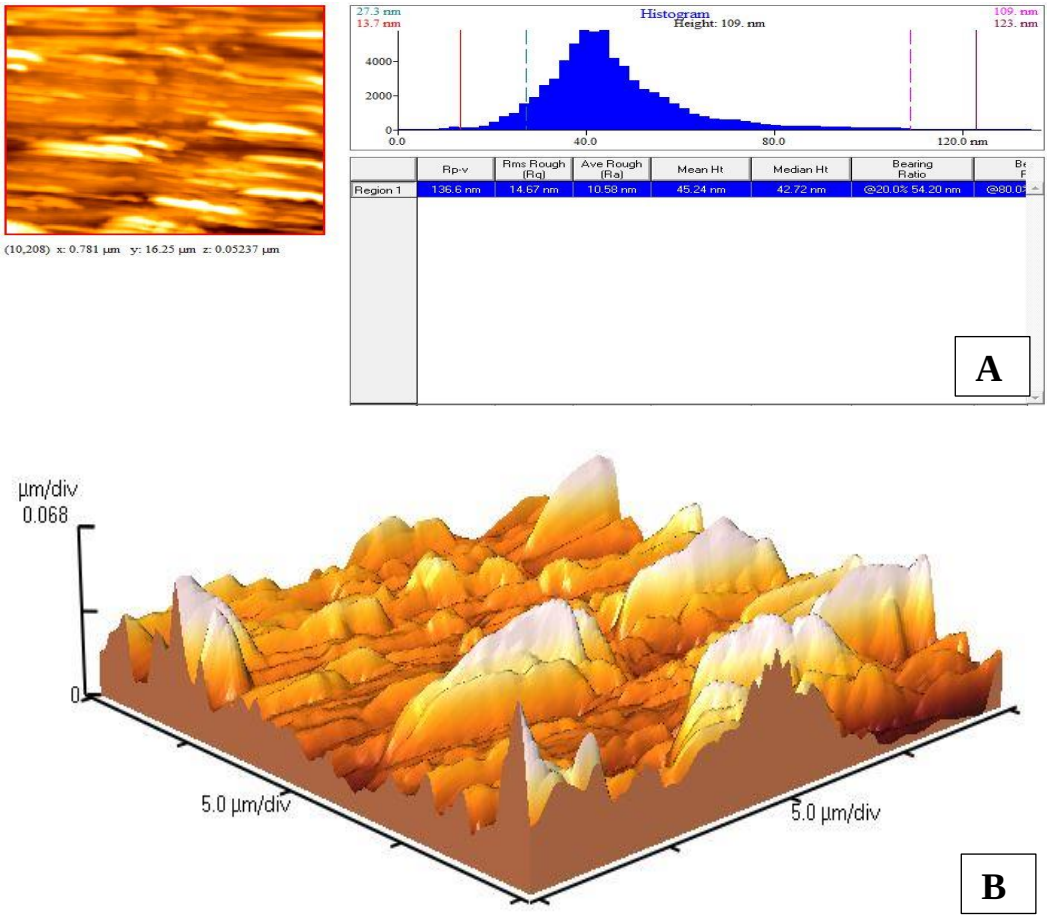

Figure 4-17: AFM Analysis, the figure Shows Topography and Granularity Cumulation Distribution report. A: Granularity and Cumulation Distribution chart. B: 3D Characterization of Biogenic AgNPs shown by *Hibiscus sabdarriffa* AgPNs

4-8: Biological Applications of Ag Silver Nanoparticles:

4-8-1: The Effect of Extract of *H.sabdarriffa* AgNPs Concentration on the Viability of the Protoscolices of the *E.granulosus*

Through the figure (4-18), significant differences have been noted between the concentrations used in the experiment. The number of concentrations is 0.1 and 0.2µg\ml, there is no significant difference between them. The concentration of 0.8 µg\ml was the best in its effect on the effectiveness of the viability of the protoscolices of the *E.granulosus*.

Figure (4-19) shows the effect of time duration on the viability of *E.granulosus* protoscolices, revealing an inverse association between decreased viability and increasing time period, After 192 hours of treatment, the viability ratio was 0.8%, compared to 85.54% after zero hours of therapy, according to the statistical analysis. The significance of the differences found was indicated. The study's results are consistent with those of a previous study Al-Musawi (2012) and Al-Hasnawi *et al.* (2021).

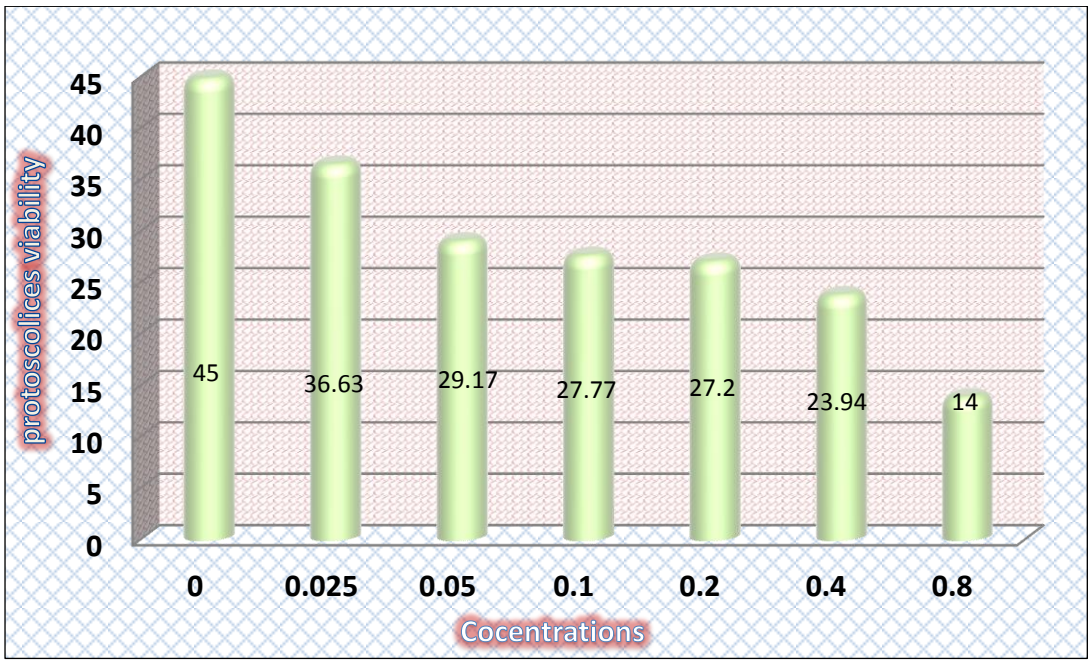

Figure 4-18**:** Effect of the Plant Extracts *H.sabdarriffa* AgNPs Concentration Factor on the Number of Protoscolices. (L.S.D Value Least Significance Differences at Level 0.05 = 3.004).

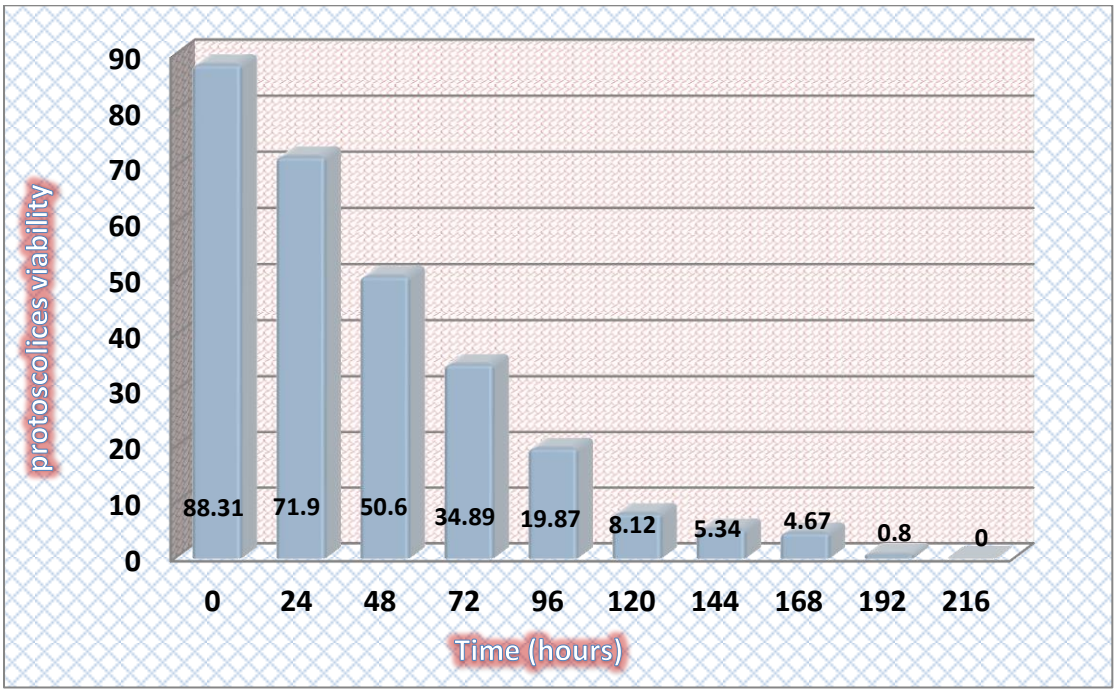

Figure 4-19: Effect of Time Duration on the Total Number of Cysts Protoscolices Treated with *H.sabdarriffa* AgNPs (L.S.D Value Least Significant Differences at Level 0.05 = 7.601).

