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Detection Cysticercus tenuicollis Isolated from Sheep and Goats with / Without Exposure to Some Plant Extracts by GC-MS and Scanning Electronic Microscope in Basrah Province, Iraq

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Abstract. For the first time, parasites were entirely analyzed in terms of their chemical composition using gas chromatography-mass spectrometry methods. This study revealed a distinct chemical molecule identified in *Cysticercus tenuicollis* and scolex fluids, which were recovered from sheep and goats, respectively. These chemical compounds comprise amino acids, organic compounds, and other compounds. Also, chemical compounds differ when exposed to two plant extracts, *Syzygium aromaticum* (Clove) and *Capparis spinosa*. While scanning electron microscope study showed.

Keywords: GC-MS, analysis of chemical compounds, Scanning Electron Microscopy, Cysticercus, *Taenia Hydatigenia*.

Introduction

Taeniidae is a family of helminths parasites of canids containing many *Echinococcus species* and *Taenia* (Dalimi *et al.*, 2006). Taenia species cause economic losses in animals and human health problems (Lavikainen et al., 2008). Thus, *Taenia hydatigenia* may infect a wide range of ruminants through its larval stage *Cysticercus tenuicollis*, which was found in ruminants, and the adult worm, which was found in dogs and other canids as the final hosts (Murat, 2005). Time, place, and month affected stray dog *T. hydatigenia* infection rates. Al-Emarah (1999) and Al-Azizz (2005) found 54.2% and 7.62% *T. hydatigenia* infections in Basrah stray dogs, respectively. Taeniasis is diagnosed by parasite morphology and molecular characteristics (Gasser *et al.*, 1999 and González *et al.*, 2006). On the other hand, plant extracts have considerable therapeutic benefits with few side effects for treating numerous infectious illnesses (Beshbishy *et al.*,2019), making medicinal plants an appealing source of novel therapeutic molecules (Batiha *et al.*, 2018).

Gas chromatography-mass spectrometry (GC-MS) is a method of identifying various chemicals in a test sample that combines the advantages of gas chromatography and mass spectrometry (Sparkman et al., 2011). Drug detection, fire investigation, environmental analysis, explosives investigation, and the identification of previously unidentified materials (such as samples taken from Mars on probe missions as early as the 1970s) are just some of the many uses for GC-MS. Also applicable to airport security for detecting drugs in bags or on individuals. Like liquid chromatography-mass spectrometry, it can identify even small chemical levels and show trace components in materials assumed to have decomposed (Jones, 2019). The "gold standard" in forensic drug identification, it can provide a test that is 100% specific for the presence of a certain compound. High temperatures of 300 C^0 employed in the GC-MS injection port (and oven) might promote thermal destruction of injected molecules, leading to false identification, even when a non-specific test can statistically predict the drug's identity.

Chromatography may separate and analyze mixtures containing various constituents, such as hydrocarbons and essential oils. It may also be used to develop biochemical pathways for the parasite life cycle, helminth infection, and host-parasite interaction to find places where parasites or diseases might be avoided or treated (Balamurugan *et al.*, 2013). Mass spectrometry is the most widely used and adaptable method for determining the identity and concentration of chemical compounds (Kadhim *et al.*, 2016). There are few references to GC-MS in the scientific literature; these references pertain to plants or chemical

compounds. In contrast, gas chromatography-mass spectrometry is a technique that performs a comprehensive analysis of a sample by separately measuring each of its constituents (Kell et al., 2005). By designing biochemical pathways such as GC-MS, we hope to shed light on the processes of helminths infection and host-parasite interaction, as well as provide targets for further parasite control or infection treatment (Estela *et al*., 2017).

Before World War II, scientists in several countries, especially Germany, worked on making the electron microscope. The dramatic increase in resolution compared to light microscopy promised to change many areas of cell biology, virology, bacteriology, mycology, and protozoan parasitology. At the same time, new methods for preparing and staining slides were developed and improved, giving scientists a better understanding of many aspects of the biology of microorganisms (Agar, 1996). Scanning electron microscopy is used to determine the surface composition of organs and tissues in the biomedical sciences (Broers, 1975 and Goldstein *et al.,* 2003). This approach has assessed cell, tissue, and organ surface topology. In material science, this method has been used to analyze all forms of natural and artificial surfaces created by fracture and abrasion (Wells and Joy, 2006).

