Chapter 14

Effect of *Capparis spinosa*, *Syzygium aromaticum* Plant Extracts on Some Bacteria and Parasites in Basrah Province/Southern Iraq

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ABSTRACT

Cysticercosis is a parasitic tissue infection caused by larval cysts of the tapeworm. These larval cysts are a major financial loss in most low-income countries. Two Plant extracts: *Capparis spinosa* and *Syzygium aromaticum* were applied as antiparasitic to *Cysticercus tenuicollis*.

Which showed that *Syzygium aromaticum* extract was more effective against *E. coli* and less effective against *S. aureus*. While *C. spinosa* extract revealed more effectiveness against *S. aureus* and less effective against *E. coli*.

SDS-page for untreated and treated cysts with plant extracts were applied to separate proteins of larval stage *Cysticercus tenuicollis*. In *vitro* and in *vivo*, plant extracts bands ranged from 17- 67 KD and 17 - 67 KD, respectively. Plant extracts of *Capparis spinosa* did not show any bands. There was a clear effect on protein cotenants of *C. tenuicollis* which was treated with two plant extracts *C. spinosa* and *S. aromaticum* in *vitro* and in *vivo* by SDS- PAGE compared with untreated.

GC-mass analysis was applied to detect a chemical compound in liquid and scolex of Cysticercus *tenuicollis* exposed to two plant extracts. And scanning electron microscopy was applied to characterize the morphological changes of scolex and membrane cyst. The result revealed differences in morphological characters between the scolex and membrane cyst during exposure to plant extract compared with control.

KEYWORDS
Capparis spinosa, Syzygium aromaticum, Plant Extracts

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INTRODUCTION

Many excellent studies about the supposed effects of plants were found without proof that they were true (Klimpe et al. 2011). As a result, it is vital to test plants again to see if they are efficient against various bacteria and parasites since many have evolved resistance to various chemotherapeutics. Recently, research has been resurgent into plant antiparasitic benefits (Schmahl et al. 2010).

Most of the animals used in the research were mice and rats; however, a few researchers used in vitro investigations or non-typical lab animals (Bryda, 2013).This highlights the need for more investigation into the effectiveness of certain plants in combatting parasites in farm animals, whereas comparatively little research has been conducted on their effectiveness against parasites in humans (Mehlhorn et al. 2011).

According to Benseghir and Seridi (2007), the *Capparis spinosa* herb is a long-lived plant that belongs to the Capparidaceae family. Vegetative components of *Capparis spinosa* have antioxidant, antifungal, antihepatotoxic, anti-inflammatory, antiallergic, antihistamine, chondroprotective, hypolipidemic, and photoprotective properties (Bonina et al. 2002). In addition, *C. spinosa* contains biogenic salts including potassium, magnesium, calcium, sodium, zinc, copper, and phosphorus, essential oils, fatty acids, steroids, glucosinolates, carotenoids, and tocopherols (Tlili et al. 2009). The protein-rich plant contains bioactive salts including potassium, magnesium, calcium, sodium, zinc, copper, and phosphorus (Rodrigo et al. 1992).

Syzygium aromaticum, also known as *Eugenia cariophylatis*, is a medium-sized tree that is added cloves. For hundreds of years, the Asian region's economy and agriculture flourished due to the clove trade and the hunt for this prized spice. Because they are employed in so many aspects of everyday life, cloves are significant to people's lives around the world (Kamatou et al. 2012).

Over generations people have utilized this plant as a medicinal and food preservation herb, mostly as an antioxidant with antibacterial qualities. The antibacterial, antifungal, antiviral, and anticarcinogenic properties of this herb have been shown by several recent investigations. Clove is particularly popular due to its antioxidant and antibacterial properties (Shan et al. 2005).

Plant Extracts

Capparis spinosa

Capparis spinosa is a xerophytic plant that grows at cooler alpine heights and dry deserts. It is a member of the Capparidaceae family (Pugnair, 1989). It has huge, white to pinkish blooms and round, meaty leaves (Ramezani et al. 2008).

There are just two species that can be found in Iraq: *C. spinosa* L. and *C. cartilaginea* (Blakelock and Townsend, 1980). Moreover, *Capparis* L. belongs to the Capparoideae subfamily (Capparidaceae) (Linnaeus, 1753; 1754). It is composed of more than 250 species from various kinds that can be found in tropical and subtropical areas like southern region of America, Europe, and Africa (Willis, 1988; Inocencio et al. 2006).