The results in table (4-7) show the interactin between different levels of concentrations of plant extract concentration and time period on the viability of Protoscolices are lost, as the viability percentage in concentration (0.8) mg/ml after 24 hours of treatment was 0%, compared to 85.54% in the control treatment. Significant differences were discovered as a result of the statistical analysis.

These results reveal the boiled water extract for *H.sabdarriffa* AgNPs is more effective due to the small size of the particles, they penetrate the wall. As for the remaining concentrations, it gradually had an effect on the effectiveness of the substrates, in addition to the length of the exposure period to the nano-extract, The higher the concentration and the longer the exposure time, the more effective against the protoscolices (Salih, 2020; Saeed, 2021).

Through present results it is noted that the effect of the extract of the *H.sabdarriffa* nanoparticle is better than the Albendazole, as the *H.sabdarriffa* nanoparticle had zero the viability of the protoscolices after 48 hours for a concentration of 0.8 µg/ml while the Albendazole had reached zero of the viability after 120 hours and at 300 mg/ml concentration.

4-8-2: Hemolysis assays

The curve present in figure (4-20) reveals the hemolysis rate is high at the highest concentration of 512µg and the hemolysis begins to decrease with a decrease in the concentration and low that is at the concentration of 64 µg Positive, undissolved blood control and Triton-negative control were used, and four concentrations were added to the blood . The rate of hemolysis after four hours was 3.8% after entering the equation at the highest concentration 512 µg down to the lowest concentration 64 µg, and the hemolysis rate was the lowest at 0.3% In this experiment, four concentrations of Nano *Hibiscus sabdarriffa* extract was used (512, 256, 128, 64) µg.

Table 4-7: Effect of interfering plant extracts *H.sabdarriffa* AgNPs concentrations on the number of protoscolices (L.S.D value least significant differences at level 0.05 = 13.2)

Extracts	*H.sabdarriffa* AgNPs						
Concentrations µg / ml / Time (hour)	**Control**	**0.025**	**0.05**	**0.1**	**0.2**	**0.4**	**0.8**
0	85.54	92.15667	89.64667	89.55667	86.84333	87.78333	86.65
24	77.94	81.26333	73.02333	73.36667	71.47333	72.81667	53.37
48	63.29	66.66667	62.72667	56.31333	57.54667	50.62	0
72	52.27	63.33333	43.62667	37.08333	32.61	28.24333	0
96	42.85	46.66667	22.60667	21.37667	21.71	0	0
120	40.60	16.18	0.051333	0	0	0	0
144	37.27	0.135667	0	0	0	0	0
168	32.7	0	0	0	0	0	0
192	17.68	0	0	0	0	0	0
216	0	0	0	0	0	0	0

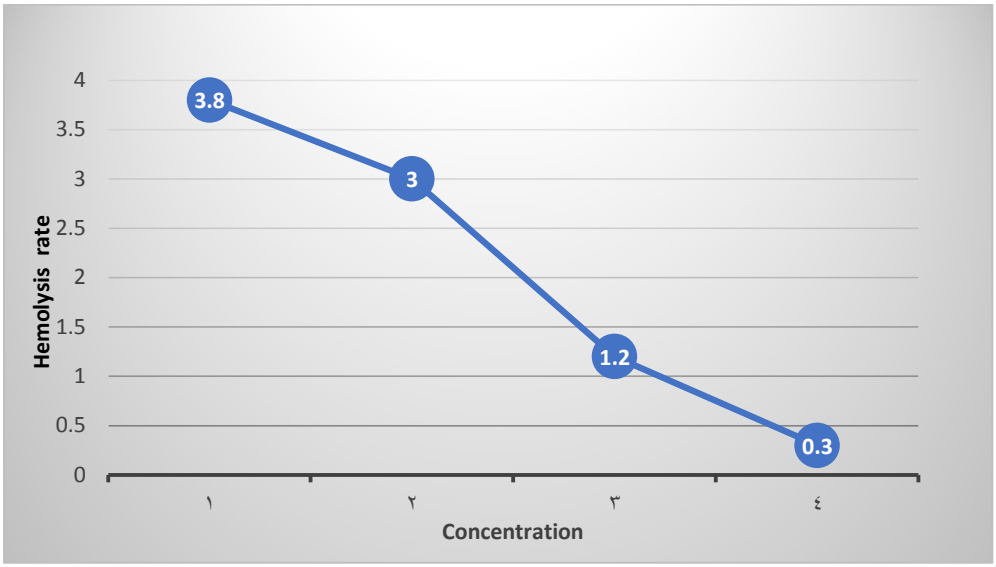

Figure 4-20: Human RBC Lysis (%) Induced by Ag NPs in Washed RBC .

The amount of haemolysis is determined by subtracting the interference of Ag NPs from haemoglobin absorbance at 550nm. There was a high difference AgNPs of 50 and 100 µg/mL observed in washed RBC, after 24 hours of incubation compared to controls. The haemolytic effect was shown to be lower in cleansed RBC than in whole blood .This is most likely related to the fact that plasma adsorption occurs in entire blood proteins on NPs can happen and have an impact, on characteristics of haemolysis (Shi *et al*., 2012).

As the use of AgNPs increases, toxicological testing is required to ensure patient safety, Metallic silver ionizes and releases ions in the bloodstream when it comes into contact with blood, which can interact with transmembrane proteins, However, silver ion formation is not the only cause of haemolysis triggered by NPs; other processes (deformability, adhesiveness, membrane vesiculation, etc.) also play a role, various studies evaluated the haemolysis induced by AgNPs (Choi *et al*., 2011). The biological effects of AgNPs on human blood cells and the possible danger of thrombosis are poorly understood (Greulich *et al*., 2011).

In recent years, *Hibiscus* leaves mucilage (HLM) has been investigated for the development of several edible sustained release dosage forms. HLM has also been shown to have antibacterial properties against Gram-positive and Gram-negative bacteria (Rm and Nair, 2018). Phytochemical elements such as alkaloids, flavonoids, phenolics, and tannins have been linked to the antibacterial activity of *H.sabdariffa* extract (Nkumah, 2015; Zahraa, *et al.,* 2020).

4-8-3: Antioxidant Test:

The antioxidant capacity of the chemical AgNPs was determined using a 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, and AgNPs were biosynthesized from *H.sabdarriffa* extract in vitro by decreasing DPPH free radicals. After 30 minutes in the dark, the absorbance (A) was measured at 517 nm after adding the nanoparticles and *H.sabdarriffa* extract to (0.1m) DPPH solution. The results revealed that nanoparticles and *H.sabdarriffa* extract had the ability to scavenge DPPH-free radicles, as evidenced by the color change from DPPH purple to yellow (Figure 4- 21).

These findings demonstrated the antioxidant activity of biosynthesized AgNPs and *H.sabdarriffa* extract in vitro, leading to a determination of the nanoparticles' and *H.sabdarriffa* extract's antioxidant competency *In vitro*.

