

Research Article

Detection *Cysticercus tenuicollis* Exposed to Novel Synthesized Compounds by GC-MS and SEM In Basrah Province, Iraq

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Abstract:

Background: The chemical makeup of plants or other organic molecules was comprehensively characterized using gas chromatography-mass spectrometry technologies (GC-MASS). However, this is the first time that a parasite has been included. **Aim of the study:** it attempts to detect the content and structure of larval stage *Cysticercus tenuicollis* by GC- MS and scanning electron microscope as a comparison method when this parasite was treated with novel Synthesized Compounds. **Materials and methods:** The slaughtered animal samples, which were sheep and goat, were collected from Basrah slaughterhouse and butchers and, after removing the cyst from the infected organ, then separated and collected the fluid and scolex. **Result and Discussion:** This study showed a chemical compound that different in both scolex and liquid of *Cysticercus tenuicollis* and novel synthesized compounds. The current study compared the scolex and the fluid of a *Cysticercus tenuicollis* after it was treated with 5 novel synthesized compounds prepared for the first time. The study showed a chemical compound that different in both scolex and liquid of *Cysticercus tenuicollis*, such as cholesterol, ethanol, sulfone, ester, Phenol, Sulfadiazine, and acids. While scanning electron microscope showed registered different morphological characters and a clear destruction in the membrane and hooks of the scolex. **Conclusion:** The study recorded a chemical compound that was different in both the scolex and liquid of *Cysticercus tenuicollis* and novel synthesized compounds and registered different morphological characters and a clear destruction in the membrane and hooks of the scolex.

Introduction

Cysticercosis is a frequent parasite disease in animals, particularly sheep and goats. It has been discovered in various districts of Iraq and harms the health of affected animals (1, 2, 3). It undermines health and the economy by destroying meat and healthy animals that people depend on. This means that livestock raising suffers a lot of material and financial losses (4, 5).

According to time, place, and monthly variation, stray dogs' *T. hydatigena* infection rates varied. For instance, the infection rates of the stray dog with *T. hydatigena* in Basrah city varied between 54.2% and 7.62%, respectively, according to (6, 7).

Condensation of substituted dibenzoyl diselenide and benzoyl selenonitrile with N-monosubstituted hydroxylamines yields several selenonitrone compounds (8, 9). In order to make benzoylselenonitrile, benzoyl chloride, and potassium selenonitrile are reacted (10, 11). At the same time, dibenzoyl diselenide is produced by the alkaline hydrolysis of substituted benzoylselenonitrile (12). As a result, chemotherapy is still utilized to treat many malignancies, and the search for novel chemotherapy drugs with fewer side effects is ongoing (13, 14). In 1817, elemental selenium was found, and Ethylselenol was the first organoselenium compound published in 1847 by (15).

GC-MASS has a long history of use in metabolic profiling of biological material (16) and is currently widely applied in biochemical (17), agricultural (18), environmental (19), and biomedical research (20) as well as a variety of industrial applications (21, 22). Data from GC-MASS is collected as a

Materials and Methods

Sample Collection

The samples of sheep and goats that had been killed were gathered from slaughterhouses and butchers. After extracting the cyst from

temporal series of mass spectral scans, with each scan consisting of a sequence of (m/z, intensity) pairs. By binning raw scans at equidistant m/z intervals, the raw data is typically processed into a two-dimensional matrix. Binned m/z intensities fill the resultant data intensity matrix, where the indices stand for scan numbers (or time points) and m/z values. Further processing can include noise smoothing, baseline correction, peak identification, alignment, normalisation, library matching, and optional display. By supplying samples with remarkable optical magnification, it has enabled scientists to better grasp microstructure; as a consequence, this technique has provided an accurate description of biological and non-biological materials at the nano scale (23). With its much higher resolution than light microscopy, electron microscopy was expected 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 (24). SEM is further used to examine various cell kinds. This approach clearly examines cell, tissue, and organ surface topology. SEM has been widely used in material science to analyse natural and artificial surfaces formed by fracture and abrasion (25).

This study aimed 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 was treated with novel Synthesized Compounds.

the diseased organ and separating it, the fluid and scolex were collected at the Veterinary Parasitology Laboratory of Basrah University's Faculty of Veterinary Medicine.

Chemicals Methods

The solvents utilized in this investigation were of the HPLC grade and were procured from commercial sources and used exactly as supplied (26). Thin-layer chromatography (TLC) was used to monetarize all reactions, which were conducted in dry settings. The

Gc-Mass Study

It is possible to combine the characteristics of compounds in a test sample using gas chromatography-mass spectrometry (28). The current study employed chemical

Scanning Electronic Microscope

Cyst was collected from randomly selected abdominal cavity cyst was washed three times in phosphate buffer saline and put in the vial as control. Some vials were put on plant extracts. After this, samples were immediately transferred to Tehran University for scanning electron microscopy and fixed in 3% (V/V) glutaraldehyde for 24 hours before being rinsed in 0.1 M PBS. The samples were

Results

In this study, 5 novel synthesized compounds were used. Liquid and scolex of *Cysticercus tenuicollis* treat with synthesized compounds. The result showed the different materials isolated by GC_ mass in scolex and liquid of the parasite *Cysticercus tenuicollis* treated with synthesized compounds. In table 1,

spots were then visible under UV light. According to(27), the interaction of elemental Se with KCN produced KSeCN. By utilising column chromatography and silica gel 60 A° with benzene/ethanol (9:1) as the elution solvent, the reaction products were purified.

compound analysis to discover what chemical compounds were present in the parasite sample. This was done at the GC-MS Lab Basrah Oil Company (Nahrn-Omer) according by (29).

centrifuged twice at 1500 rpm for 5 min. The pellets were suspended in 1% (V/V) Osmium tetroxide (OsO₄) that had been made at room temperature after the supernatant was removed. The samples were then dehydrated in ethanol and acetone, dried for an hour at the critical point in a Polaron E 3000 device, and lastly gold-coated (30).