The current investigation aims to detect the content and structure of larval stage *C. tenuicollis* by GC- MS and scanning electron microscope as a comparison method when this parasite with or without treated with two plant extracts *Syzygium aromaticum* (Clove) and *Capparis spinosa*.

Materials and Methods

Sample collection

The slaughtered animal samples, sheep and goat, were collected from slaughterhouses and butchers. After removing the cyst from the infected organ then separated, collected the fluid and scolex at the Veterinary Parasitology Laboratory in the College of Veterinary Medicine at Basrah University.

Preparation Plants Extract:

Capparis spinosa and *Syzygium aromaticum* are two plant species gathered from the Basrah Province's Natural Plant Nursery. These plants were cleaned, dried, and then ground by a mortal. Next, 100 grams of the plant were placed in a beaker with 200 ml of distilled water and stirred with a magnetic stirrer for 30 minutes. After that, the precipitator was separated from the plant using a centrifuge at 3000 rpm for 15 minutes. Finally, the plant was distributed on glass plates and put inside an oven set to 60°C to dry. Finally, the weight of the dried plant was determined (Hamza, 2005). In order to create 200g of ground samples for the extraction soxhlet technique, the dried clove bud samples were roughly crushed using a grinder (Guntero *et al.*, 2017).

GC-MS study

In the current experiment, The Basrah Oil Company used gas chromatography-mass spectrometry (GC-MS) analysis to identify the chemical components contained in the parasite sample (Nahran-Omer). The GC-MS technique combines the chemical characteristics of a test sample (Kell *et al*., 2005).

Procedure:

After washing, the cyst from the diseased organ was frozen for one day before being ground in a mortar until it became liquid, at which point it was promptly sent to the Basrah Oil Company. In this process, a gas chromatograph connected to a mass spectrometer and a Shimadzu QP 2010 GC system is employed. It was outfitted with a fused silica capillary column (DB5MS) (length 30m, diameter 0.32 mm, film thickness 0.25 µm, 95% methyl poly siloxane, and 5% phenyl) Helium gas. In split mode, 1 l of sample was injected into the capillary column while keeping the temperatures of both the injector and the detector at 40 °C. The temperature of the column was set to 40 °C for 5 minutes, then it was raised at a rate of 28 °C per minute until it reached 280 °C (Al-Ataby, 2022).

Scanning Electronic Microscope

Procedure:

Cysts were collected from randomly selected abdominal cavities, washed three times in phosphate buffer saline, and placed in vials as controls, and some vials were placed on plant extracts. Following this, samples were immediately transferred to Tehran University for scanning electron microscopy and fixed for 24 hours in 3% (V/V) glutaraldehyde before being rinsed in 0.1 M PBS. After that, the samples were centrifuged twice for 5 minutes at 1500 rpm. After removing the supernatant, the pellets were suspended in Osmium tetraoxide at a concentration of 1% (V/V) at room temperature (OsO4). After being washed in ethanol and acetone, drying for an hour in a Polaron E 3000 at the critical point, and then coated with gold, the samples were dehydrated (Galindo *et al.,* 2008).

Result

In this work, *Capparis spinosa* and *Syzygium aromaticum*, as two different types of plant extracts, were used. The results showed that the percentage of Phenol with *Capparis spinosa* and *Syzygium aromaticum* is equal. In contrast, the alcohol showed a high in *Syzygium aromaticum*as compared to *Capparis spinosa*. However, *Capparis spinosa* has a high percentage of Ketone. Also, *Syzygium aromaticum* contains a high percentage of organic acid, cyclic compounds, ester, and unsaturated fatty acids compared with *Capparis spinosa.* On the other hand eugenol, aldehyde, steroids, and saturated organic compound present in *Syzygium aromaticum* only and did not found in *Capparis spinosa* (Table 1)

Table 1. Percentage of chemical compounds in Capparis spinosa and Syzygium aromaticum