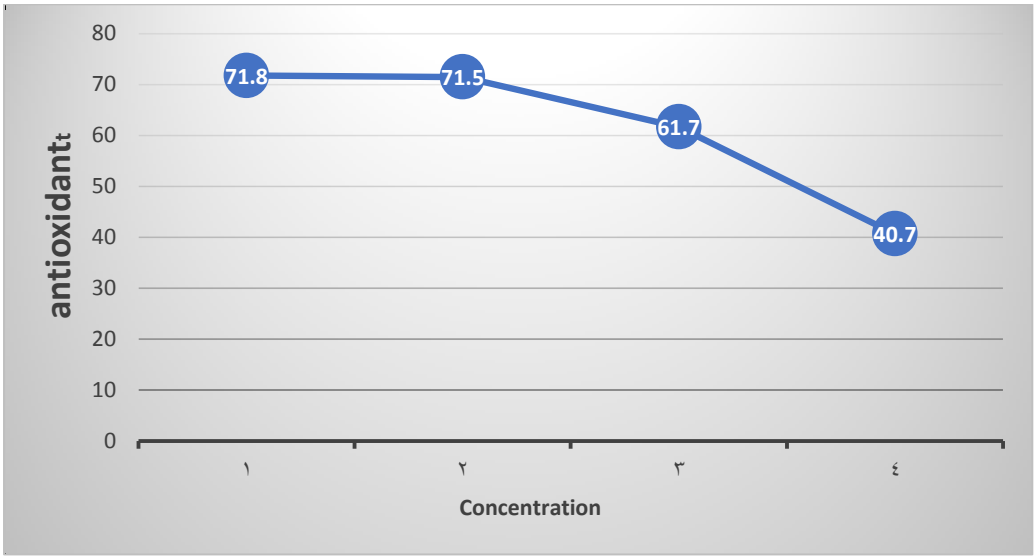

Figure 4-21: Identification of Antioxidant Activity of Biosynthesized AgNPs and *Hibiscus sabdarriffa* Extract.

The largest effect was found in the mixture of DPPH with Silver biosynthesized from *H.sabdarriffa* extract at a concentration of 2mg/ml (71.8) %, while the smallest inhibition titer found in the mixture of DPPH with *H.sabdarriffa* extract with silver from *H.sabdarriffa* extract at concentration 1mg/ml (40.7) %.

The varying scavenging potentials of the compounds could be explained by the various processes engaged in radical-antioxidant interactions. Antioxidants work not just by scavenging free radicals, but also by limiting the generation of free radicals (Niki, 2010; Xing *et al*., 2015).

The one,1-diphenyl-2-picrylhydrazyl scavenging activity of nanoparticles increased as their concentration rose, as evidenced by the increased percentage of the DPPH effect. Increasing the concentration of AgNPs had a greater effect because DPPH absorbs and donates more electrons (Kanipandian *et al.*, 2014; Bhakya *et al*., 2015).

4-9: Molecular Detection by PCR for 18SrRNA ITS1 and ITS2 gene for *E.granulosus*

The results of the PCR technique show the success of all DNA amplification processes extracted from the protoscolices and the germinal layer of the ITS1 and ITS2 genes in detecting the presence of the parasite. The results appeared as a positive result in ITS1 in some samples of sheep hydatid cysts numbered (1, 3, 4, 5 and 8) at molecular weight (100-1100) base pair as shown in figure (4-22). Negative results in humans (2, 6 and 7) after performing electrophoresis on the agarose gel, ITS1 appeared in sheep samples but did not appear in human samples.

Figure (4-23)shows the presence of the diagnostic gene for (ITS1 and ITS2) gene, ITS1 gene at the molecular weight (1100bp) and ITS2 gene at the molecular weight (750bp). The sample (1 and 2) of human hydatid cysts, ITS1 had negative results in the sample (1) and positive results for ITS2. But the sample (3 and 4) sheep hydatid cysts had positive results for ITS1 for the sample (3) and ITS2 for the sample (4). This study is consistent with the study of (Abdul-Kazim and Al-Mayali 2020) to detect the presence of the parasite using ITS1 gene.

It is clear that the nucleotide sequences of the samples under study selected (H220224-023_O12_A2_AF and H220224-023_O14_B1_BF) had similarities with the numbered Indian sample ID: KX434757.1 with minor genetic variations as shown in figures (4-24) and (4-25).

Table (4-8), shows that the first sample numbered H220224-023_O12_A2_AF has a symmetry ratio of 98.87% for seven variations: G\A at position 5; -\A at position 10; C\A at position 11; A\C at position (80, 109), G\C at position 174; and A\G at position 162 are the transition mutation, whereas the second sample numbered H220224-023_O14_B1_BF, the similarity ratio was 99.68% with two variants, only

one A\G at position 521 and A\C at position 559 which are transition mutations.

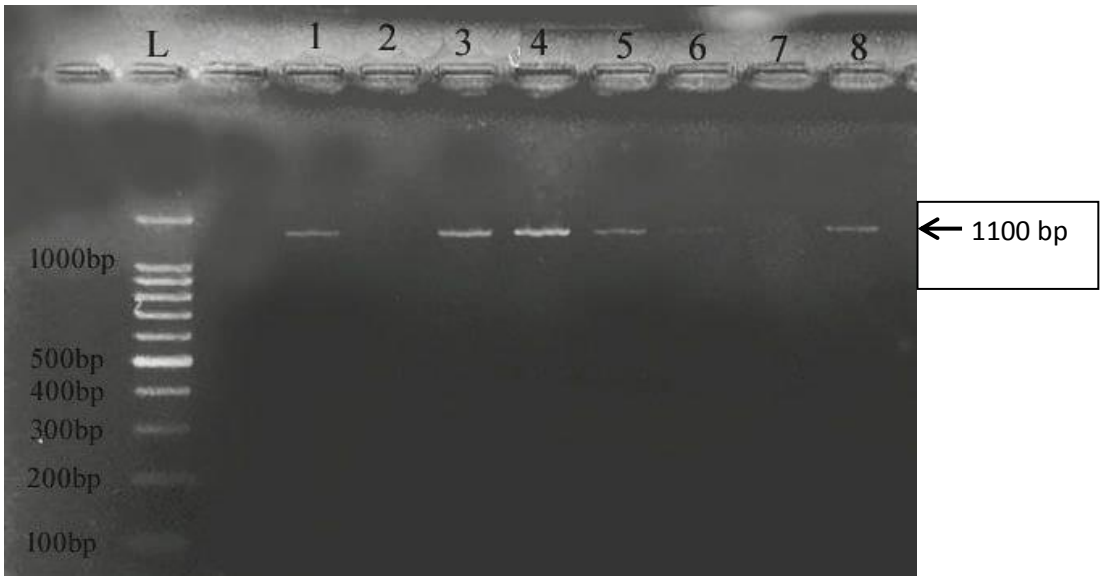

Figure 4-22: Banding patterns of ITS1 gene in hydatid cysts, L; molecular size marker (100 bp); ITS1 gene in samples of sheep hydatid cysts numbered (1, 3, 4, 5 and 8) at molecular weight (100-1100bp), (2, 6 and 7) in human after perform an electrophoresis on the agarose gel.

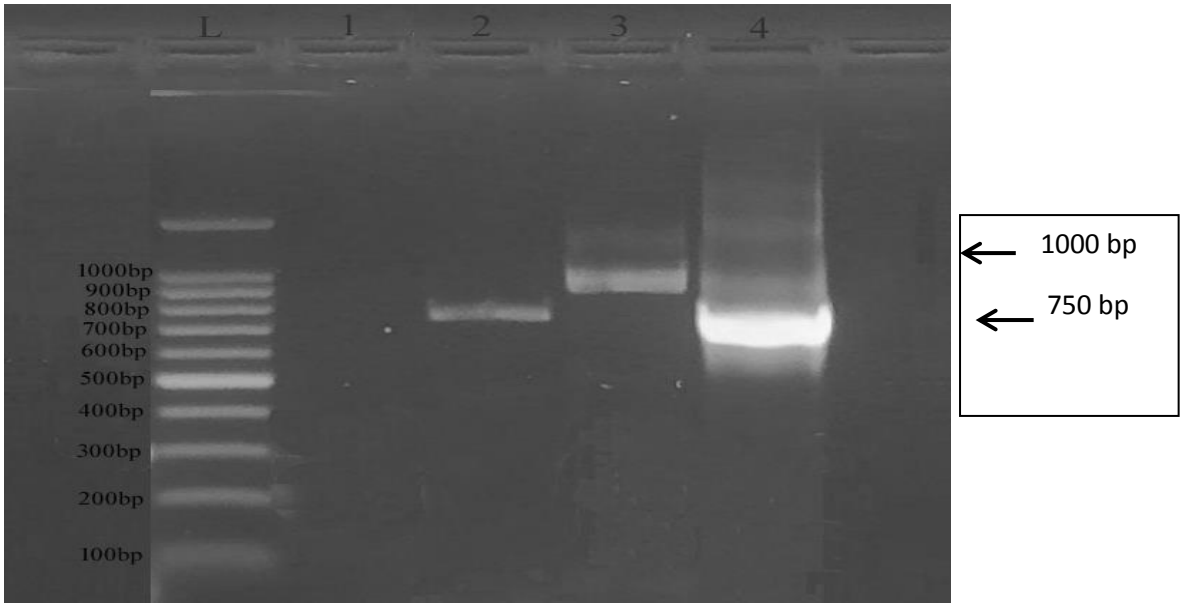

Figure 4-23: Banding Patterns of ITS1 and ITS2 Gene in Hydatid Cysts, L; Molecular Size Marker (100 bp), Line 1 and 2 in Human Hydatid Cysts, 3 and 4 Sheep Hydatid Cysts.