Phenol, Sulfone, and Aldehyde are present in the liquid and not present in scolex. In addition, Organic acid, Cyclic compound, Ester, and Amine register high percentages in Scolex treatment with synthesized compound 1 compared with liquid treatment. In contrast, the percentage of Alcohol is high in liquid treatment with synthesized compound 1 compared with scolex treatment. (Table 1).

Table 1: Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis* treated with synthesized compound 1

| chemical compounds | Liquid treatment with compound 1 | Scolex treatment with compound 1 |
|--------------------|----------------------------------|----------------------------------|
| Phenol | 20.212 | 0 |
| Alcohol | 84.651 | 35.164 |
| organic acid | 226.795 | 285.636 |
| Cyclic compound | 23.752 | 47.735 |
| Ester | 83.604 | 141.937 |
| Amine | 12.632 | 18.889 |
| Sulfone | 10.011 | 0 |
| Aldehyde | 44.547 | 0 |

The percentage materials that were identified by GC_ mass in the scolex and fluids of the parasite *Cysticercus tenuicollis* treated with synthesized compound 2 recorded in table 2, Phenol, Ketone, Sulfone, Aldehyde, and Glycerin that found just in liquid treatment with synthesized compound 2 as compared with Scolex treatment. In contrast, Saturated fatty acid and Cholesterol were found just in scolex

treatment with synthesized compound 2 compared with liquid treatment. Also, this table shows a high percentage of Organic acid, a Cyclic compound, in Scolex treatment with synthesized compound 2 compared with liquid treatment during Alcohol a high percentage in liquid treatment with synthesized compound 2 compared with scolex treatment. (Table 2) .

Table 2: Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis* treated with synthesized compound 2

| chemical compounds | Liquid treatment with compound 2 | Scolex treatment with compound 2 |
|----------------------|----------------------------------|----------------------------------|
| Phenol | 37.564 | 0 |
| Alcohol | 20.501 | 5.105 |
| organic acid | 38.212 | 119.336 |
| Ketone | 44.485 | 0 |
| Cyclic compound | 19.622 | 48.075 |
| Sulfone | 6.356 | 0 |
| Aldehyde | 10.09 | 0 |
| Saturated fatty acid | 0 | 24.87 |
| Glycerin | 6.754 | 0 |
| Cholesterol | 0 | 23.364 |

Table (3) is shown the low percentage of Alcohol in Scolex treatment with synthesized compound 3 compared with liquid treatment. In contrast, Organic acid and Ester show a higher percentage in Scolex treatment with synthesized compound 3 than in liquid

treatment. On the other hand, Ketone and Amine were found only in liquid treatment with synthesized compound 3, while Cyclic compound and Cholesterol were found only in scolex treatment with synthesized compound 3 (Table 3) .

Table 3: Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis* treated with synthesized compound 3

| chemical compounds | Liquid treatment with compound 3 | Scolex treatment with compound 3 |
|--------------------|----------------------------------|----------------------------------|
| Alcohol | 25.891 | 5.142 |
| organic acid | 89.758 | 280.715 |
| Ketone | 20.421 | 0 |
| Cyclic compound | 0 | 19.287 |
| Ester | 62.299 | 204.636 |
| Amine | 38.242 | 0 |
| Cholesterol | 0 | 32.364 |

The materials that were separated by GC_ mass from the parasite *Cysticercus tenuicollis* in scolex and liquid treated with synthesized compound 4 are presented in table (4). Scolex treatment contains a high percentage of Alcohol, Ester, and Cholesterol, while liquid treatment with synthesized

compound 4 consists of a high percentage of Organic acid and Amine. Cyclic compound, Sulfone, Aldehyde found just in liquid treatment with synthesized compound 4 while Ketone was found in scolex treatment with synthesized compound 4 (Table 4).

Table 4: Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis* treated with synthesized compound 4

| chemical compounds | Liquid treatment with compound 4 | Scolex treatment with compound 4 |
|--------------------|----------------------------------|----------------------------------|
| Alcohol | 20.594 | 32.889 |
| organic acid | 414.366 | 357.292 |
| Ketone | 0 | 9.058 |
| Cyclic compound | 19.937 | 0 |
| Ester | 137.637 | 235.314 |
| Amine | 29.597 | 29.209 |
| Sulfone | 9.198 | 0 |
| Aldehyde | 11.234 | 0 |
| Cholesterol | 32.342 | 32.349 |

Table (5) shows Organic acid and Cyclic compounds with a high percentage in liquid treated with synthesized compound 5 compared with scolex treatment. At the same time, Alcohol has a high percentage in scolex treated with synthesized compound 5

compared with liquid treatment. Furthermore, Ester, Saturated fatty acid, and Cholesterol were found only in liquid treated with synthesized compound 5, while scolex treated was not available. (Table 5).

