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SDS-Page Analysis Of *Cysticercus tenuicollis* In Vitro And In Vivo In Basrah Province Southern Iraq

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Abstract

The larval stage *Cysticercus tenuicollis* was isolated from slaughtered sheep and goats at the Basrah slaughterhouse with 30 samples. In vitro, 16 samples, 2 control, and 14 were treated with plant extracts and novel chemical compounds. In vivo 14 samples,1 control and 13 were treated with plant extracts and novel chemical compounds.. SDS- page electrophoresis using the gel as separation gel of larval stage *Cysticercus tenuicollis*. Lanes (C) control, (A) *Syzygium aromaticum*, (B) *Capparis spinosa*, novel acylselenourea, i.e., 4-Nitro-N-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) carbamoselenoyl) Benz amide (**1**), 4-Methyl-N-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) carbamoselenoyl) Benz amide (**2**), 4-Nitro-N-((4-nitrophenyl) carbamo selenoyl) Benz amide (**4**), N-(2,6-dioxo-1,2,3,6-tetrahydropyrimidine-1-carbonoselenoyl)-4-nitrobenzamide (**5**) and novel acylthiourea 4-Methyl-N-((4-(N-(pyrimidin-2-yl) sulfamoyl phenyl) carbamothioyl) Benz amide (**3**). The rats were administered plant extracts and synthetic compounds orally via mouth for 15 days, Then the rats were dissected, the cysts were isolated, and their SDS page was measured. The results found in in vitro, all plant extracts, and synthesized compounds bands ranged from 67 to 17KD. In contrast, the protein bands of cysts varied from 67 to 17KD in vivo whereas *Capparis spinosa* and the synthetic chemical (5) showed no bands.**:** This study recorded a clear effect on protein cotenants of *C. tenuicollis* which was treated with two plant extracts *C. spinosa* and *S. aromaticum*, and five novel chemically synthesized compounds in vitro and in vivo by SDS- PAGE as compared with untreated.

Keywords*: Capparis spinose; Cysticercus tenuicollis; SDS-PAGE; Syzygium aromaticum.*

1.Introduction

Taenia hydatigenia's larval stage, *Cysticercus tenuicollis,* has a significant economic impact on agriculture, animal husbandry, and veterinary research (1; 2; 3). The metacestodes, or larval stage, are found in many domestic and wild intermediate ruminants, while the adult worms live in dogs' and foxes' tiny intestines (4). (5) found that *T. hydatigena cysticerci* cause significant morbidity and mortality in livestock. Researchers from all over the world have found that cysticerci are common in sheep, including (6) with 16.7%, (7) with 34.2% in goats and 21.4% in sheep, and (8) with 33.3% in goats. *Serodiagnosis* is frequently the only way to diagnose human and animal *Taeniid cestode* larvae infections, which are economically significant. (9 and 10).

The long-lived shrub *Capparis spinosa* belongs to the genus Capparis (11). The plant's leaves and stems have been found to have several biological activities, including: "antioxidant, antifungal, antihepatotoxic, anti-inflammatory, antiallergic and antihistaminic, chondroprotective, hypolipidemic, and photoprotective" activities (12).

While *Syzygium aromaticum* is regarded as a valuable spice, it is mainly processed into clove oil, which is widely used in medicine due to its antioxidant, antibacterial, antinociceptive, antiviral, and anesthetic effects attributable to the presence of eugenol as a significant ingredient (13). Organoselenium compounds are building blocks in producing several naturally occurring and physiologically active chemicals (14and15). Several reactions have documented numerous synthetic techniques for producing nitrones and organoselenium compounds (16 and 17). Since 1937, acylselenourea derivatives have been manufactured by reacting KSeCN with acyl halides to make organoseleno nitrile, then adding the appropriate amine in the appropriate organic solvents to form the desired molecule with the formula RC(O)NHC(Se)NR'R (18). Organoseleno nitrile and organoisoseleno nitrile were used as precursor in organoselenium chemistry (19and 20).

Aim of the study: The current investigation attempts to determine the effect of two plant extracts *Capparis spinosa* and *Syzygium aromaticum*, and five novel chemically synthesized compounds on the *Cysticercus tenuicollis* in vitro and in vivo by SDS- PAGE.

2.Materials and Methods

2.1. Collection *Cysticercus tenuicollis*

Fourteen samples of *C. tenuicollis* were obtained from laboratory rats infected with the eggs of *Taenia hydatigenia*, which had been isolated from adult worms in the gut of a stray dog, and sixteen samples were isolated from sheep and goats (21).

The rats were divided into 7 groups (3 for each group), leaving 2 for control, the rats were administered plant extracts and synthetic compounds orally via mouth for 15 days, Then the rats were dissected, the cysts were isolated, and their SDS page was measured.