Table 4-8: The NCBI-BLAST Homology Sequence Identity (%) of ITS2 Gene between local *E.granulosus* local Isolates and NCBI-BLAST submitted *E.granulosus* isolates.

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Identities
H220224-023_O12_A2_AF.	Transition	5	G\A	ID: KX434757.1 *E.granulosus* from India	98.87%
	Transition	10	-\A		
	Transition	11	C\A		
	Transition	80	A\C		
	Transition	109	A\C		
	Transition	174	G\C		
	Transition	162	A\G		
H220224-023_O14_B1_BF	Transition	521	A\G		99.68%
	Transition	559	A\C		

Echinococcus granulosus isolate VT-ASg 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

Sequence ID: KX434757.1 Length: 619 Number of Matches: 1

Range 1: 1 to 619 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1103 bits(597)	0.0	612/619(99%)	1/619(0%)	Plus/Plus

```
Query  29   ATTAGTGTG-CTCGCAGACTGCTTTGAACGTCGACATCTTGAACGCATATTGCGGCCATG  87
Sbjct  1    ....A....AA.............................................  60

Query  88   GGCTTGCCTGTGGCCACGTATGTCCGAGCGTCGGCTTATGAACCATCAATGTGTGCAATG  147
Sbjct  61   ..................C...........................C...........  120

Query  148  AGCGGTGGCTGGGGAGAGTGCGGTGCCGTCCCGTGCCGTGGGGCGCGGTGGGTAGAGCGT  207
Sbjct  121  .........................................C...........G......  180

Query  208  GTGCTGGTTGGCTGGCTCACGGTGCGGACTGGCGTGGCTTCTCACTAGGTGTGCTGGTGC  267
Sbjct  181  ............................................................  240

Query  268  TGTCGAATTCGGTGGCGTGGAGTTTGCGGTTGTGCTGCTGCAGTGGCCGCAGTGCGGTCA  327
Sbjct  241  ............................................................  300

Query  328  GCCGTTGCGCTGTGCTGTGGCGTTGATGCGCGTGTACGGCAGTTGTGCCAGTAGGTTGGC  387
Sbjct  301  ............................................................  360

Query  388  GGGTGGTGATGCGGTTGCAGTCTTCGCAGTCCACGGACCGTGGCCCAGTGTGCGAATGGG  447
Sbjct  361  ............................................................  420

Query  448  CAGgtgtatgtgtgtgtgtaggtgtgAGCACGCTGCACACGTTATGTGGATTGTGGATGC  507
Sbjct  421  ............................................................  480

Query  508  TGTGGCGGGTGGGGCGTGCTTTCTTCTCTCGCTCGCCGCAGGCACTTGCATCGTTGTGCT  567
Sbjct  481  ............................................................  540

Query  568  GCGTTGCACGTGTAATGCGATGGGTGGGAGGGTGCGCGTCACGCCCCGCCTCGCCTAGCC  627
Sbjct  541  ............................................................  600

Query  628  TTGCCTTGATTGACTCGTT  646
Sbjct  601  ...................  619
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Figure 4-24: Alignment of the First Sample Numbered H220224-023_O12_A2_AF with DNA Nitrogenous Base Sequences of *E.granulosus* from India has ID: KX434757.1

Echinococcus granulosus isolate VT-ASg 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

Sequence ID: KX434757.1 Length: 619 Number of Matches: 1

Range 1: 1 to 619 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1133 bits(613)	0.0	617/619(99%)	0/619(0%)	Plus/Plus

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Query  28   ATTAATGTGAATCGCAGACTGCTTTGAACGTCGACATCTTGAACGCATATTGCGGCCATG  87
Sbjct  1    ............................................................  60

Query  88   GGCTTGCCTGTGGCCACGTCTGTCCGAGCGTCGGCTTATGAACCATCACTGTGTGCAATG  147
Sbjct  61   ............................................................  120

Query  148  AGCGGTGGCTGGGGAGAGTGCGGTGCCGTCCCGTGCCGTGGCGCGCGGTGGGTGGAGCGT  207
Sbjct  121  ............................................................  180

Query  208  GTGCTGGTTGGCTGGCTCACGGTGCGGACTGGCGTGGCTTCTCACTAGGTGTGCTGGTGC  267
Sbjct  181  ............................................................  240

Query  268  TGTCGAATTCGGTGGCGTGGAGTTTGCGGTTGTGCTGCTGCAGTGGCCGCAGTGCGGTCA  327
Sbjct  241  ............................................................  300

Query  328  GCCGTTGCGCTGTGCTGTGGCGTTGATGCGCGTGTACGGCAGTTGTGCCAGTAGGTTGGC  387
Sbjct  301  ............................................................  360

Query  388  GGGTGGTGATGCGGTTGCAGTCTTCGCAGTCCACGGACCGTGGCCCAGTGTGCGAATGGG  447
Sbjct  361  ............................................................  420

Query  448  CAGgtgtatgtgtgtgtaggtgtgAGCACGCTGCACACGTTATGTGGATTGTGGATGC  507
Sbjct  421  ............................................................  480

Query  508  TGTGGCGGGTGGGGCGTGCTTTCTTCTCTCGCTCGCCGCAAGCACTTGCATCGTTGTGCT  567
Sbjct  481  ...........................................G................  540

Query  568  GCGTTGCACGTGTAATGCGATGGGTGGGAGGGTGCGCGTCACGCCCCGCCTCGCCTAGAC  627
Sbjct  541  ............................................................C.  600

Query  628  TTGCCTTGATTGACTCGTT  646
Sbjct  601  ...................  619
```

Figure 4-25: Alignment of the Second Sample Numbered H220224-023_O14_B1_BF, with DNA Nitrogenous base Sequences of *E.granulosus* from India has ID: KX434757.1

4-10: Phylogenetic tree of *E.granulosus*:

Figure (4-26) shows phylogenetic tree analysis based on ITS2 gene partial sequence in local *E.granulosus* isolates that are used for genotyping analysis. After matching this sample with the samples from the gene bank.

The study reached the identification of a sample of hydatid cyst at the molecular level using the region ITS, and the matching percentage was 99% with the sample which has ID: KX434757.1named *E.granulosus* from India and this is recognized through the alignment drawn to compare it.

The phylogenetic tree was constructed using the Maximum Likelihood method and Tamura-Nei model tree method) in (MEGA X version). The *E.granulosus* isolate from the second sample numbered H220224-023_O14_B1_BF from human in figure (4-26) were showed genetic variation related to NCBI-BLAST *E.granulosus* isolates at genetic changes when compared to the other samples under study that appeared in the phylogenetic tree, besides the samples that registered with the NCBI Blast in diameter by drawing 0.1 came with very little genetic variation and close to the recorded international sample