Table 5: Percentage of chemical compounds in liquid and scolex of *cysticercus tenuicollis* treated with synthesized compound 5

| chemical compounds | Liquid treatment with compound 5 | Scolex treatment with compound 5 |
|----------------------|----------------------------------|----------------------------------|
| Alcohol | 15.32 | 32.496 |
| organic acid | 137.445 | 97.151 |
| Cyclic compound | 19.383 | 19.604 |
| Ester | 44.468 | 0 |
| Saturated fatty acid | 24.871 | 0 |
| Cholesterol | 32.342 | 0 |

Scanning electronic microscope

It was primarily beneficial for detecting cystic membranes and was used to characterize the outer surface and hooks. The current investigation included samples of *Cysticercus* and *Scolex* fluids isolated from slaughtered

sheep and goats. The sample was treated with compounds 1,2,3,4, and 5. The result showed different morphological characters and a clear destruction in the membrane and hooks of scolex.

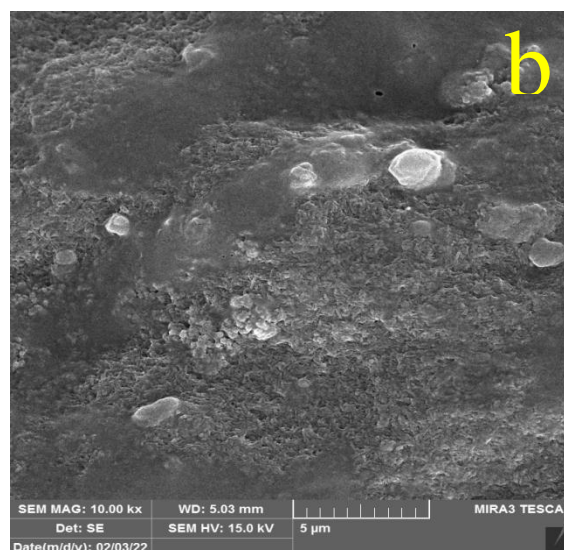
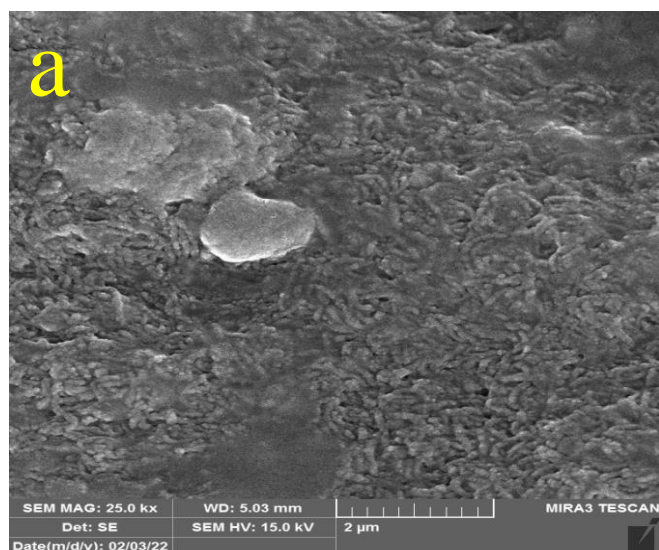


Fig.6 (a, b): Scanning electron Microscope of Scolex of *C. tenuicollis* treated with synthesized compound 1

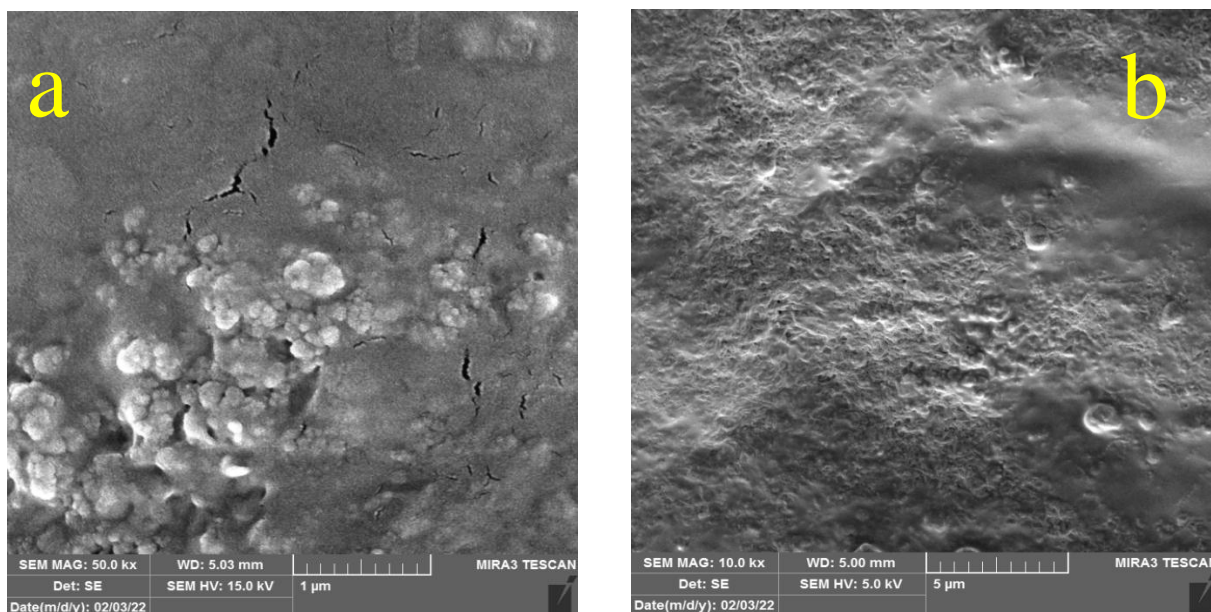


Fig.7 (a, b): Scanning electron Microscope of Membrane of *C. tenuicollis* treated with synthesized compound 1