Chemical compounds	*C. spinosa*	*S. aromaticum*
Phenol	11.244	11.244
Alcohol	5.875	20.505
Eugenol	0	30.505
Organic acid	44.357	72.188
Ketone	7390.23	235.344
Cyclic compound	32.622	99.849
Salt	0	0
Ester	76.675	152.23
Amine	31.25	15.321
Sulfone	0	0
Unsaturated compound	35.927	211.848
Aldehyde	0	40.722
Steroids	0	70.026
Saturated organic compound	0	63.629

Table (2) compares chemical compounds found in liquid and scolex *cysticercus tenuicollis* showing Phenol, Alcohol, Cyclic compound, Salt, Amine, Sulfone, and Lidocaine found in liquid of *Cysticercus tenuicollis*. In contrast, Eugenol, Ketone, and Cholesterol are found in scolex of *Cysticercus tenuicollis.* Also, there is a high percentage of organic acid, Ester, and saturated fatty acid in scolex compared with liquid, while Aldehyde has a high percentage in liquid compared to scolex.

.Table 2. Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis*

Chemical compounds	Liquid	Scolex
Phenol	40.323	0
Alcohol	33.625	0
Eugenol	0	164.1
Organic acid	99.363	638.301
Ketone	0	276
Cyclic compound	42.386	0
Salt	18.182	0
Ester	83.604	482.2
Amine	5.767	0
Sulfone	24.396	0
Aldehyde	82.99	6.136
Saturated fatty acid	106.71	692.2
Lidocaine	23.307	0
Cholesterol	0	546.2

Liquid and scolex of *Cysticercus tenuicollis* treated with *Capparis spinosa and Syzygium aromaticum* respectively, found that Phenol had a high percentage in Liquid treatment with *C. spinosa* as compared with Scolex treatment with *C. spinosa*. In contrast, Phenol has a higher percentage in Scolex treatment with *S. aromaticum*as than Liquid treatment with *S. aromaticum.* In addition, Alcohol, Cyclic compound, and Ester register high percentages in Scolex treatment with *C. spinosa*as compared with other treatments. At the same time, Amine has a high percentage in liquid treatment with *C. spinosa*as compared with other treatments.

The percentage of Eugenol was low in Scolex treatment with *C. spinosa* and high in liquid treatment with *S. aromaticum* also, organic acid had a low percentage and was high in Liquid treatment with *C. spinosa.*

Ketone registers similar ratios between liquid, scolex, which treatment with *C. spinosa*, in treatment with *S. aromaticum* ketone found in liquid only. On the other hand, salt, a saturated organic compound found in liquid and scolex treatment with *C. spinosa*, Salt high in liquid, while saturated organic compound is high in scolex.

Furthermore, Sulfone, Glycerin was found just in liquid treatment with *C. spinosa* and *S. aromaticum*, sulfone was high in liquid treatment with *C. spinosa*, while Glycerin was high in liquid treatment with *S. aromaticum.* Dyes were only found in liquid treatment with *C. spinosa*. Vinylfuran was only found in Scolex treatment with *C. spinosa* and *S. aromaticum.* Vinylfuran was high in Scolex treatment with *C. spinosa*, while unsaturated compounds were only found in Scolex treatment with *C. spinosa*. In liquid treatment with *C. spinosa*, there is a high percentage of aldehyde, but not in Scolex treatment with *S. aromaticum*. On the other hand, there is a high percentage of saturated fatty acid in Scolex treatment with *S. aromaticum.* Finally, Cholesterol a high percentage liquid treatment with *S. aromaticum* and not found in Scolex treatment with *S. aromaticum*

Table 3. Percentage of chemical compounds in liquid and scolex of cysticercus tenuicollis treated with Capparis spinosa and Syzygium aromaticum