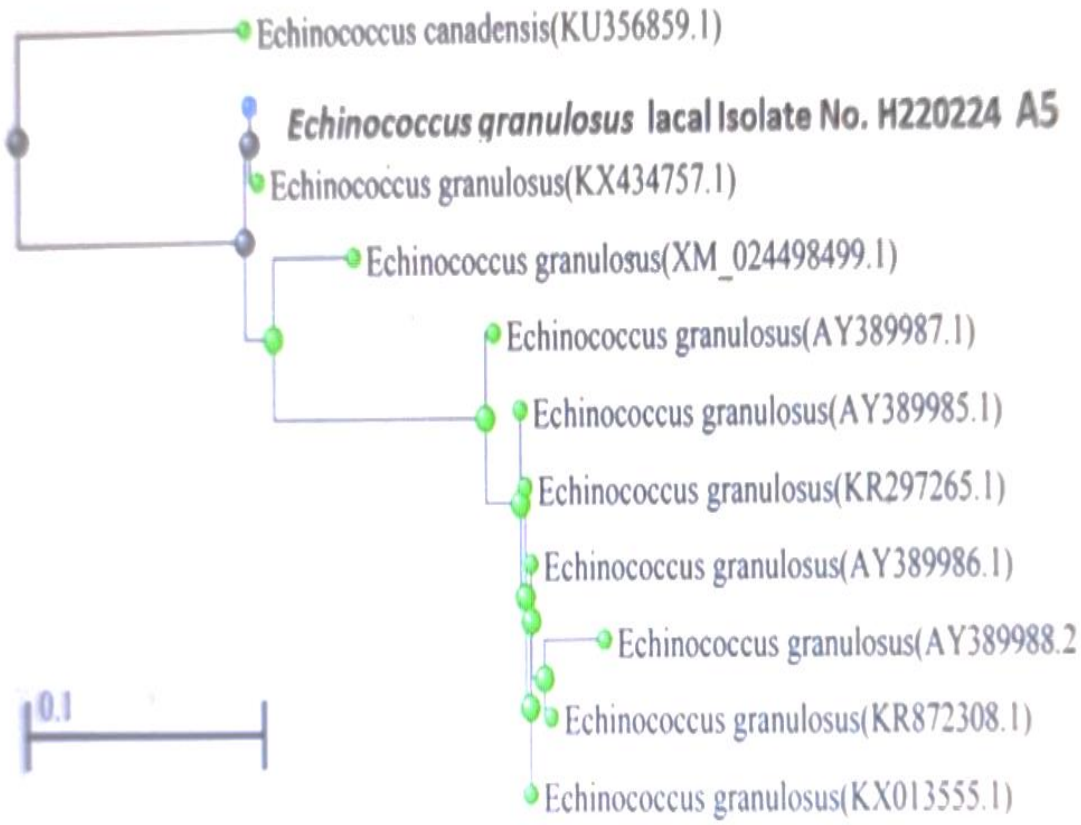

Figure 4-26: Phylogenetic Tree of Isolates Samples in the Current Study and Comparison with Another International Isolates are Registered in NCBI.

The current study of *E.granulosus* constantly undergoing genetic alteration through the appearance of mutations in the region ITS2. Genetic variation is present, and genetic evolution is clear within the species under study. For example, the samples approved in drawing the evolutionary tree showed something indisputable that there is an internal development that leads to variation, so it becomes clear, for example, that *E.granulosus* (KU356859.1) differs greatly from the rest of the samples deposited in the gene bank, *E.granulosus* (XM-024498499.1) is different from the rest of the isolates and from our isolate diagnosed in *E.granulosus* H220224 no.2, and even the samples that appear in one group,*E.granulosus*(XM024498499.1,AY389987.1,AY389985.1,KR297265.1,AY389986.1,AY389988.2, KR872308.1, KX013555.1) appear to be one group, but there is genetic variation inside it, so they are not on one line.

The reason for the difference in the results may be due to the difference in the size of the samples, their number, origin and time of collection, the difference in geographical areas, the nature of animal feeding in those areas, or the presence of dogs near the abattoirs and butcher's shops that slaughter or sell a specific type of these animals (sheep, cattle) and thus contaminate water and vegetables by the feces of infected dogs, in addition to the customs and traditions of the inhabitants of those areas and the level of health awareness they have, and the extent of the spread of intermediate and final hosts to them, or it may be due to the adaptation of specific strains in those areas more than others (Al-Hasnawi *et al*., 2021). Gareh *et al*. (2021) emphasized that the goal of studying genetic evolution was to identify the forms of *E.granulosus* that have spread throughout the country, which is crucial information in the fight against this animal illness.

The nature of the environment, temperature and climatic conditions all play a role in the emergence of such variations in the samples. Therefore, the current study showed that the ITS sequence analysis is simple, fast and

very reliable and can be used as an effective tool for identifying and classifying the studied species. It also provided a lot of useful genetic information about the studied species for the management of genetic assets. High Efficiency (Weidong *et al*., 2019).

Chapter Five

Conclusions

&

Recommendations

5-1 :- Conclusions

1- The aqueous extract of the chosen plant confirmed that they may be used as a viability detection stain, it appeared that the stain extracted from *Crocus sativus* was the best herbal stain compare with other natural dyes.

2- The aqueous extract of *Hibiscus sabdarriffa* became the handiest at the viability of protoscolices (In-vitro), followed by *Brassica oleracca*, *Beta vulgaris* and *Crocus sativus*.

3- The boiled water extract of *Hibiscus sabdarriffa* has better parasitic efficacy than Albendazole.

4- Silver nanoparticles that are synthesized from *Hibiscus sabdarriffa* are safe and reliable and they could be taken into consideration as a brand new antihelminthic agent.

5- *Hibiscus sabdarriffa* AgNPs show sporicidal activity on hydatid cyst protoscolices, which affects the parasite's in vitro survival.

6- When PCR genes were amplified, DNA isolated from protoscolices performed better than DNA extracted from the germinal layer.

7- The study emphasized parasite's presence and has a similar percentage was 99% with a sample which has ID: KX434757.1 named *E.granulosus* from India and by recognized through the alignment drawn to compare it.

5-2 :- Recommendations

1. conducting a study in vivo employing active substances derived from the *Hibiscus sabdarriffa*, such as an investigation on white mice experimentally infected with *E.granulosus*
2. Finding a treatment for stray dogs and security dogs, as well as regular examination for them.
3. urging researchers to develop a vaccine that is effective against Hydatid cystic diseases or treatments for both humans and animals that can help reduce the risk of infection and stop the life cycle by keeping dogs from eating dead animals or burying or burning infected animals that have been killed in abattoirs.
4. Informing people about the parasite's life cycle and the role of dogs in the disease transmission.

5. The Synthesis of gold nanoparticles and examination of their impact on the hydatid cyst of the *Echinococcus granulosus*.
6. Studying how *Hibiscus sabdarriffa* AgNPs affect the parasite's molecular structure and gene expression using the polymerase chain reaction method would aid in developing new strategies around the role that this extract plays.

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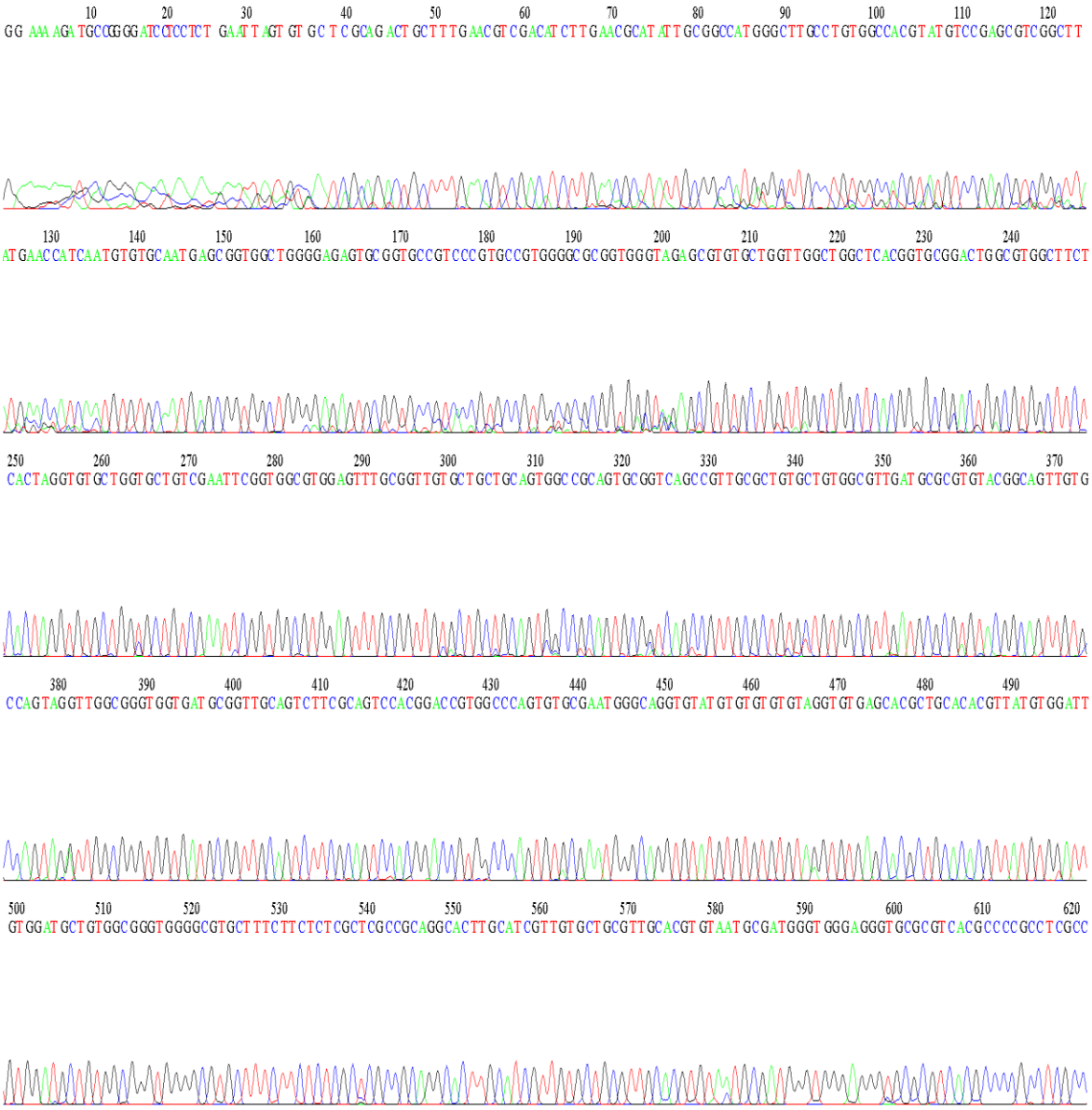
Appendix