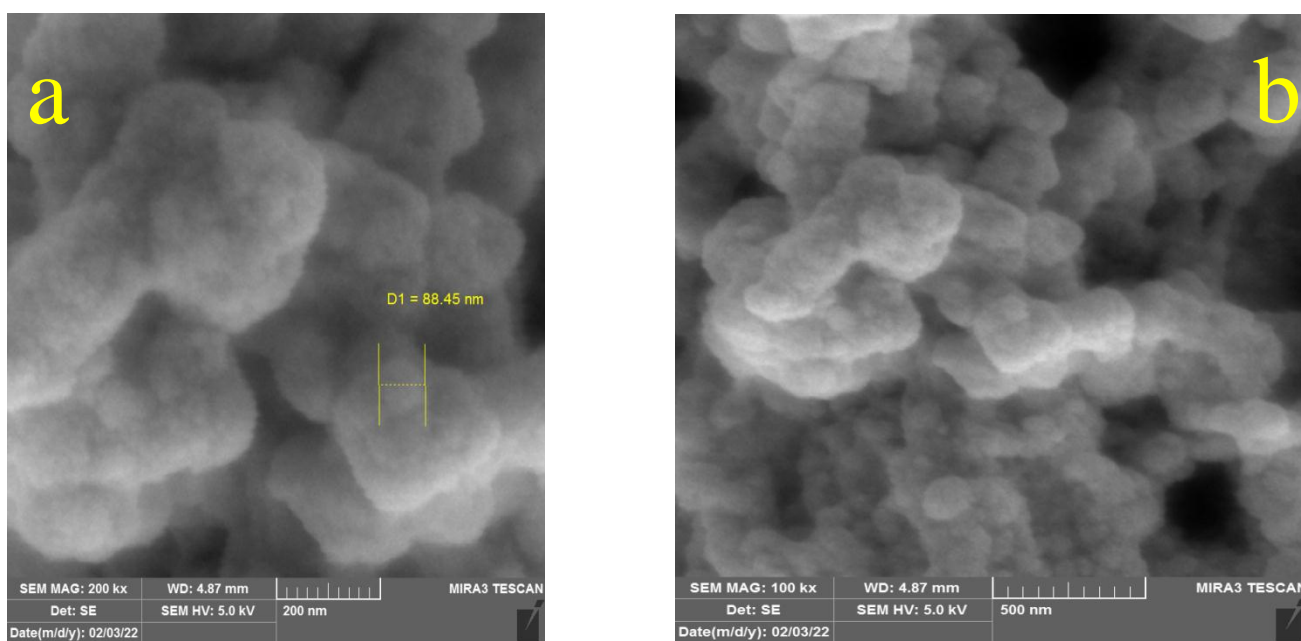


Fig.8 (a, b): Scanning electron Microscope of Scolex of *C. tenuicollis* treated with synthesized compound 2

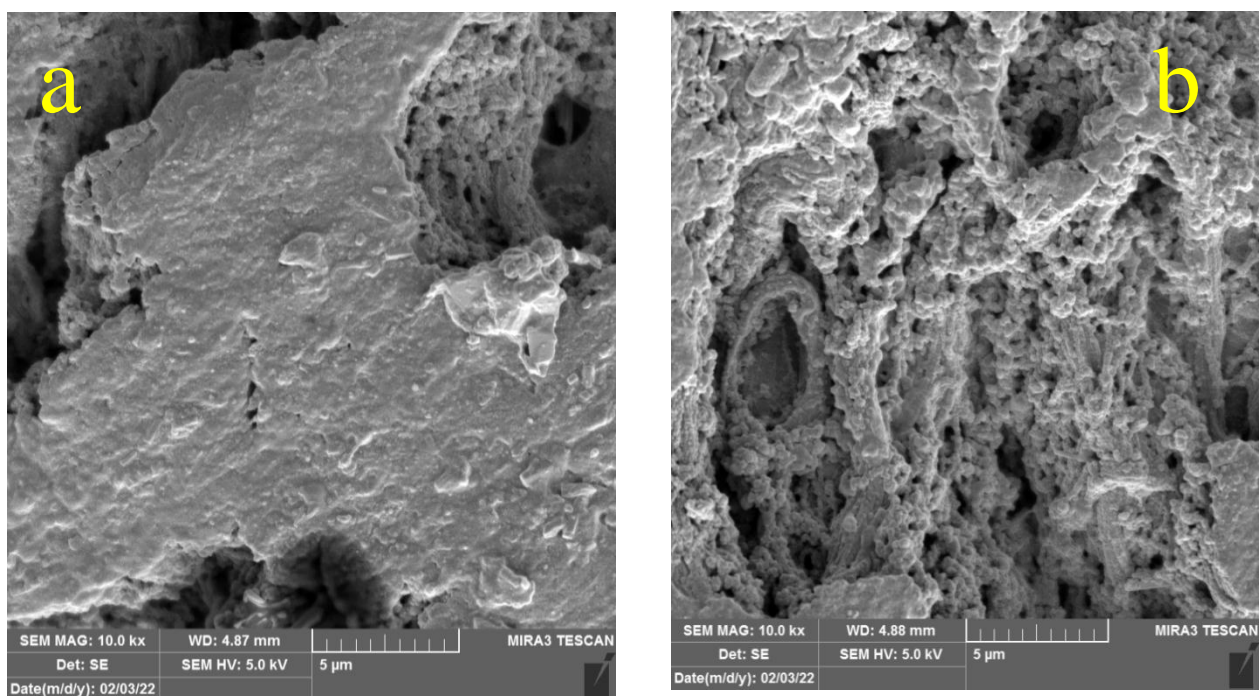


Fig.9 (a, b): Scanning electron Microscope of Membrane of *C. tenuicollis* treated with synthesized compound 2