The many beneficial parts of *C. spinosa*, from its young shoots and flower buds to its leaves and seeds, are used in medicine, cooking, and cosmetics (Aliyazicioglu et al. 2013). Different parts of *C. spinosa* are employed in traditional medicine for treating various diseases (Eddouks et al. 2004; Mishra et al. 2007). Arab traditional medicine treats spleen, stomach, skin, ear, kidney, and hepatic disorders using *C. spinosa* leaves, roots, and buds (Sher and Alyemeni, 2010 Tlili et al. 2011). It has traditionally been used to treat paralysis, convulsions, and gum disease. The fruits have long been used to treat diabetes, migraines, fever, and rheumatism (Rivera et al. 2003; Jiang et al. 2007). Iranian traditional medicine has used C. spinosa for antimalarials, diuretics, and tonics for years (Miraldi et al. 2001; Ahvazi et al. 2011; Mosaddegh et al. 2012). *C. spinosa* leaves treat analgesia, hemorrhages, rheumatics, and inflammation (Tliliet al. 2011). Asthmatics and coughers may benefit from *C. spinosa* (Jiang et al. 2007).

C. spinosa has different pharmacological performances like anti-hepatotoxic, antioxidant, anti-diabetic, anti-parasite, anti-bacterial, anti-fungal, anti-sclerosis, immunostimulant, anti-cancer. *C. spinosa* has been used in phytomedicine for anti-inflammatory and anti-arthritic properties (Tlili et al. 2011; Al-Said et al. 1998; Feng et al. 2011). Canapés in traditional economies include pickled flower buds, immature fruits, and shoots with olives, cheese, and other vegetables (Sher et al. 2010). The GC MASS analysis also found chemical components in *C. spinosa's* roots, fruits, and seeds that support caper's use in traditional medicine (Sher and Alyemeni, 2010).

Fig. 1: *Capparis spinosa* plant

Syzygium aromaticum

Syzygium aromaticum or clove is the dried flower bud of a plant in the Myrtaceae family that is native to the Maluku islands of Indonesia but is now widely cultivated elsewhere across the world (Batiha et al., 2019). The clove tree's commercial element is its leaves and buds, and flower buds grow four years after planting. After that, they are picked before they bloom either by hand or with a natural phytohormone (Diego and Wanderley,2014). Clove trees are evergreens that grow 10–20 m. It has large oval leaves and numerous terminal clusters of crimson blooms. Clove buds start pale and become green. When they are ready for collection, their color changes to brilliant crimson. The length of a clove, when plucked, ranges from 1.5 to 2 cm. Its calyx consists of four sepals that spread out and four petals that remain closed, creating a small ball in the center. Cloves, which have a powerful perfume and a spicy, pungent flavor, are used to flavor various foods, particular meats, and baked goods (Yousif, 2015).

The flower species is well-known, and the warm area is home to the carnation tree, which is tiny and evergreen and produces a wide variety of crimson blossoms. Before the drought, the flower buds are green or red, and they change into a screw-shaped, easily fractured structure (Azeredo and Soares, 2013). Clove is a moderate variety of cinnamon with an

astringent, anti-bulging aroma. Its oil is therefore widely recognized for its medicinal and spasticity properties, as well as its efficacy in treating tooth pain (Lane et al., 1991). South-east Asians have utilized clove oil for thousands of years as a cure for most tropical ailments. It also lowers Nematoda survival by 50% compared to the control group due to more dead eggs (Meyer et al. 2008).

S. aromaticum is regarded as one of the most valuable spices; it is mostly processed into clove oil, which is widely used in medicine due to its antioxidant, antibacterial, antinociceptive, antiviral, and anesthetic characteristics linked to the presence of eugenol as its predominant ingredient (Cortés-Rojaset al., 2014). According to Teles et al. (2021), *S. aromaticum's* essential oil exhibits antimicrobial, antioxidant, and antitrypanosomal activity, with eugenol (53.23%) serving as the primary material that was confirmed. This results from the chemical elements included in its plant-based products, such as flavonoids and total phenolic compounds.

Fig. 2: *Syzygium aromaticum*

Preparation of Plant Extraction

Plants of *C. spinosa* and *S. aromaticum* were purchased from a natural plant nursery located in the Basrah area. As previously said, they were cleaned, dried, and ground with mortal (Hamza, 2005).

For *C. spinosa* extraction, 100 gm plant leaves were combined with 200 ml ethanol in a beaker for 30 min with a magnetic stirrer and centrifuged at 3000rpm for 15 min. After that, the plant material was placed on glass plates and oven-dried at 60°C (Hamza, 2005).

Condensed solvent (dichloromethane) was added to a distillation flask and 200g of the plant material for *Syzygium aromaticum* was put in a Soxhlet thimble holder (Wenqiang et al. 2007).

GC-Mass Analysis

GC-Mass analysis was done briefly, the cyst isolated from the affected organ was transferred to the Basrah Oil Company lab for GC-Mass analysis.It was 30 m long, 0.32 mm wide, and 0.25 m thick fused silica capillary column (DB5MS). It was composed of phenyl and 95% methyl polysiloxane. The sample was injected into the capillary column in split mode, maintaining a 40°C temperature differential between the injector and detector. For the first 5 min, the column was heated at 40 °C and then it was heated at 28 ℃ each min until it reached its final temperature at 280 °C (Al-Ataby, 2022).