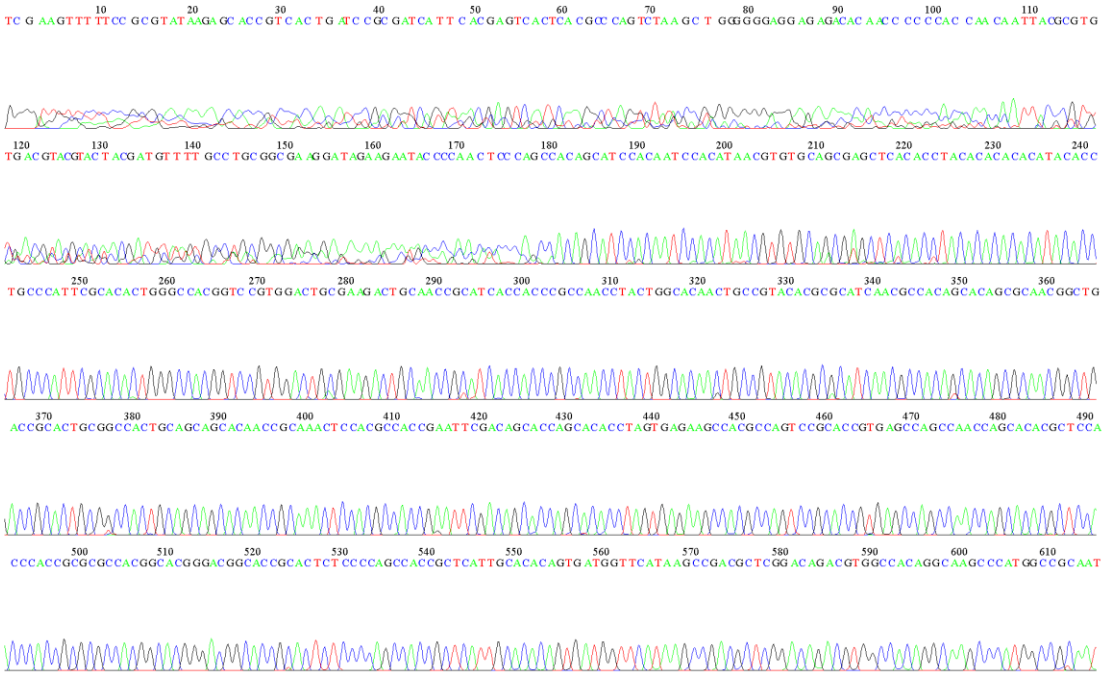
Appendix (1)

The genetic sequence sent by the Macrogen Company in South Korean before the give the sequence number for registration on the samples under study.



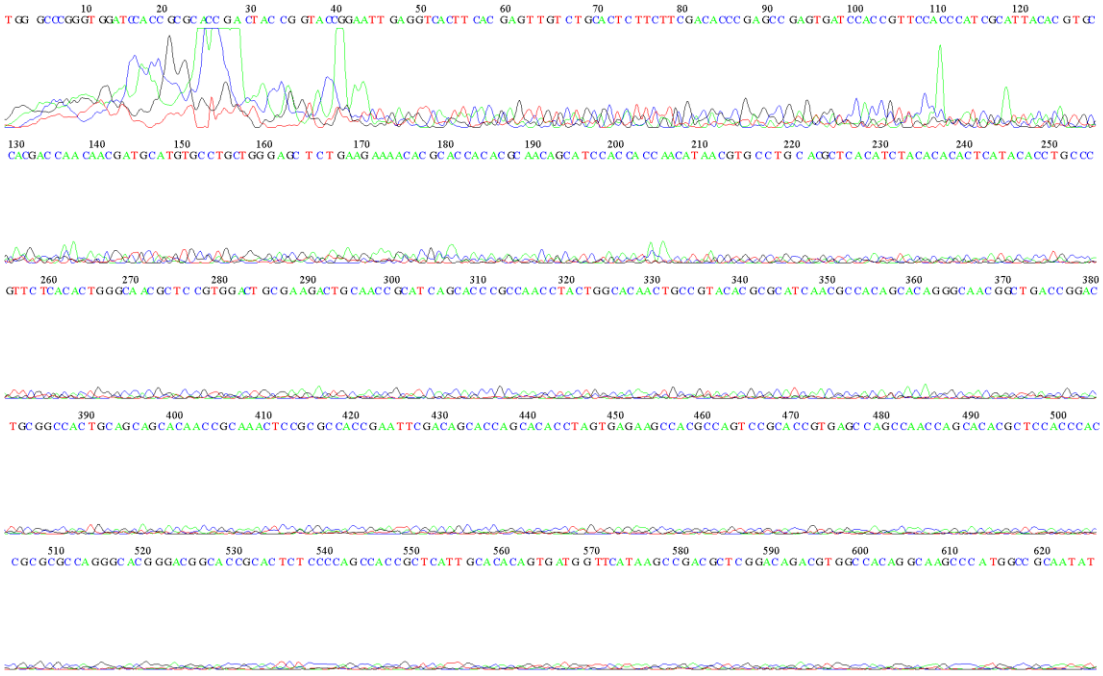
Appendix (2)

The genetic sequence sent by the Macrogen Company in South Korean before the give the sequence number for registration on the samples under study.



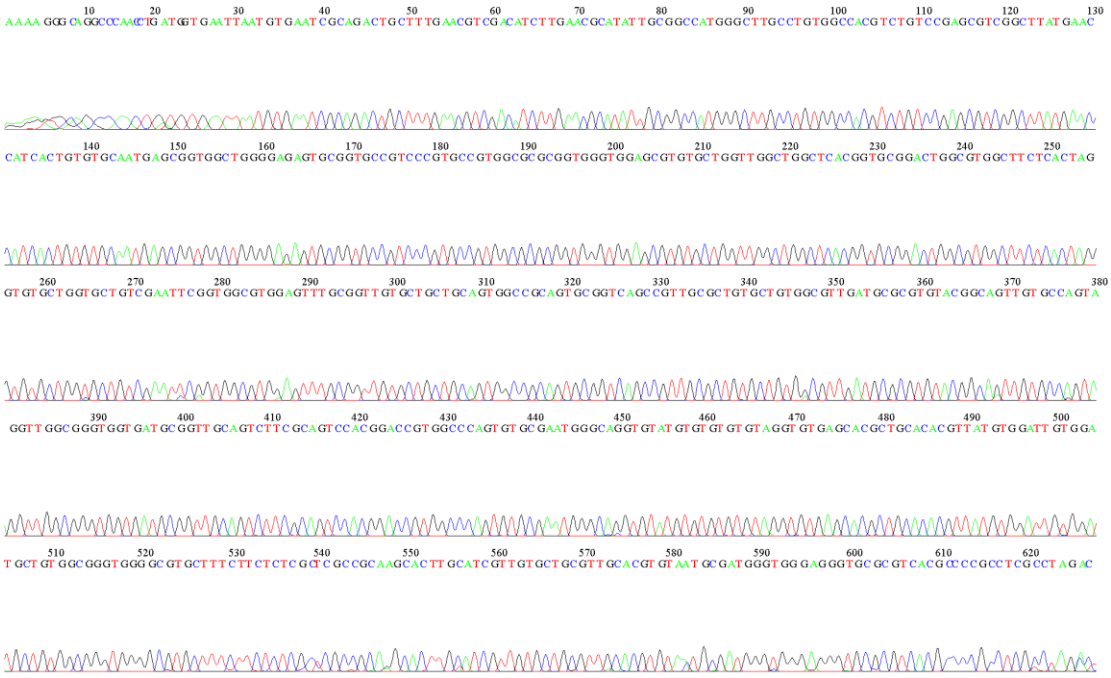
Appendix (3)

The genetic sequence sent by the Macrogen Company in South Korean before the give the sequence number for registration on the samples under study.