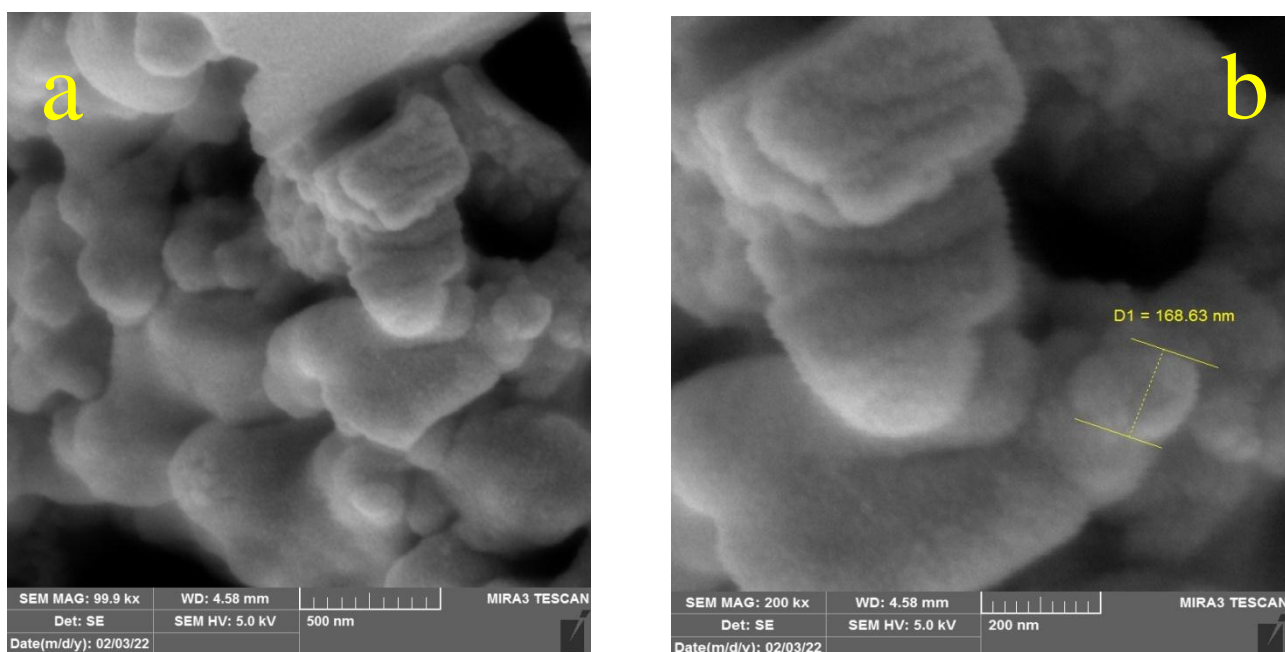


Fig.10 (a, b): Scanning electron Microscope of Scolex of *C. tenuicollis* treated with synthesized compound 3

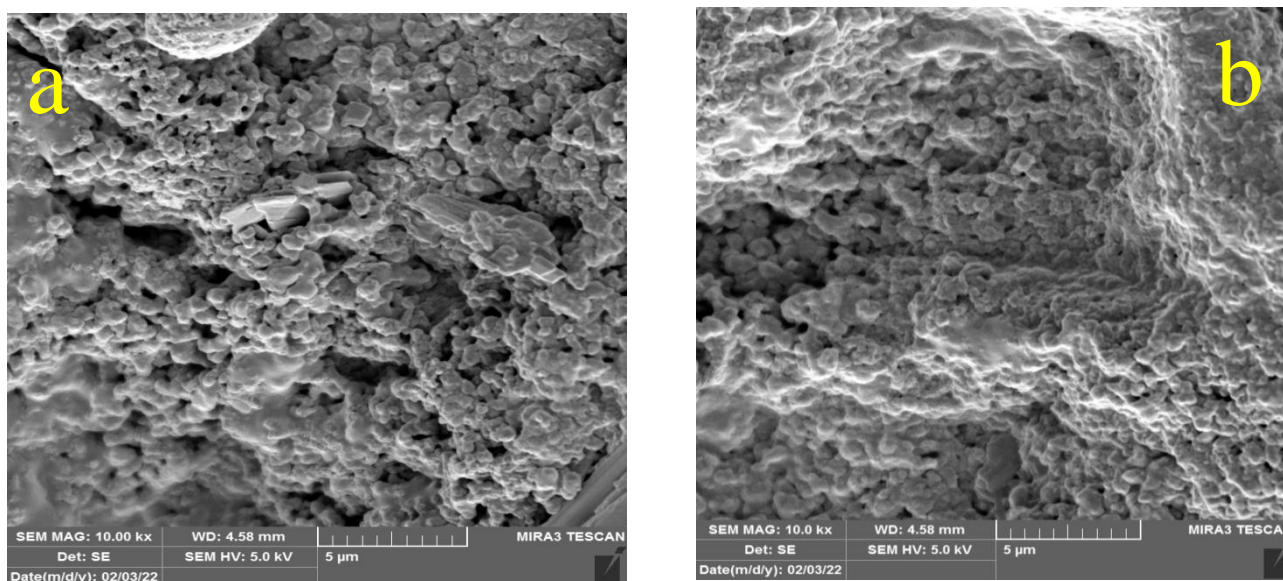


Fig.11(a, b): Scanning electron Microscope of Membrane of *C. tenuicollis* treated with synthesized compound 3