Chemical compounds	Liquid treatment with *C. spinosa*	Scolex treatment with *C. spinosa*	Liquid treatment with *S. aromaticum*	Scolex treatment with *S. aromaticum*
Phenol	39.029	8.635	6.851	80.164
Alcohol	59.884	70.134	7.323	9.728
Eugenol	9.8	9.68	21.762	9.722
Organic acid	397.241	335.076	24.878	177.392
Ketone	63.768	69.871	7.824	0
Cyclic compound	27.59	66.032	7.626	10.061
Salt	25.803	13.92	0	0
Ester	331.605	411.842	33.712	171.451
Amine	53.86	37.363	6.386	0
Sulfone	6.596	0	6.358	0
Unsaturated compound	0	8.156	0	0
Aldehyde	53.718	14.281	6.978	0
Steroids	0	0	0	0
Saturated organic compound	11.984	14.326	0	0
Saturated fatty acid	14.522	0	9.7	235.225
Glycerin	6.907	0	17.214	0
Dyes	6.711	0	0	0
Vinylfuran	0	6.346	0	6.247
Cholesterol	39.933	39.955	58.005	0

Scanning electronic microscope

It is especially effective for detecting cystic membranes, which were used to characterize the surface and hooks. The current investigation includes samples from the liquid of Cysticercus and scolex isolated from slaughtered sheep. Some samples were normal (Figs. 9, 10, 11, 12, 13), but some were treated with extracts *Syzygium aromaticum* and *Capparis spinosa*. It can be shown different morphological characters and evident destruction in the membrane and hooks of scolex. Furthermore, the *Syzygium aromaticum* has higher effects on the cyst than *Capparis spinosa* (Figs. 14, 15, 16, 17, 18).