Gas chromatography mass spectrometry (GC-Mass) combines the properties of substances inside a test sample, as described by (Kell et al., 2005). The current study used GC-Mass analysis to identify the parasite sample's constituent chemicals. The sample was transferred to Basrah Oil Company lab to analyses the sample (Nahran-Omer).

The study used two distinct plant extracts, *C. spinosa* and S. aromaticum, and found that both extracts exhibited a similar amount of Phenol. The alcohol content, in contrast, was highest in *S. aromaticum* in contrast with *C. spinosa* is present. Nevertheless, *C. spinosa* contains a substantial number of ketones. Compared to *C. spinosa*, S. aromaticum possesses more organic acid, cyclic compounds, ester, and unsaturated fatty acids. Conversely, *C. spinosa* did not contain eugenol, aldehyde, steroids, or saturated organic compounds, which were exclusively detected in *S. aromaticum*.

Disc Diffusion Method

There are two different types of plant extract were used: *S. aromaticum*and *C. spinosa* against two types of bacteria: *S. aureus* and *E. coli* in six concentrations:1,5, 10,25, 50 and 100 mg/ml for each plant extract. The findings indicated that S. aromaticum exhibited a greater antibacterial effect against *E. coli* than *S. aureus*. Conversely, *C. spinosa* demonstrated a higher antibacterial effect against *S. aureus* than against *E. coli*, as shown in Tables (1 and 2).

Table 1: *Syzygium aromaticum* extract's antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli* bacterial isolates

Syzygium aromaticum	Types of bacteria	viability cm Inhibition zone (cm)					
		1 mg/ml	5 mg/ml	10 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
	Staphylococcus aureus	2.4	2.4	2.6	3	2.4	0
	Escherichia coli	2.3	2.5	2.7	2.7	2.9	3.1

Table 2: *Capparis spinosa* extract's antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli* bacterial isolates

Capparis spinosa	Types of bacteria	viability cm Inhibition zone (cm)					
		1 mg/ml	5 mg/ml	10 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
	Staphylococcus aureus	0	1.4	1.4	1.3	1.3	1.2
	Escherichia coli	0	1.2	1.2	1.3	1.3	1.3

Bacterial Viability by Primary Tissue Culture

After being stained with acridine orange stain, *S. aureus* (A) is treated with *S. aromaticum* (a), *C. spinosa* (b), in the Figure (3). The dark red cells in these Figure represent the pancreatic cancer cells that were treated to plant extracts *S. aromaticum* and *C. spinosa*.

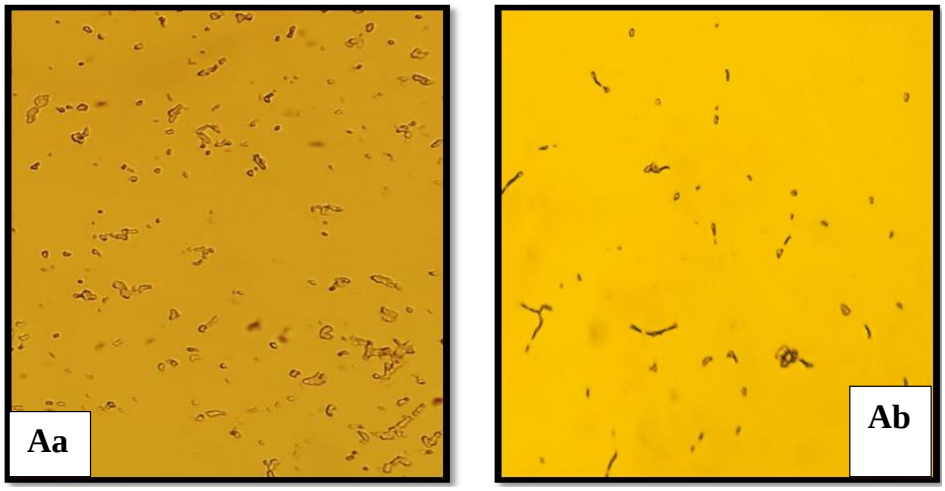

Fig. 3: Bacterium A (*S. aureus*) treat with (Aa, Ab)

A: Bacterium (*S. aureus*), a: *Syzygium aromaticum*(Cloves), b:*Capparis spinosa*

While the Figures (Ba, Bb) depict bacteria *B. cereus*(B)being treated with *S. aromaticum*(a), *C. spinosa*(b), after being staining acridine orange stain. These Figure depict the amount of pancreatic cancer cells that were exposed to plant extracts *S. aromaticum* and *C. spinosa*, with the cells being dark red.