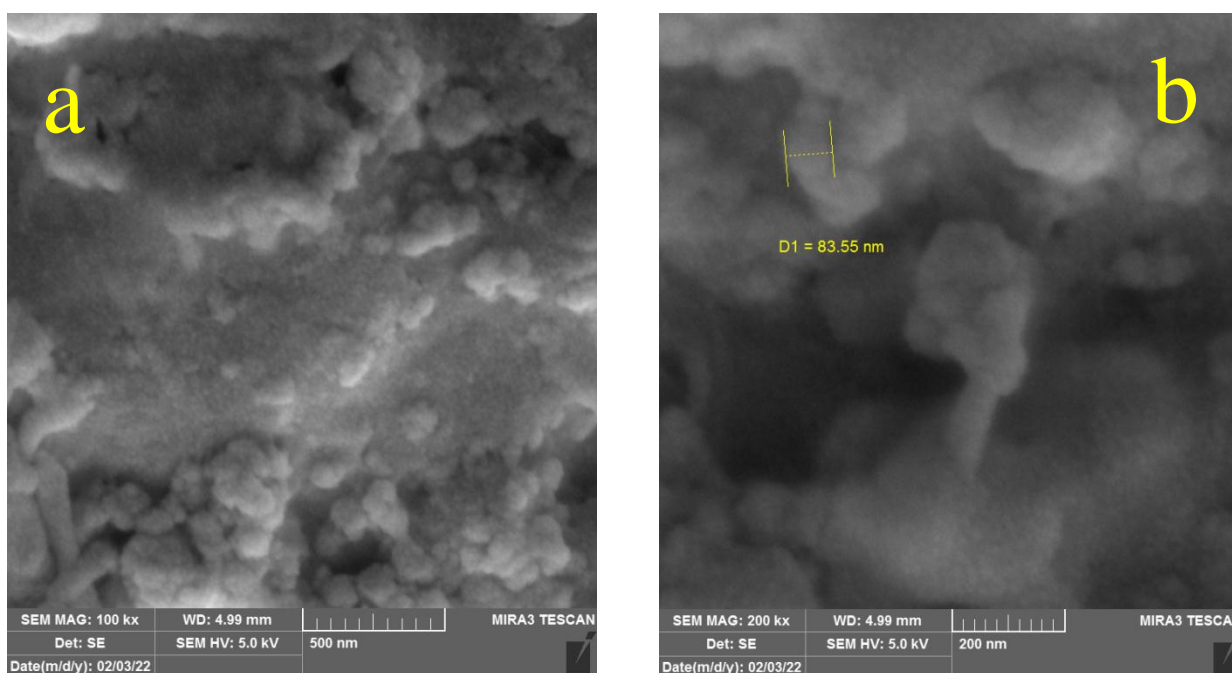


Fig.12 (a, b): Scanning electron Microscope of Scolex of *C. tenuicollis* treated with synthesized compound 4

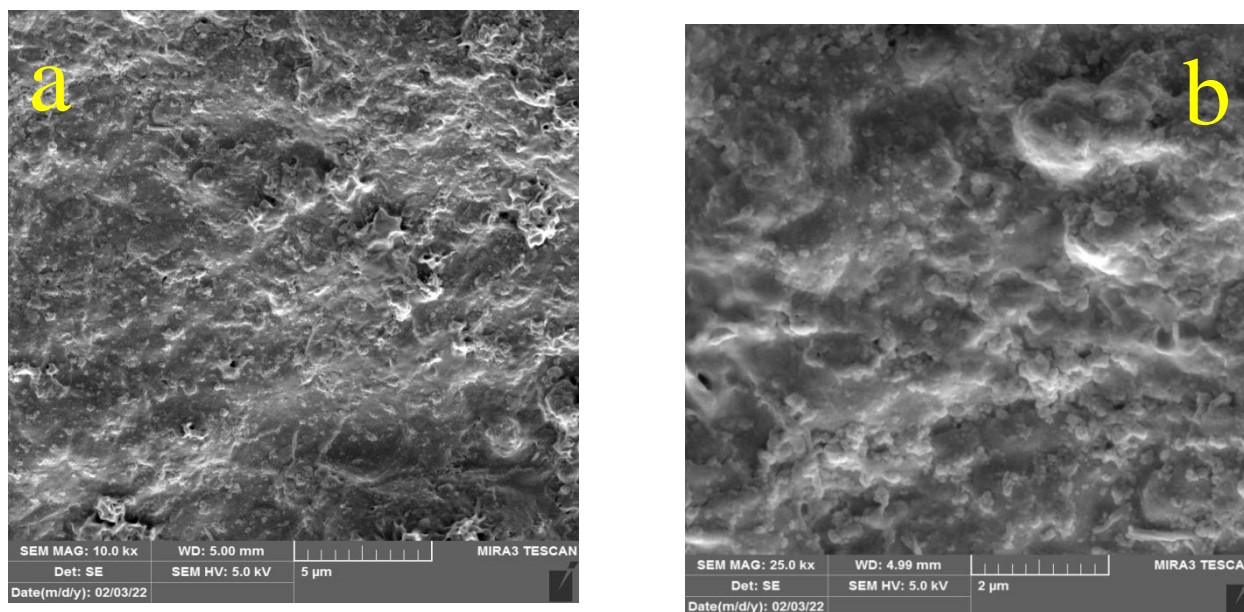


Fig.13 (a, b): Scanning electron Microscope of Membrane of *C. tenicollis* treated with synthesized compound 4