2.2. Preparation of Plant Extraction

Under this study *Capparis spinosa* and *Syzygium aromaticum*, two types of plants were extracted,

washed, dried, and then ground by mortal in accordance following the procedure by (22).

2.3. Extraction of *Capparis spinose*

One hundred grams of plant material were put into a beaker with 200 ml of ethanol. The mixture was stirred with a magnetic stirrer for 30 minutes. After that, the precipitator was separated from the plant material using a centrifuge at 3000 rpm for 15 minutes. The plant material was then spread out on glass plates and dried in an oven at 60°C (22).

Figure (1): *Capparis spinosa* plant

2.4. Extraction of *Syzygium aromaticum*

Using a grinder, the dried clove was roughly crushed before being separated into 200g of ground samples for the extraction process. In a typical Soxhlet apparatus, plant material is inserted in a thimble-holder and a distillation flask is filled with condensed, fresh solvent (Dichloromethane). Soxhlet is a traditional technique that is used to assess the efficacy of various solid-liquid extraction procedures. When the thimble-holder solution overflows, a siphon aspirates it and empties it back into the distillation flask, delivering the extracted solutes into the bulk liquid. In the solvent flask, distillation is employed to separate the solute from the solvent. Fresh solvent is returned to the plant solid bed, while the material that has been dissolved remains in the flask. The procedure is repeated until the extraction is complete. Matrix characteristics, particle size, and internal diffusion all play crucial roles in Soxhlet extraction and may even be the limiting step (23). A Soxhlet extractor was used to put the sample in. Dichloromethane and the boiling chip granules were put in a round flask. The extraction process took 6-7 hours at a temperature of 70-80C^0. After filtering, a rotating vacuum evaporator evaporated the solvent from the crude extract. Then, the crude extract was measured out, and it was placed in a vial for future examination.

2.5. Preparation of synthesis compounds

The HPLC grade chemicals and solvents used in this study were purchased from commercial sources and utilized exactly as supplied, Solvents were dried using the prescribed technique by (24). All reactions were conducted under dry circumstances, quantified using thin-layer chromatography (TLC), and the spots were

seen under ultraviolet light. KSeCN was prepared from the reaction of elemental Se with KCN according to (25). Column chromatography was used to purify the reaction products using silica gel 60 A° and benzene/ethanol (9:1) as the elution solvent.

2.6.SDS-PAGE(Polyacrylamide gel electrophoresis)

The isolated *C. tenuicollis* were kept in a liquid nitrogen container and then removed to homogenize. Three hundred microliters of RIPA lysis Buffer were added to about 0.5 g of cysticercus cyst. Then the supernatant of samples was collected after 5000 g for 5 min. After that, samples were mixed with a 5X loading sample buffer. They were loaded to 10 % SDS-PAGE gel and then allowed to run on the gel using electrophoresis buffer for 45 min at 200 volts using Bio Rad power pack Electrophoresis power supply (UK). After electrophoresis, gels were washed with distal water and incubated in fast Coomassie blue staining solution overnight on the orbital rocker. The next day, the stain was changed with distain solution for 2 h to clear all protein bands based on the molecular weight of each protein according to the method (26 and 27).

2.7. Statistical Analysis

The Statistical Analysis System- SAS (28) program was used to detect the effect of different factors in study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means in this study (29).