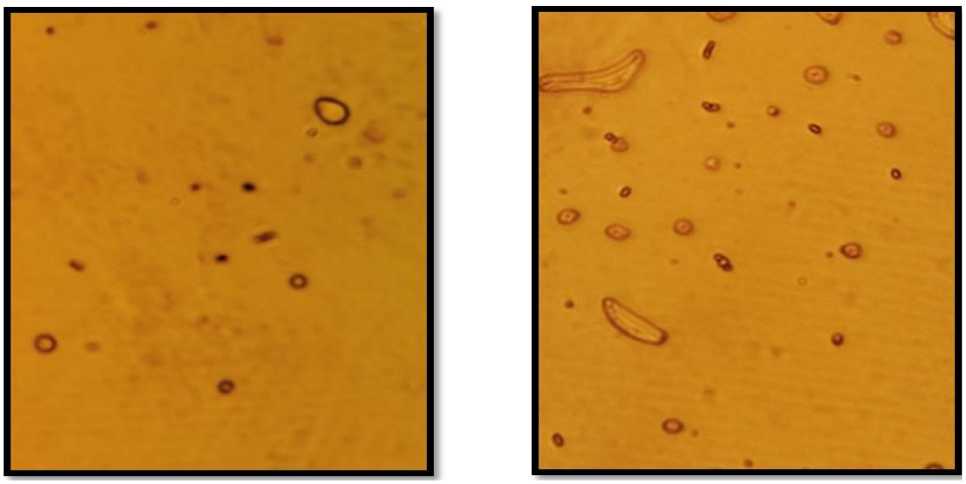

Fig. 4: Bacterium B (*B. cereus*) treat with (Ba, Bb)

B: Bacterium (*B. cereus*), a: *Syzygiumaromaticum*(Cloves), b:*Capparis spinosa*.

Additions shown are the *E. coli* (C) treated with *S. aromaticum*(a), *C. spinosa* (b), after being stained with acridine orange stain in Fig. 5. The cells' dark red color denotes the pancreatic cancer cells that were treated to plant extracts *S. aromaticum* and *C. spinosa*.

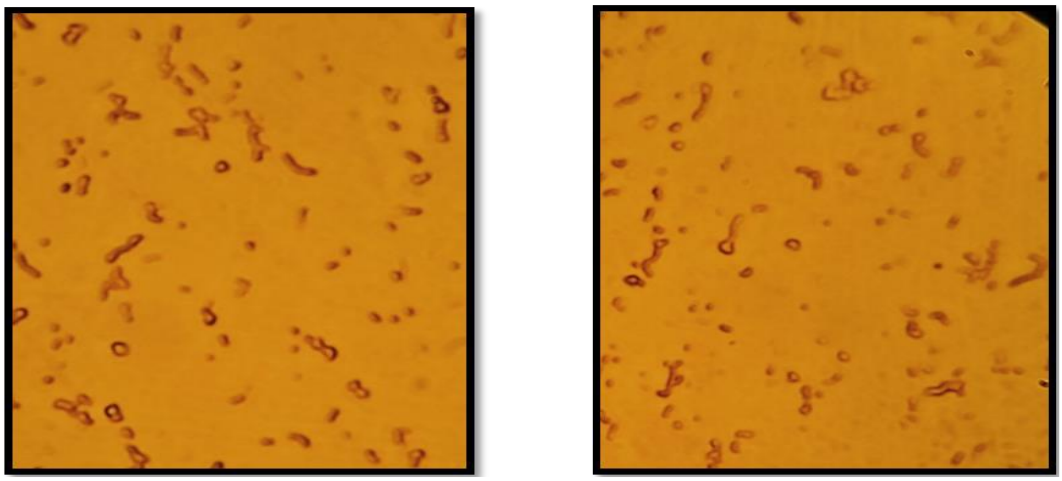

Fig. 5: Bacterium C (*E. coli*) (Ca, Cb)

C: Bacterium (*E. coli*), a: *Syzygium aromaticum*(Cloves), b:*Capparis spinosa*

Additionally, the treatment of *S. typhimurium* (D) with *S. aromaticum* (a), *C. spinosa* (b) after staining them with acridine orange stain is explained in Figures (6). These figures illustrate how many dark red pancreatic cancer cells were exposed to the plant extracts *S. aromaticum* and *C. spinosa*.