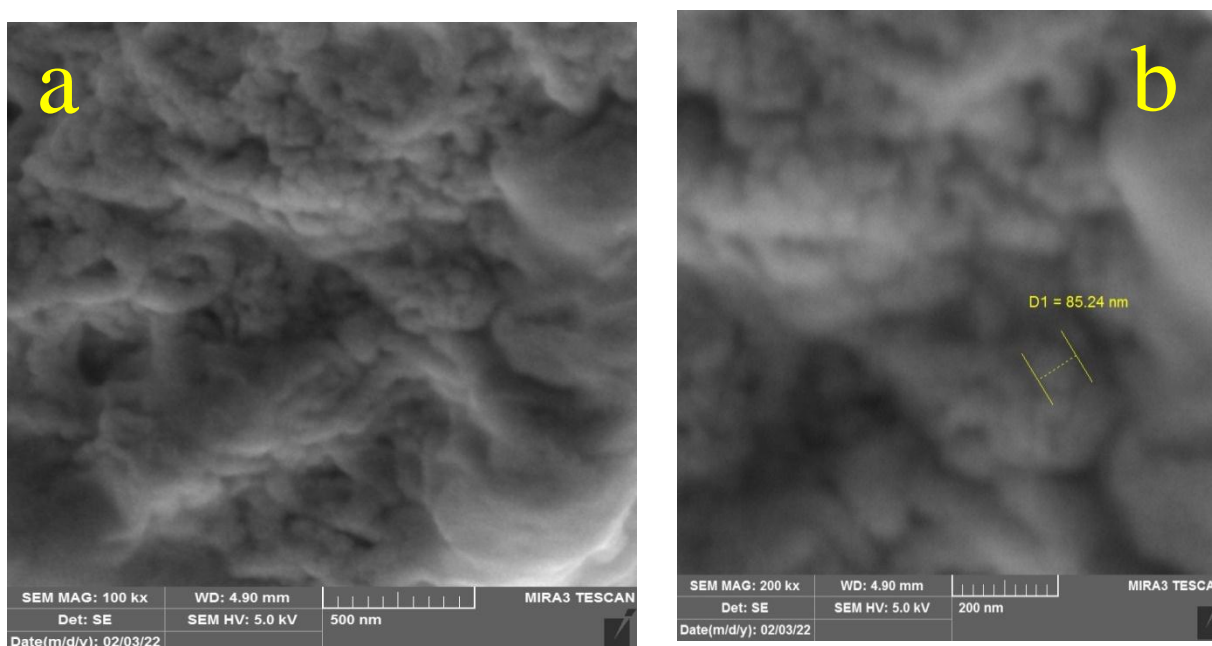


Fig.14(a, b): Scanning electron Microscope of Scolex of *C. tenuicollis* treated with synthesized compound 5

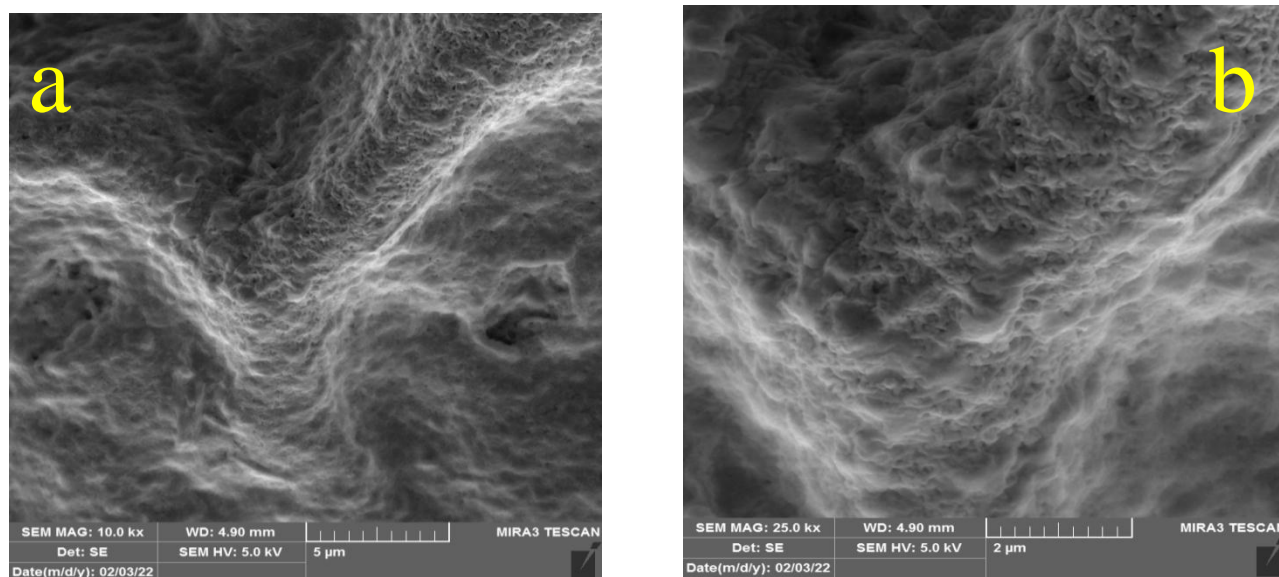


Fig.15 (a, b): Scanning electron Microscope of Membrane of *C. tenuicollis* treated with synthesized compound 5