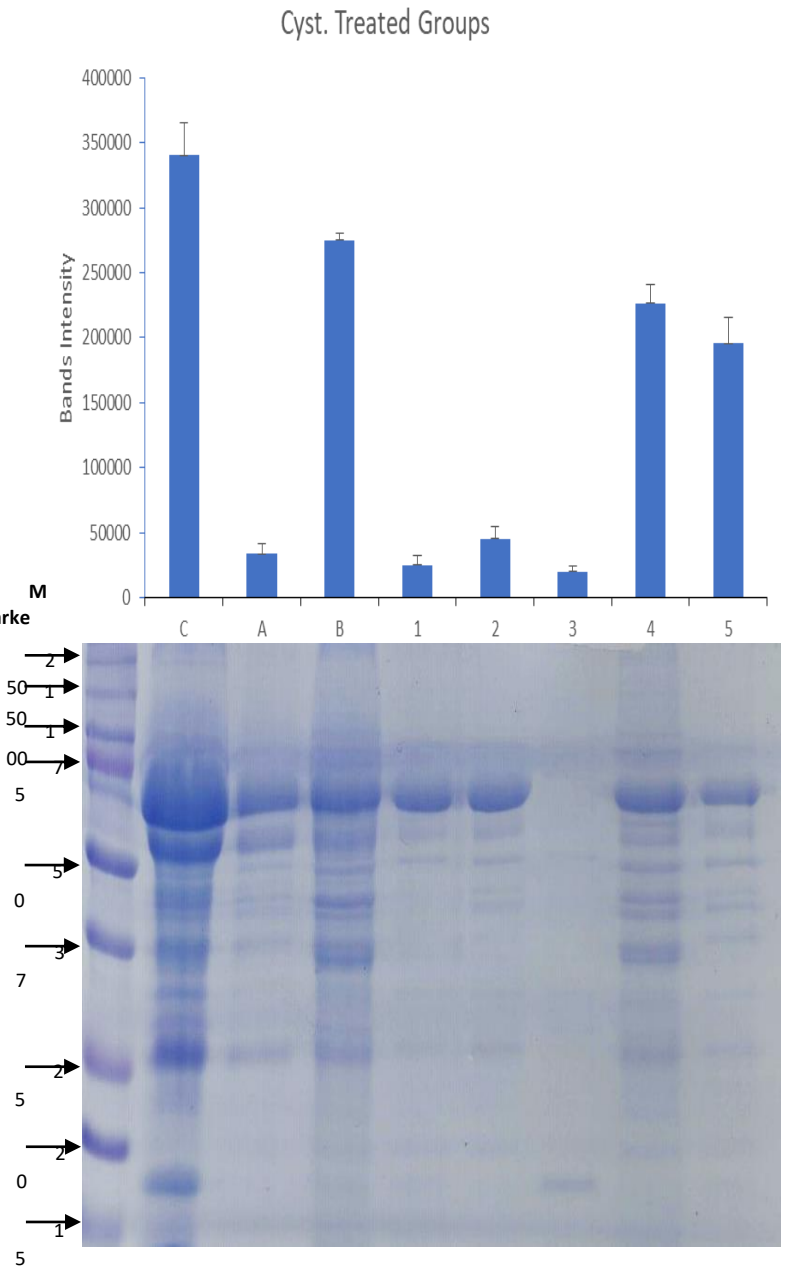


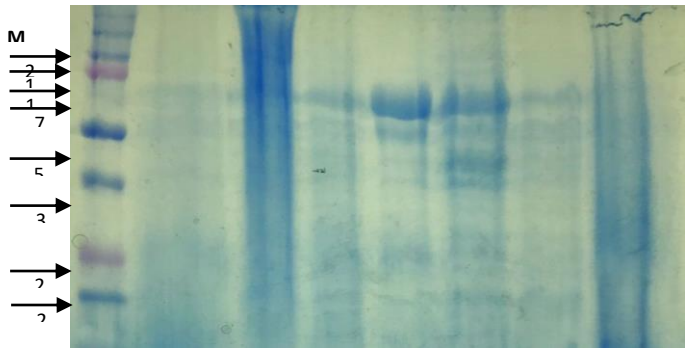
Figure 3: SDS-PAGE separation gel of *Cysticercus tenuicollis*. Lanes (C) control, *Syzygium aromaticum*(A), *Capparis spinosa*(B), novel chemical compounds (1), (2), (3), (4), (5). Twenty-five microliters of each sample were loaded per well. Coomassie Blue staining was performed.

Table no 1 P value for each group in comparison with Control group

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.*	95% Confidence Interval	
						Lower Bound	
Tukey HSD	C1	A1	306596.000*	18974.015	.000	240905.10	
		B1	65423.333	18974.015	.051	-267.57	
		1	315188.667*	18974.015	.000	249497.77	
		2	295368.000*	18974.015	.000	229677.10	
		3	320470.667*	18974.015	.000	254779.77	
		4	113794.333*	18974.015	.000	48103.43	
		5	144894.333*	18974.015	.000	79203.43	

**P* value for each group in comparison with Control group

In vivo, the protein bands of control cysts varied between 17 and 250 KD. While in cysts treated with *Syzygium aromaticum*(A) the protein bands ranged from 37 to 67 KD, and in cysts treated with *Capparis spinosa* (B), no protein bands were found. In cysts treated with novel chemical compound (1), the protein bands ranged from 17 to 67 KD. In cysts treated with a novel chemical compound (2) in cysts treated with a unique chemical substance, the protein bands varied from 26 to 67 KD (3) Protein bands ranged in size from 20 to 67 KD in cysts treated with a new chemical substance (4) The protein bands varied between 20 and 67 KD. However, no protein bands were found in cysts treated with novel chemical compound (5) (Fig. 4).



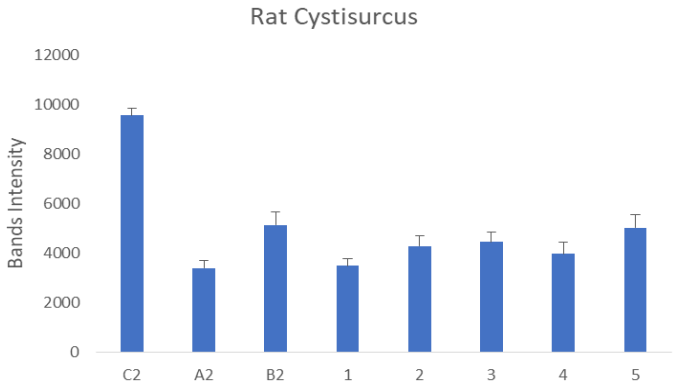


Figure 4: SDS-PAGE separation gel of *Cysticercus tenuicollis*. Lanes (C) control, *Syzygium aromaticum*(A), *Capparis spinosa*(B), novel chemical compounds (1), (2), (3), (4), (5). Twenty-five microliters of each sample were loaded per well. Coomassie Blue staining was performed.