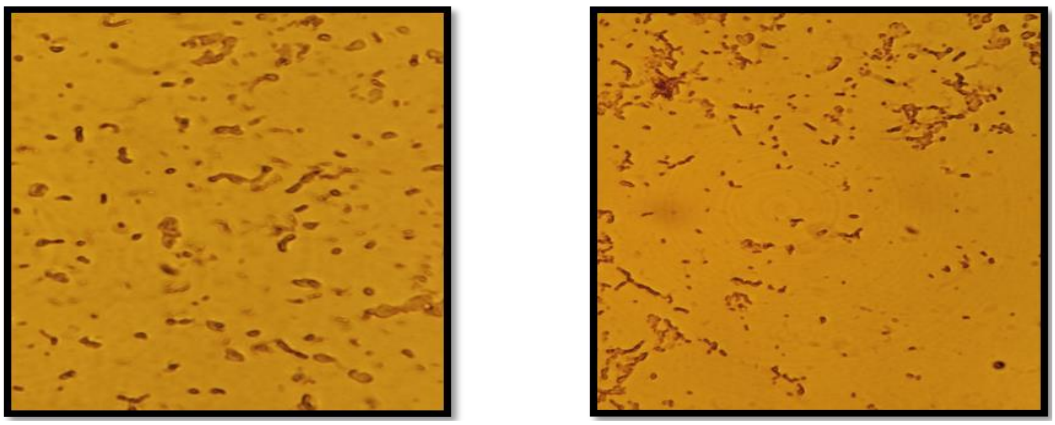

Fig. 6: Bacterium D (*Salmonella typhimurium*) (Da, Db). D: Bacterium (*Salmonella typhimurium*), a: *Syzygium aromaticum*(Cloves), b:*Capparis spinosa*.

Table 3: Bacterial viability % using acridine orange

Bacterial strain	Extracts			Controls		
	A	B	Control cell suspension	Cell suspension/no bacteria	Cell suspension/no extract	bacterial broth
A (*S.aureus*)	40	28.5	83.3	66.6	81.8	0
B (*B. cereus*)	16.6	52.3	71.4	69.2	70	0
C (*E. coli*)	27.2	54.5	33.3	60	66.6	0
D (*S. typhimurium*)	38.8	37.5	64.2	81.8	63.6	0

According to a Table (3) which displays the cell count after staining acridine orangereveals that extract *C. spinosa* and compound 4 are the most effective against the four different species of bacteria. The effectiveness of *S. aromaticum* against S. aureus was higher than that of *B. cereus*, whereas the effectiveness of *C. spinosa* against *E. coli* was lower than that of *S. aureus.*

SDS-PAGE (Polyacrylamide gel electrophoresis)

In the present study, SDS-PAGE analysis was carried out *C. tenuicollis* in vitro (Fig. 7,8) and in *vivo* (Fig.9,10).

SDS-PAGE In Vitro

In vitro, the size of the protein bands seen in control cysts ranged from 17 to 250 KD. These were 62, 55, 26, and 17KD in cysts treated with *S. aromaticum* (A) and *C. spinosa* (B) the protein bands varied from 26 to 67 KD. (Fig.7) and the same as in (Fig. 8).

In *vivo*, the protein bands of control cysts varied between 17 and 250 17KD. While in cysts treated with *S. aromaticum*(A) the protein bands ranged from 37 to 67 KD, and in cysts treated with *C. spinosa*(B), no protein bands were found (Fig. 9, 10).

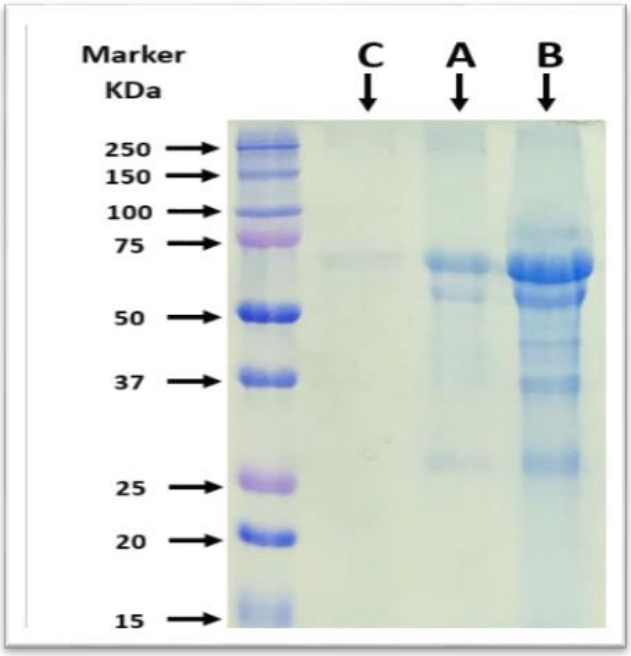

Fig. 7: SDS-PAGE separation gel of (*Cysticercus tenuicollis*). Lanes (C) control, *Syzygium aromaticum*(A), *Capparis spinosa*(B).