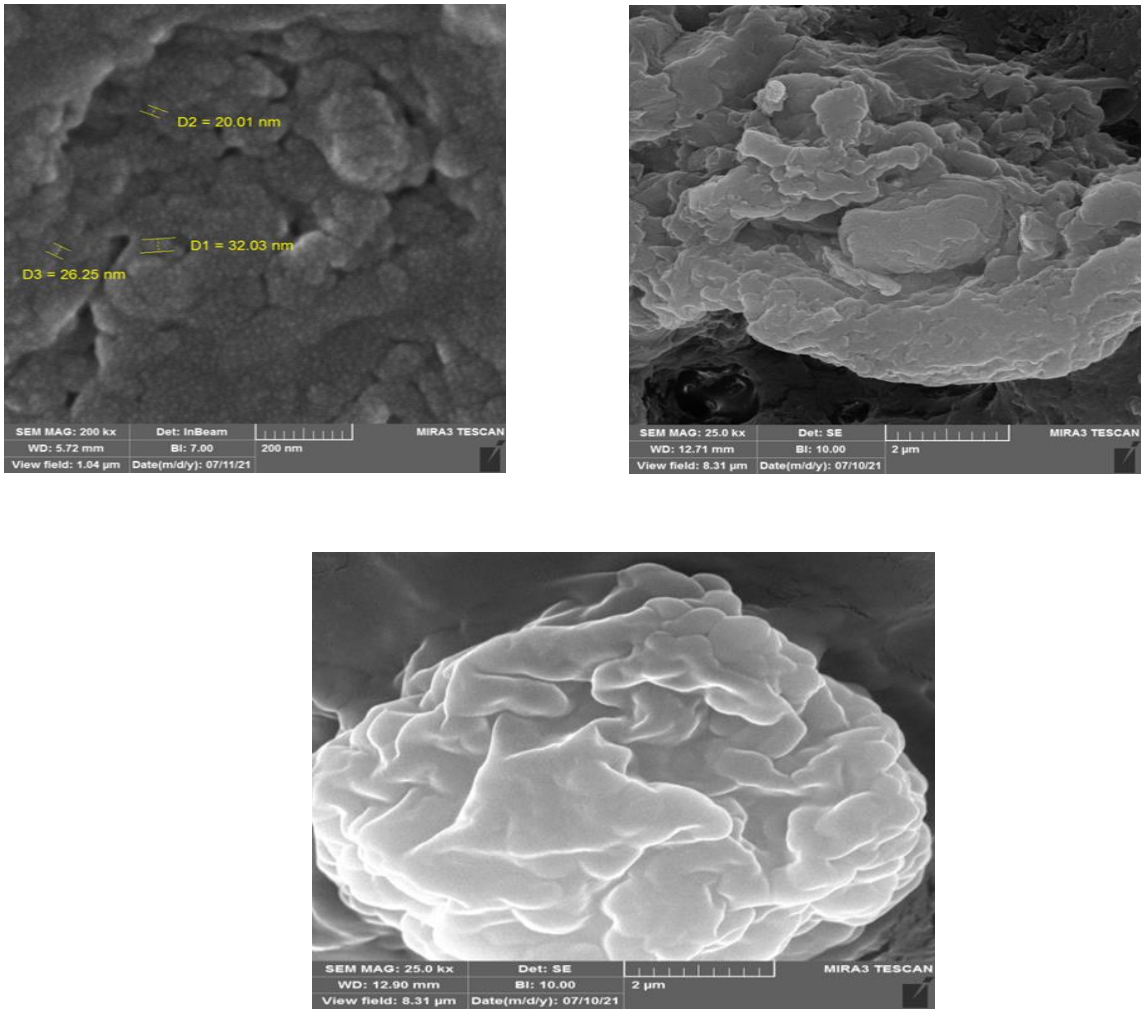

Figure (9, 10, 11). Scanning electron Microscope of Scolex of *C. tenuicollis* untreated

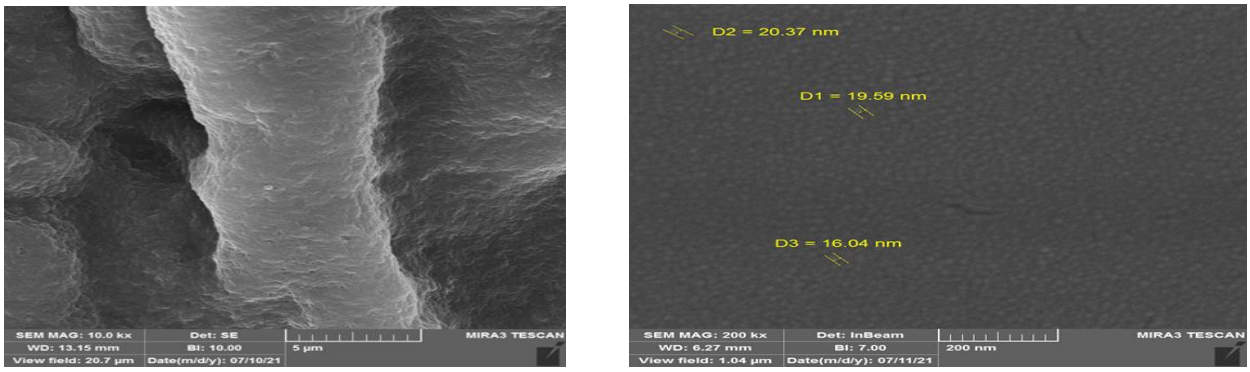

Figure (12, 13). Scanning electron Microscope of Cyst of C. tenuicollis untreated