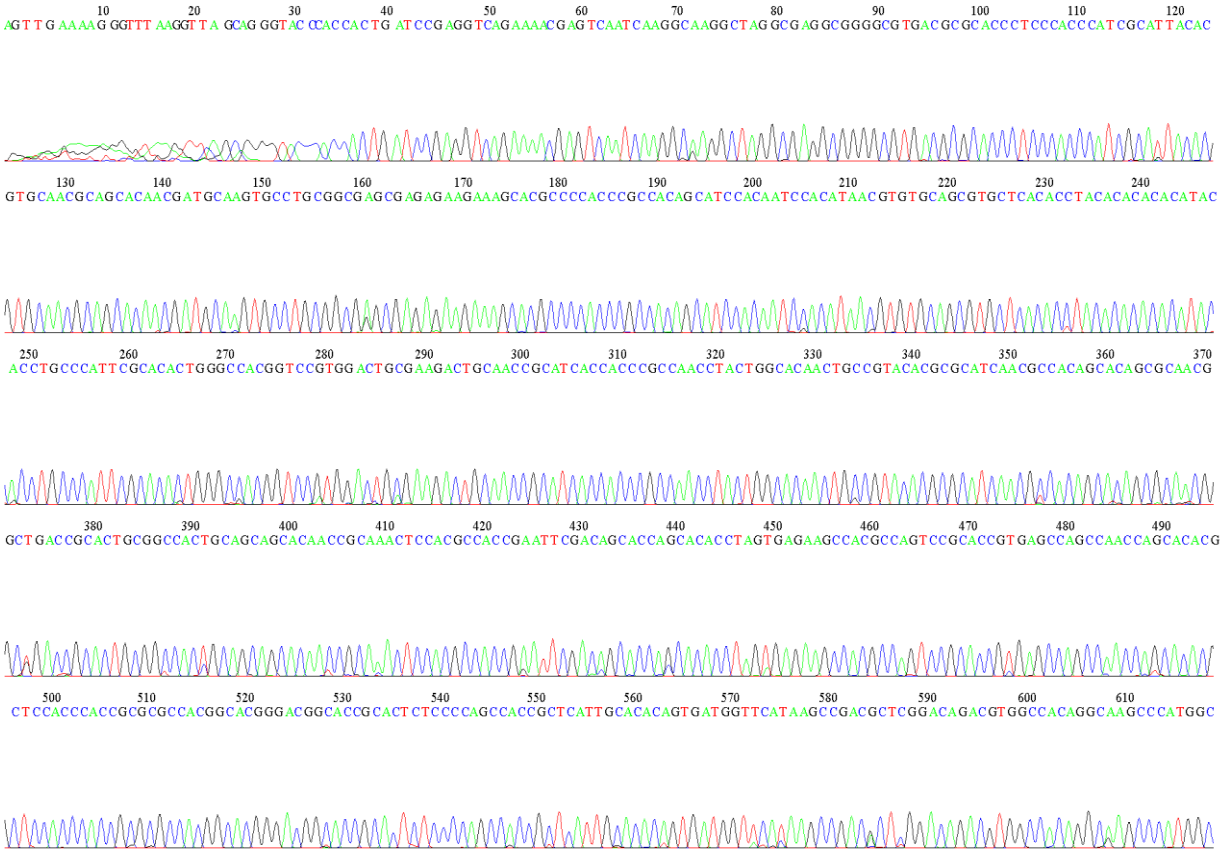
Appendix (4)

The genetic sequence sent by the Macrogen Company in South Korean before the give the sequence number for registration on the samples under study.



Appendix (5)

The genetic sequence sent by the Macrogen Company in South Korean before the give the sequence number for registration on the samples under study.



Appendix (6)

Echinococcus granulosus isolate VT-ASg 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

Sequence ID: KX434757.1 Length: 619 Number of Matches: 1

Range 1: 1 to 524 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
902 bits(488)	0.0	514/526(98%)	4/526(0%)	Plus/Minus

```
Query  141  TGCCTGCGGCGAAGGATAGAAG-AA-TACCCCAACTCCCAGCCACAGCATCCACAATCCA  198
Sbjct  524  ............GC..G.....A..GC..G..C-.A...-.....................  467

Query  199  CATAACGTGTGCAGCGAGCTcacacctacacacacacatacacCTGCCCATTCGCACACT  258
Sbjct  466  ................T.....................................  407

Query  259  GGGCCACGGTCCGTGGACTGCGAAGACTGCAACCGCATCACCACCCGCCAACCTACTGGC  318
Sbjct  406  ............................................................  347

Query  319  ACAACTGCCGTACACGCGCATCAACGCCACAGCACAGCGCAACGGCTGACCGCACTGCGG  378
Sbjct  346  ............................................................  287

Query  379  CCACTGCAGCAGCACAACCGCAAACTCCACGCCACCGAATTCGACAGCACCAGCACACCT  438
Sbjct  286  ............................................................  227

Query  439  AGTGAGAAGCCACGCCAGTCCGCACCGTGAGCCAGCCAACCAGCACACGCTCCACCCACC  498
Sbjct  226  ............................................................  167

Query  499  GCGCGCCACGGCACGGGACGGCACCGCACTCTCCCCAGCCACCGCTCATTGCACACAGTG  558
Sbjct  166  ............................................................  107

Query  559  ATGGTTCATAAGCCGACGCTCGGACAGACGTGGCCACAGGCAAGCCCATGGCCGCAATAT  618
Sbjct  106  ............................................................  47

Query  619  GCGTTCAAGATGTCGACGTTCAAAGCAGTCTGCGATTCACATTAAT  664
Sbjct  46   ..........................................  1
```

Appendix (7)

Echinococcus granulosus isolate VT-ASg 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

Sequence ID: KX434757.1 Length: 619 Number of Matches: 1

Range 1: 1 to 574 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
854 bits(462)	0.0	541/578(94%)	9/578(1%)	Plus/Minus

```
Query  101  CACCGTTCCACCCATCGCATTACACGTGCCACG-ACCAACAACGATGCATGTGCCTGCTG  159
Sbjct  574  ....C.C.....................A...C.G.-...........A........G.  516

Query  160  GGAGC-TCTGAAGAAAACACGCACCACACGCAACAGCATCCACCACCAACATAACGTGCC  218
Sbjct  515  C....GAGA.......G.....C....C...C...........A.T.C..........--  458

Query  219  TGCA-C--GCTCACATCTACACACACTCATACACCTGCCCGTTCTCACACTGGGCAACGC  275
Sbjct  457  ....G.GT.......C..........A.............A...G..........C...G  398

Query  276  TCCGTGGACTGCGAAGACTGCAACCGCATCAGCACCCGCCAACCTACTGGCACAACTGCC  335
Sbjct  397  ..............................C.............................  338

Query  336  GTACACGCGCATCAACGCCACAGCACAGGGCAACGGCTGACCGGACTGCGGCCACTGCAG  395
Sbjct  337  ............................C.............C.................  278

Query  396  CAGCACAACCGCAAACTCCGCGCCACCGAATTCGACAGCACCAGCACACCTAGTGAGAAG  455
Sbjct  277  ...................A......................................  218

Query  456  CCACGCCAGTCCGCACCGTGAGCCAGCCAACCAGCACACGCTCCACCCACCGCGCGCCAG  515
Sbjct  217  ............................................................C  158

Query  516  GGCACGGGACGGCACCGCACTCTCCCCAGCCACCGCTCATTGCACACAGTGATGGTTCAT  575
Sbjct  157  ............................................................  98

Query  576  AAGCCGACGCTCGGACAGACGTGGCCACAGGCAAGCCCATGGCCGCAATATGCGTTCAAG  635
Sbjct  97   ............................................................  38

Query  636  ATGTCGACGTTTCAAAGCAGTCTGCGATTCACATTAAT  673
Sbjct  37   ...........-..........................  1
```

Appendix (8)

Echinococcus granulosus isolate VT-ASg 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