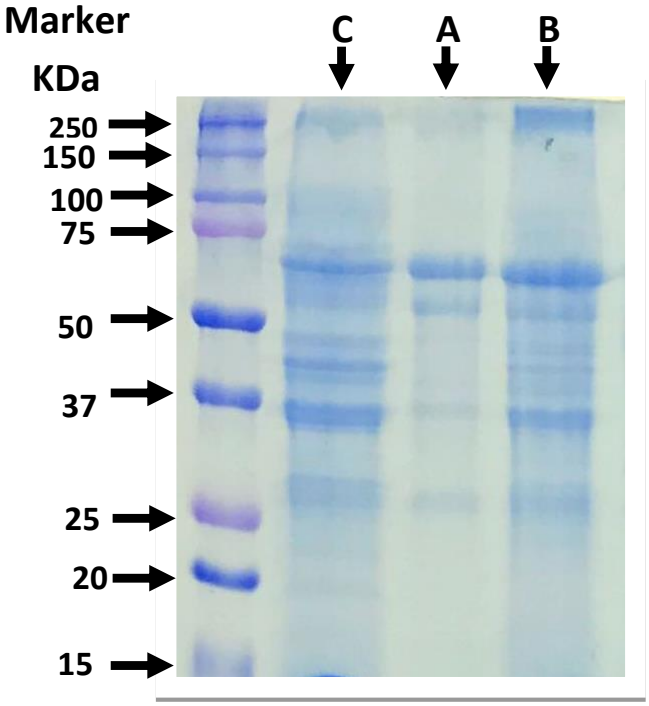

Fig. 8: SDS-PAGE separation gel of (*Cysticercus tenuicollis*). Lanes (C) control, *Syzygium aromaticum*(A), *Capparis spinosa*(B).

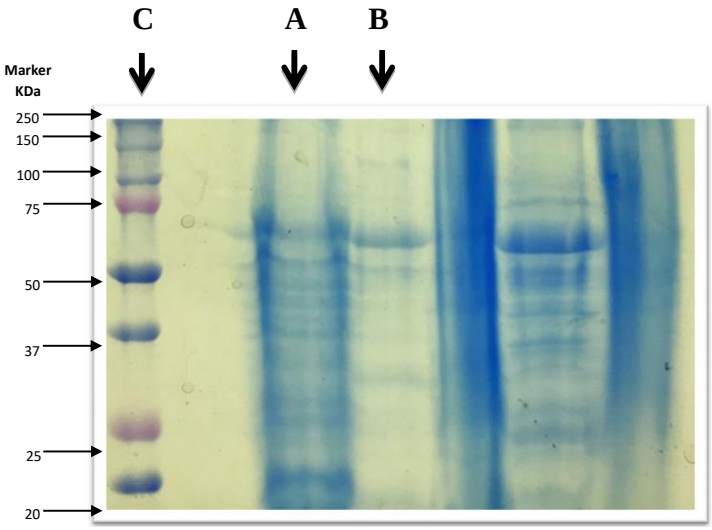

Fig. 9: SDS-PAGE separation gel of (*Cysticercus tenuicollis*). *Syzygium aromaticum*(A), *Capparis spinosa*(B).

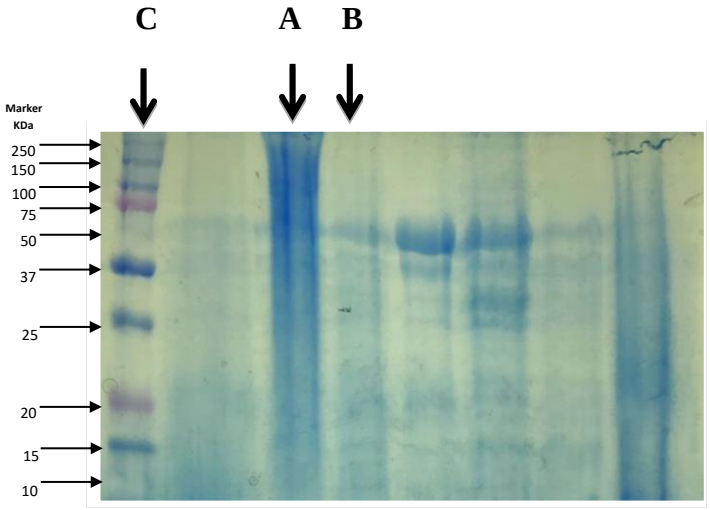

Fig. 10: SDS-PAGE separation gel of (*Cysticercus tenuicollis*). *Syzygium aromaticum*(A), *Capparis spinosa*(B).

Scanning Electron Microscope (SEM)

The technique is especially useful for detecting cystic membranes, which were formerly employed to characterise the surface and hooks. Samples from the membranes of scolex and cysticercus that were extracted from butchered sheep are used in the present study. Figures 11a, b, c, d, and 12a, b show that some samples were normal, while others received treatment with the plant extracts *S. aromaticum* and *C. spinosa*. It displays several morphological traits in addition to clear degradation of the scolex membrane and hooks. Moreover, *S. aromaticum* affects the cyst more than *C. spinosa* (Fig. 13 a, b, 14–15 a, b, 16).