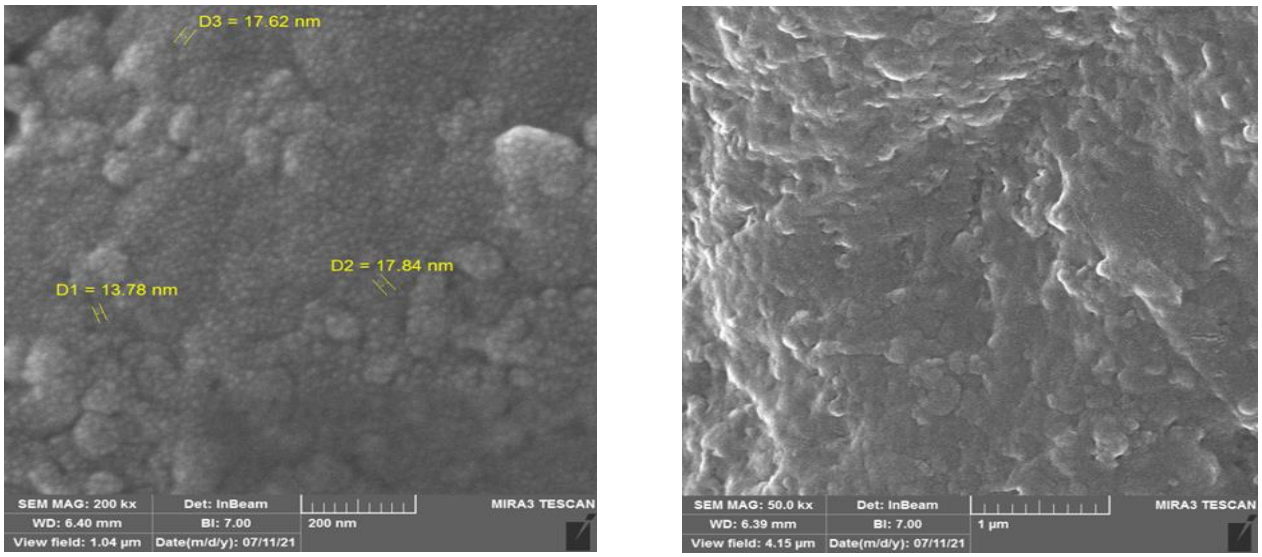

Figure (14, 15). Scanning electron Microscope of Cyst of C. tenuicollis treated with Syzygium aromaticum

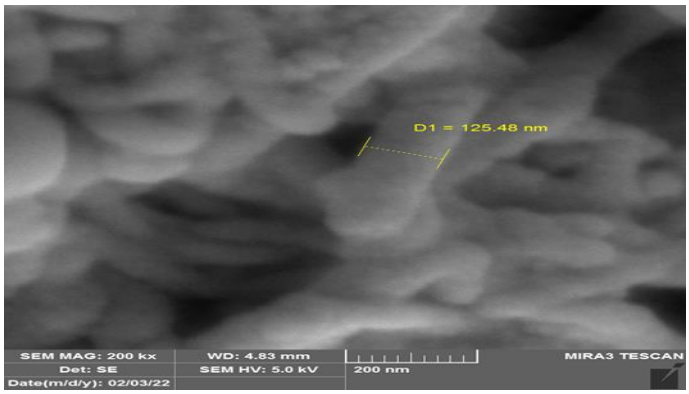

Figure 16. Scanning electron Microscope of Scolex of C. tenuicollis treated with Syzygium aromaticum

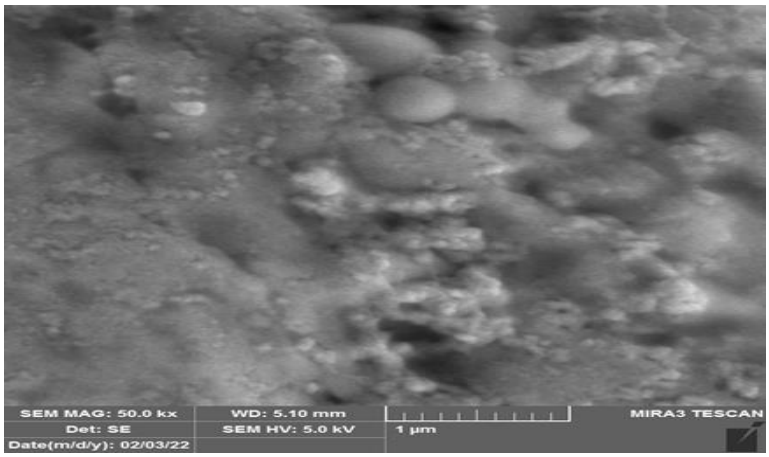

Figure 17. Scanning electron Microscope of Cyst of C. tenuicollis treated with C. spinosa