Discussion

C. tenuicollis larvae cause a serious herbivore disease, which has a global spread, and hepatitis cysticercosis, a disease manifestation, appears in infected sheep and severe infections, larval migration destroy hepatic cells, causing severe inflammation that could be deadly (31). Because to the high costs associated with the widespread condemnation of offal that contains *C. tenuicollis* larvae, this parasite has gained more attention in recent years, especially in resource-poor countries (32).

Four of the selenourea compounds were made using a one-pot procedure: reacting $KSeCN$ with the properly substituted acid chloride, adding the correct ammine, and then adding dry THF. In dry THF, one thiourea chemical was synthesized in one pot from $KSCN$ and 4-Methylbenzoyl chloride. Compounds (1, 4, and 5) showed two bands, one strong between $(1527 \text{ and } 1535 \text{ cm}^{-1})$, and the other medium between $(1324 \text{ and } 1350 \text{ cm}^{-1})$, which were attributed to the asymmetrical and symmetrical stretching vibrations of the NO_2 group, respectively (33, 34). Compounds (1,2,4, and 5) have two medium bands, with the stretching contribution of $C=Se$ being responsible for the second band between $(678-725 \text{ cm}^{-1})$ and the first band between $(1111-1280 \text{ cm}^{-1})$ (34,

35). Compound (3) exhibits a noticeable band at (1103) that is caused by the $C=S$ bond's stretching vibration (35). These findings corroborate the synthesis of the examined chemicals and are in good agreement with previously published values.

The present study is thought to be the third one done in Iraq on parasites using Gas Chromatography-Mass Spectrometry. These methods were used to get a complete picture of the chemical makeup of plants or other organic compounds, and they showed the compound name, formula, and length of each compound's solution and what made them different. Chromatography has a wide range of uses and is mostly used for the separation and analysis of mixtures with several components (36). In Iraq there was no study about parasite by GC-Mass, and in Basrah city a three studies on *Echinococcus granulosus* (37) and *Monezia expansa* (38). This study showed a chemical compound that different in both scolex and liquid of *C. tenuicollis* and novel synthesized compounds. The current study compared the scolex and the fluid of a *C. tenuicollis* after it was treated with 5 novel synthesized compounds prepared for the first time. The study showed a chemical compound that is different in both scolex and liquid *C. tenuicollis*, such as

cholesterol, ethanol, sulfone, ester, Phenol, Sulfadiazine, and acids.

Synthesized compounds 1,2 register Phenol in liquid treatment with these synthesized compounds. Alcohol registered a high percentage in liquid treatment with synthesized compounds 1,2 and 3, while synthesized compounds 4 and 5 were a high percentage in scolex treatment with these synthesized compounds. On the contrary, Organic acid was found to have a high percentage in scolex treatment with synthesized compounds 1,2, and 3. In contrast, synthesized compounds 4 and 5 were a high percentage in liquid treatment with these synthesized compounds. Furthermore, the Cyclic compound registered a low percentage in liquid treatment with synthesized compound 4 while registering a high percentage in scolex treatment with synthesized compounds 1,2,3, and 5. In addition, Ester found a high percentage in scolex treatment with synthesized compounds 1,3, and 4. In contrast, a high percentage in liquid treatment with synthesized compound 5. Also, Amine presents a high percentage in scolex treatment with synthesized compound 1 while a high percentage in liquid treatment with synthesized compounds 3 and 4.

On the other hand Sulfone, Aldehyde is just found in liquid treatment with synthesized compounds 1,2, and 4. Ketone was found in liquid treatment with synthesized compounds 2,3 while in scolex treatment with synthesized compound 4.

In synthesized compound 2, Saturated fatty acid was present in the scolex treatment

with synthesized this compound, while in synthesized compound 5 presented in liquid treatment with this compound. Also, in synthesized compound 2, Glycerin was found to only in liquid treatment with this compound.

In addition, Cholesterol was registered in scolex treatment with synthesized compounds 2 and 3, while synthesized compound 5 was registered in liquid treatment with this compound. However, in synthesized compound 4, Cholesterol was found in both scolex and liquid, and a high percentage was registered in scolex treatment.

Using concentrated electron beams to magnify objects, the scanning electron microscope (SEM) may provide information on topography, morphology, and composition (39). The SEM data on *Cysticercus* is a significant improvement over light microscopy, and the detailed morphological features found here, especially with regard to the hooks (attachment organs) and body surface, can help explain how the parasite attaches to and absorbs nutrients from its host. Observations on clinical features of parasite manifestation and risk to human health suggest zoonotic implications(40). This study recorded samples from a liquid of *Cysticercus* and scolex isolated from slaughtered sheep and goats. Samples of *C. tenuicollis* treated with 5 synthesized compounds. The result registered different morphological characters and clear destruction in the membrane and hooks of the scolex.

Conclusion:

This study recorded a chemical compound that was different in both the scolex and liquid of *C. tenuicollis* and novel synthesized compounds and registered different morphological characters and a clear destruction in the membrane and hooks of the scolex.

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