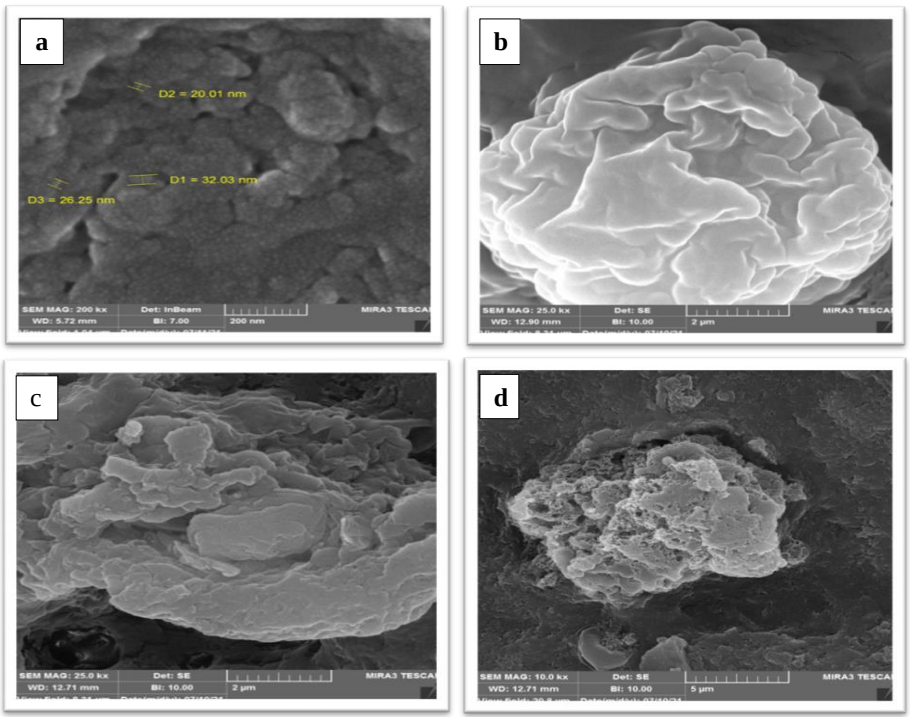

Fig. 11 a, b, c, d: Scolex scanning electron microscopy of untreated *C. tenuicollis*

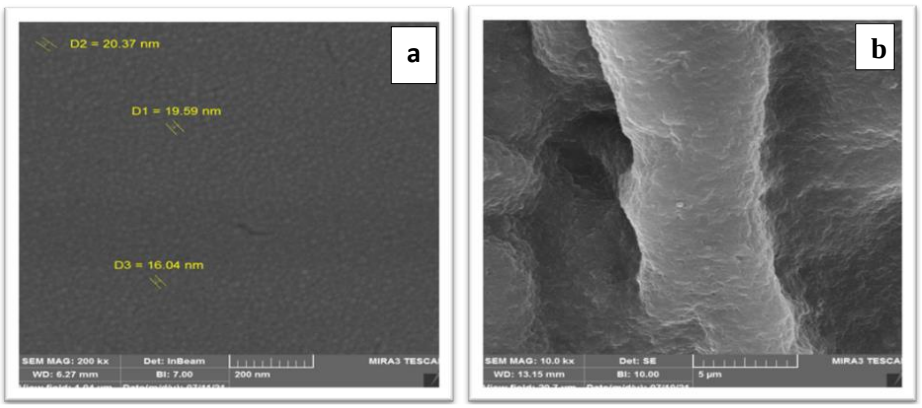

Fig. 12 a, b: Scolex scanning electron microscopy of *C. tenuicollis* untreated

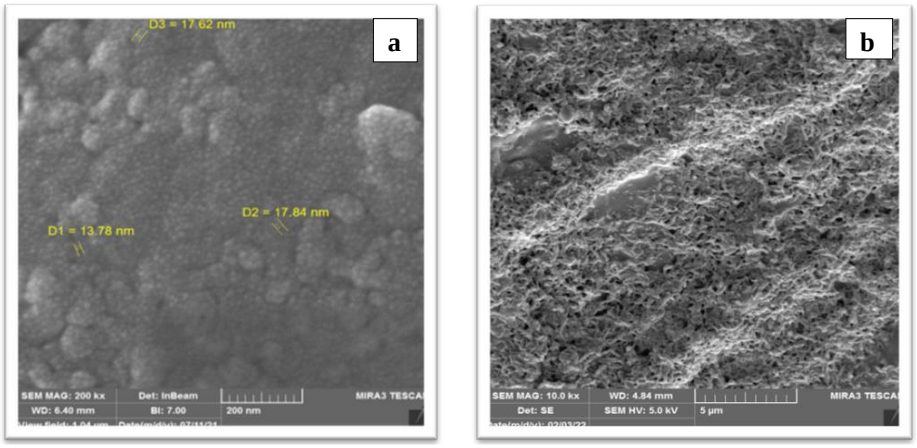

Fig. 13 a, b: Scanning electron microscopy of a *C. tenuicollis* cyst treated with *Syzygium aromaticum*