4. Discussion

Specific protein or nucleic acid components can be separated using the straightforward, quick, and compassionate protein analysis technique of electrophoresis (30).

In this work used S. aromaticum and C. spinosa plant extracts, as well as novel synthetic compounds created in accordance with (31).

Protein bands in cysts were measured to be between 17 and 67 KD in vivo. However, the plant extracts Capparis spinosa and a recently synthesized chemical compound (5) did not display any protein bands. This may be because some protein bands occurred abundantly and were visible in the studied cestodes, whereas others were in low abundance and were hardly detectable. Notably, all examined cestode species and cyst compartments contained the 67-, 55-, and 23-KDa proteins, respectively. When compared to other studies of a similar kind, such as (32 and 33), their findings were comparable in some aspects but dissimilar in others. Some cestodes' high-molecular-weight protein complexes can dissociate under decreasing circumstances into two or more subunits (34). This may also explain why some SDS-PAGE samples include tiny proteins while others do not.

Furthermore, certain protein locations contribute to the development of several cestode infections in animal hosts (35). Also, SDS-PAGE analysis of dialyzed *C. tenuicollis* cyst fluid, cyst membrane, and cyst scolex revealed a total of 37 bands, including 3 bands in the fluid, 22 bands in the membrane, and 12 bands in the scolex (36). On the other hand, a study by (37) resulted that SDS-page electrophoresis showed clear two protein bands found in scolex and multi bands in the gravid segment with high molecular weight. This can have

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Rat Cyst vs treat

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD	C2	1	6156.000*	587.375	.000	4122.42	8189.58
		1	4426.000*	587.375	.000	2392.42	6459.58
		2	6072.667*	587.375	.000	4039.09	8106.25
		2k	5272.667*	587.375	.000	3239.09	7306.25
		4	5112.667*	587.375	.000	3079.09	7146.25
		4k	5582.667*	587.375	.000	3549.09	7616.25

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Rat Cyst vs treat

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD	C2	A2	6156.000*	587.375	.000	4122.42	8189.58
		B2	4426.000*	587.375	.000	2392.42	6459.58
		1	6072.667*	587.375	.000	4039.09	8106.25
		2	5272.667*	587.375	.000	3239.09	7306.25
		3	5112.667*	587.375	.000	3079.09	7146.25
		4	5582.667*	587.375	.000	3549.09	7616.25
		5	4529.333*	587.375	.000	2495.75	6562.91

explained that the structure plays a good role in each part; the scolex with hooks, and suckers want a high protein for moving and attaching and the gravid segment with eggs want protein to complete their maturating of it. While other samples were without any bands. Furthermore, (38) found, by SDS-page and western blotting, that the fluid antigen from *C. tenuicollis* had two unique bands with molecular weights of 38 and 42.5 KD, which may be significant proteins for *T. hydatigena* diagnosis in dogs. (39) discovered protein bands ranging from 260 to 23KD in the inner membrane of *T. hydatigena* cysts, including the Scolex. These were as follows: 260, 150, 130, 67, 55, 35, and 23KD. And (40) confirmed that the plant *Cyperus longus* kills Protoscolices of *Echinococcus granulosus* because it has active compounds called polyphenols. These polyphenols break the cellular membrane of the parasite and the protein and lipids it has because they can make hydrogen bonds between hydroxyl groups and nitrogenous compounds, which makes the proteins stick together.

Date palm plants subjected to Cd and Pb generated 20 protein bands with molecular weights from 18 to 73 using SDS-PAGE protein analysis. Three protein bands with molecular weights of 73; 58, and 44 KD were detected in all treatments. The band with a molecular weight of 33 KD disappeared at the highest Pb dosage (276 mg/kg), and a newly expressed polypeptide with a molecular weight of 18 KD was discovered (41). The SDS-PAGE results of proteins retrieved from the leaves of the 17 date palm seeded strains revealed a substantial variance in protein banding pattern, with the amount and location of bands varying significantly between the tested strains (42).

Conclusions: This study recorded a clear effect on protein cotenants of *C. tenuicollis* which was treated with two plant extracts *C. spinosa* and *S. aromaticum*, and five novels chemically synthesized compounds in vitro and in vivo by SDS- PAGE as compared with untreated.

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