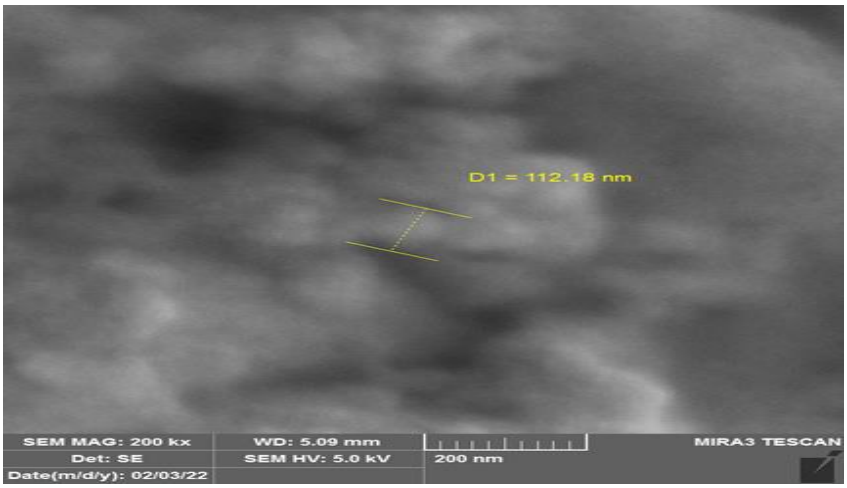

Figure 18. Scanning electron Microscope of Scolex of C. tenuicollis treated with C. spinosa

Discussion

The larvae stage of *T. hydatigenia*, a frequent parasite of pigs, cattle, buffalo, yak, sheep, goats, camel, horses, and humans, is called *C. tenuicollis* (Singh *et al*., 2015). This disease affects the under-a-year pig, with no effective treatments and a significant fatality rate. Since its first occurrence, *C. tenuicollis* has been documented in many nations worldwide (Sissay *et al*., 2008 and Ma *et al*., 2014).

The current study is considered the third in Iraq to use GC-MS to analyze parasites. Previous studies have used these techniques to characterize the chemical makeup of plants and other organic compounds and identify each compound's name, formula, and retention time. The current study uses GC-MS to analyze parasites. It is widely known that essential oils, hydrocarbons, and the three components above of GC-MS may all be separated and analyzed using chromatography (Balamurugan *et al.*, 2016).

There was no GC-MS investigation on parasites in Iraq, although three studies on *Echinococcus granulosus* (Al-Ataby *et al.*, 2020) and *Monezia expansa* were conducted in Basrah (Atyah, 2022).

In Iraq, there was no study about parasites by GC-MS, and in Basrah city a, three studies on *Echinococcus granulosus* (Al-Ataby *et al*., 2020) and *Monezia expansa* (Atyah, 2022). This study showed a chemical compound that different in both scolex and liquid of *C. tenuicollis* and the plant extracts *Syzygium aromaticum* and *C. spinosa*. The present investigation found cholesterol, oil, alkaline material, and amino acid in fluids, some of which benefit chemical compounds used in soap and cosmetics and others that might ignite and irritate the skin. The result by GC-MS about some plants such as *Syzygium aromaticum* and *Capparis spinosa* recorded compounds like Copaene, Caryophyllene oxide, Ursolic aldehyde, 4-Azido-3-bromobenzene, Methyl 3-amino-4, 5-dimethoxy benzoate and Caryophyllene oxide. Dynamic systems not constrained by the crystal lattice can also be studied using EM. These conformational changes, which occur over 10-1000 ms, may be studied using EM by flash freezing the sample at various points throughout a biological event (Chen and Frank, 2016). This might help track any conformational changes that happen when a big macromolecular complex form or when a ligand binds. Time-resolved EM has helped us understand the ribosome's catalytic cycle (Dashti *et al.*, 2014). Presently, research is being performed to enhance the mixing/spraying of the sample onto the grid before its plunge freezing (Feng *et al.*, 2017). Future drug development efforts will be aided by the capacity to capture biological systems at various time points, which provides critical insights into the mechanism of action of numerous complexes and the adaptability of inhibitor binding sites.

This study recorded samples from the liquid of Cysticercus and scolex isolated from slaughtered sheep. Some of the samples were normal, but some were treated with plant extracts *Syzygium aromaticum* and *Capparis spinosa*. The result showed different morphological characters and apparent destruction in the membrane and hooks of scolex. Furthermore, the *Syzygium aromaticum* showed a higher effect on the cyst than *Capparis spinosa*.

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