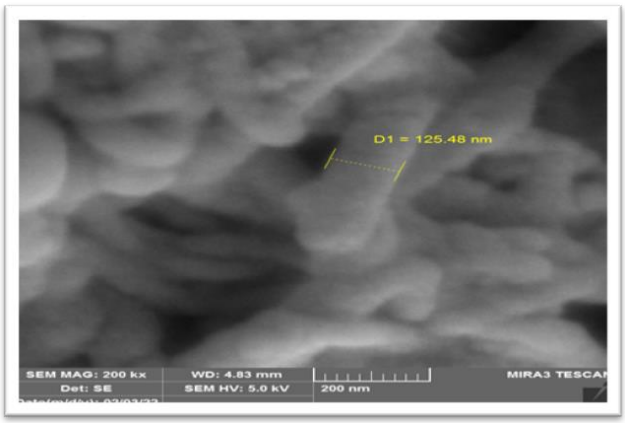

Fig. 14: Scolex scanning electron microscopy of *C. tenuicollis* treated with *Syzygium aromaticum*

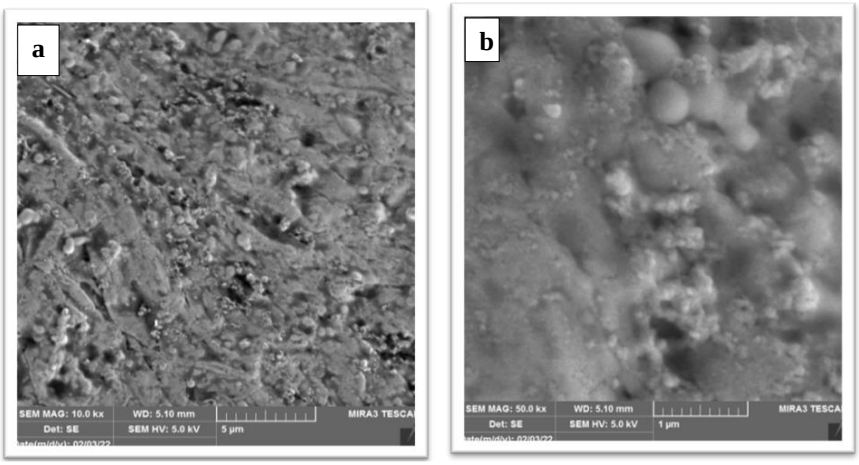

Fig. 15 a, b: Scanning electron microscopy of *C. tenuicollis* cysts that had been treated with *C. spinosa*

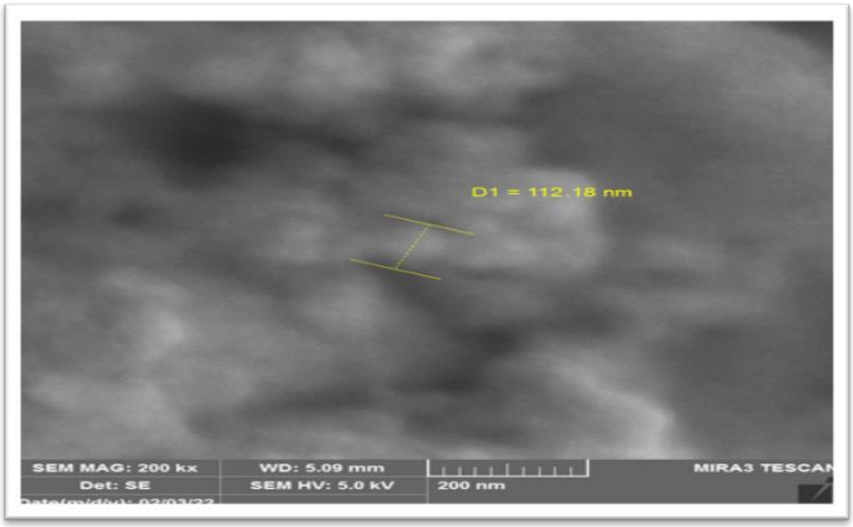

Fig. 16: Scolex scanning electron microscopy of *C. tenuicollis* treated with *C. spinosa*

Conclusions

Plant extracts showed significant antibacterial and antioxidant action against *S. aureus* and *E. coli*, making them suitable for therapy.

A apparent effect on protein cotenants of *C. tenuicollis*, which was treated with two plant extracts (*C. spinosa* and *S. aromaticum*) in *vitro* and in *vivo* by SDS- PAGE compared with untreated.

A clear destruction of the scolex, membrane, and liquid of C. tenuicollis was identified using plant extract to compare with untreated cysts.

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