Sequence ID: KX434757.1 Length: 619 Number of Matches: 1

Range 1: 1 to 619 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1144 bits(619)	0.0	619/619(100%)	0/619(0%)	Plus/Minus

```
Query  55   AACGAGTCAATCAAGGCAAGGCTAGGCGAGGCGGGGCGTGACGCGCACCCTCCCACCCAT  114
Sbjct  619  ............................................................  560

Query  115  CGCATTACACGTGCAACGCAGCACAACGATGCAAGTGCCTGCGGCGAGCGAGAGAAGAAA  174
Sbjct  559  ............................................................  500

Query  175  GCACGCCCCACCCGCCACAGCATCCACAATCCACATAACGTGTGCAGCGTGCTcacacct  234
Sbjct  499  ............................................................  440

Query  235  acacacacacatacacCTGCCCATTCGCACACTGGGCCACGGTCCGTGGACTGCGAAGAC  294
Sbjct  439  ............................................................  380

Query  295  TGCAACCGCATCACCACCCGCCAACCTACTGGCACAACTGCCGTACACGCGCATCAACGC  354
Sbjct  379  ............................................................  320

Query  355  CACAGCACAGCGCAACGGCTGACCGCACTGCGGCCACTGCAGCAGCACAACCGCAAACTC  414
Sbjct  319  ............................................................  260

Query  415  CACGCCACCGAATTCGACAGCACCAGCACACCTAGTGAGAAGCCACGCCAGTCCGCACCG  474
Sbjct  259  ............................................................  200

Query  475  TGAGCCAGCCAACCAGCACACGCTCCACCCACCGCGCGCCACGGCACGGGACGGCACCGC  534
Sbjct  199  ............................................................  140

Query  535  ACTCTCCCCAGCCACCGCTCATTGCACACAGTGATGGTTCATAAGCCGACGCTCGGACAG  594
Sbjct  139  ............................................................  80

Query  595  ACGTGGCCACAGGCAAGCCCATGGCCGCAATATGCGTTCAAGATGTCGACGTTCAAAGCA  654
Sbjct  79   ............................................................  20

Query  655  GTCTGCGATTCACATTAAT  673
Sbjct  19   ..................  1
```

الخلاصة:-

يمثل مرض الكيس العدارية عبئاً مالياً في العراق لأنه يقلل من إنتاجية الأغنام والماعز والأبقار والإبل من خلال ترك أعضائها المصابة غير صالحة للاستهلاك البشري، مما يتسبب في فقدان الوزن وسوء الحالة الصحية. وهو من أكثر الأمراض حيوانية المصدر انتشارًا بين البشر والحيوانات ويتطور في عدة أعضاء، وأهمها الكبد والرئة ككيس عداري في العديد من العوائل، بما في ذلك البشر. اذ يسبب العديد من المضاعفات التي قد تؤدي إلى الوفاة. ولا توجد الآن أدوية آمنة وفعالة لهذا الطفيل قيد الاستخدام، فالبحث جار لإيجاد مثل هذه العلاجات.

خلال الفترة من تشرين الثاني ٢٠٢١ إلى آيار٢٠٢٢، تم جمع ٢٤ عينة من الأكياس العدارية (١٤ عينة بشرية و١٠ عينات من الأغنام). جمعت عينات البشر من محافظة القادسية ومستشفى النجف العامة والخاصة وكذلك مستشفيات محافظات بابل وبغداد والقادسية وتم تخزينها في وسط استزراع كريب رنكر وسوائل الكيس (٤: ١) لحين الاستعمال. وللمقارنة، تم عمل عدة تركيزات من ألبيندازول وبعض المستخلصات النباتية المختارة. تلقت مجموعة السيطرة مليلتر واحد من الماء المقطر اما باقي المجاميع زودت بتراكيز المستخلص والدواء المذكور أعلاه في ثلاث مكررات. تم تحديد معدلات صلاحية الرؤيسات الأولية لمدة (٠، ٢٤، ٤٨، ٧٢، ٩٦، ١٢٠، ١٤٤، ١٦٨، ١٩٢، ٢١٦) ساعة وتم تقييم حيويتها باستخدام صبغة إلايوسين

في هذا الدراسة، تم إنتاج صبغات طبيعية من *Hibiscus sabdarriffa* L.، *Brassica oleracea L. var. capitata*، *Beta vulgaris* L. and *Crocus sativus* L.، وتم الحصول على أفضل النتائج من الصبغة الطبيعية المستخرجة من المستخلص النباتي *Crocus sativus* عند استخدامها لتحديد حيوية الأوالي، اذ أظهرت أن الأوالي الحية كان لها لون أبيض شفاف وتميزت الميتة باللون الأصفر، في حين كانت مصبوغة باللون الأخضر للاوالي الحية والاحمر للميتة عند مقارنتها بصبغة الايوسين القياسية.

اختبرت فاعلية المستخلصات النباتية المحددة أعلاه بتركيز (١٠٠، ٢٠٠، ٣٠٠ مجم / مل) على الرؤيسات الأولية، ومقارنة فعالية المستخلصات النباتية مع علاجات ألبيندازول بتركيز (١٠٠، ٢٠٠، ٣٠٠) مجم / مل. وتبين ان مستخلص الكجرات له تأثير أفضل من المستخلصات النباتية الأخرى قيد الدراسة وكذلك البيندازول لذلك كان سببا لاختياره لتخليق جزيئات الفضة النانوية منه لاختبار التأثير البيولوجي ضد الطفيلي.

خُلقت جزيئات الفضة النانوية من نبات *H.sabdarriffa*، وتم تقييم فعاليتها ضد الرؤيسات الأولية لـ *E.granulosus* جنبًا إلى جنب مع العلاج بالألبيندازول في المختبر. واختبر تأثير تراكيز مختلفة من جسيمات النانوية من مستخلص نبات *H.sabdarriffa* (٠.٠٢٥ ، ٠.٠٥، ٠.١، ٠.٢، ٠.٤، ٠.٨ ملغم / مل) وعلاقتها بموت الرؤيسات الأولية *E.granulosus* في فترات زمنية مختلفة في المختبر لـ (٠ ، ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ، ١٢٠ ، ١٤٤ ، ١٦٨ ، ١٩٢ ، ٢١٦) ساعة. تم فقدان الرؤيسات الأولية ، حيث كانت نسبة الصلاحية في التركيز ٠.٨ مجم / مل بعد ٢٤ ساعة من العلاج ٠٪، مقارنة مع ٨٥.٥٤٪ في معاملة السيطرة.

من خلال النتائج الدراسة الحالية لوحظ أن تأثير مستخلص جسيمات *H.sabdarriffa* النانوية أفضل من علاج البيندازول لأن جسيمات *H.sabdarriffa* المتناهية في الصغر كان لها القابلية بقتل الأوالي بعد ٤٨ ساعة بتركيز ٠.٨ ميكروغرام / مل بينما وصل ألبيندازول صفر من الصلاحية بعد ١٢٠ ساسعة وبتركيز ٣٠٠ مجم / مل. لذا كشفت نتائج مستخلص الماء المغلي من جسيمات *H.sabdarriffa* النانوية فعاليتها ضد حيوية الاوالي ويمكن استخدامها كبديل للعلاج الكيميائي في علاج عدوى الأكياس العدارية.

للدراسة الجزيئية، تم اختيار ١٨ عينة من الأكياس العدارية (٩ عينات بشرية و٩ عينات من الأغنام) للفحص الجزيئي لتحديد أكثر السلالات المشوكة الحبيبية الشائعة في مقاطعات الفرات الوسطى، وكذلك لدراسة التسلسل الجيني للسلالات، ارسم شجرة النشوء والتطور وقارنها بالسلالات المسجلة في-NCBI BLASTلتحديد السلالات الأكثر شيوعًا للإنسان والأغنام في محافظات الفرات الوسطى. تم استخدام الكبد والرئتين لجمع عينات كيس عداري لهذه الدراسة.

عُزل الحمض النووي من الطبقة جرثومية، وتم تضخيم الناتج باستخدام تقنية PCR المتسلسل في الجين 18S rRNA ضمن منطقة Internal Transcribed Spacer (ITS1وITS2) ، الذي له وزن جزيئي (٧٥٠ و١٠٠-١١٠٠) أزواج قاعدية. تم مطابقة العينات المدروسة مع العينات المثبتة في بنك الجينات. وجد أن هناك عينات مطابقة لعينات بنك الجينات بنسب مختلفة. الأكياس العدارية البشرية التي اختارت لرسم الشجرة التطورية، حيث كانت النسبة المئوية للمطابقة ٩٩٪ مع عينة تحمل المعرف KX434757.1 : تحت مسمى *E.granulosus* من الهند والتي تم التعرف عليها من خلال المحاذاة المرسومة لمقارنتها .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل / كلية العلوم للبنات
قسم علوم الحياة

تصنيع الفضة النانوية باستخدام بعض المستخلصات النباتية وتأثيرها على حيوية طفيلي الاكياس المائية(المشوكة الحبيبية) *Echinococcus granulosus*

رسالة مقدمة الى

مجلس كلية العلوم للبنات / جامعة بابل، وهي جزء من متطلبات
نيل درجة ماجستير في العلوم / علوم الحياة
من قبل

رضاب محمود شاكر

بكالوريوس علوم الحياة، كلية العلوم، جامعة بابل، (٢٠٠٧)

بإشراف

الأستاذ الدكتور
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الأستاذ الدكتور
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