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Effect of Some Plant Extracts on the Activity of Protease Enzyme from *Staphylococcus aureus* Which Isolated from Clinical Samples

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ABSTRACT
In this study, the effect of some plant extracts of capper and licorice plants was studied. These two plants were extracted, and after that three concentrations of these two plants were attended by 30%, 60 and 90%. The effect of these two plants on the activity of protease was studied. The effectiveness of the protease enzyme. The effect of the concentration of 90% of both plants gave a greater effect compared to the rest of the concentrations. The effect of cappers was more compared to licorice. The effect of using plant extract on reducing the activity of protease by Staph bacteria, and the concentration was 90% more effective in reducing the effectiveness of this enzyme

Keywords: protease activity, plant extract ,capper, licorice.
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INTRODUCTION

Staphylococcus aureus (S. aureus) is a Gram-positive bacterium belongs to the Staphylococcus genus, which is widely disbursed innature. S.aureus is a frequent pathogen inclined to a variety of animals and humans, and it can purpose a variety of serious infections, including dairy cow mastitis, endometritis, urinary tract infection.[1,2] Extracellular serine protease (SP) structures mediate rapid defense responses to tissue damage and pathogen assault in vertebrates and invertebrates. [3,4] Upon consciousness of aberrant or microbial surfaces, proteolytic processing sequentially prompts serine proteases and their homologs to finally convert prophenoloxidases (proPOs) toPOs in insects.[5,6] POs catalyze the formation of reactive compounds and melanin to kill and sequester pathogens.[7,8] Proteases are the crew of enzymes most used commercially, having a wide range of functions in meals biotechnology.[9,10] They are succesful of altering the molecular size, hydrophobicity, and the uncovering of polar groups in proteins.[11,12]

MATERIAL AND METHODS

Collection of bacterial samples

20 scientific samples were gathered from Ramadi Teaching Hospital which had been from quite a number sources including: burns, wounds, UTI infections, These samples had been amassed by sterile cotton swabs. While urinary tract infections (UTI) samples were gathered by way of a sterile container.

Bacterial isolation and identification

Bacterial isolates have been subjected to a variety of cultural and biochemical tests for identification of these isolates.[13,14]

Preparation of ethanol extract of Capparis spinosa Plant

The fantastic and most fantastic method to extracting the lively organic cloth crude from compounds in the plant used the Soxhlet apparatus. two used the plant and grinding or crushing by means of a blender apparatus into powder, taken 50 g of plant powder and placed internal Thumble, 250 mL of ethanol then positioned 95% after extraction, the extraction manner used to be carried out within 24 hours and then focused by way of a rotary evaporator, Then put in frozen under -20 ° until used.[15,16]

Protease production

Protease endeavor used to be assayed through mixed 1.8 ml of casein substrate answer and 0.2 ml of supernatants from in a single day cultures of bacterial growth, and incubated in the water bath at 37°C for 20 min. The reaction was once stopped by the addition of 3 ml of 5% Trichloroacetic acid (TCA), and then centrifuged at 2500 rpm for 20 min. The manipulate was once organized the usage of the equal steps besides for the addition of TCA reagent earlier than supernatants, then absorbance was measured at 275 nm.[17,18]

$$\text{Enzyme activity (U/ml)} = \frac{\text{Absorbance at 275 nm}}{(0.001)(0.2)(20)}$$

Study the effect of plant extracts on protease activity

100 µl of plant extract was added to tubes which contain (10 ml of nutrient broth + 100 µl of the inoculum containing 1×10^8 CFU/ml of Staphylococcus bacterial growth), then incubated for 24 h at 37°C. After incubating period, these cultural growth used to detection of protease activity.

RESULT AND DISCUSSION

Table (1) Effect of cappers extract on the activity of protease enzyme from Staphylococcus bacteria

Concentration	Protease activity U/ml				
	Frequency	Frequency	Frequency	Frequency	Frequency
30 % of capper extract	55	56	54	55	50
60 % of capper extract	44	42	46	44	45
90 % of capper extract	23	21	20	22	23
Control	155	150	156	157	156

Table (1) shows the effect capper extract on the activity of protease enzyme. The results showed significant differences for the effect of capper plant extract on the effectiveness of protease enzyme compared to the efficacy of control where the effect of using capper extract on the low efficacy of this enzyme has given treatment that The concentration of the extract was 90%, the best result compared to the rest of the treatments and control.

Table (2) ANOVA table of Effect of cappers extract on the activity of protease enzyme from Staphylococcus bacteria

ANOVA

activity of protease (U/ml)

Between Groups	(Combined)		50344.600	3	16781.533	18646.148	.000
	Linear Term	Contrast	40723.240	1	40723.240	45248.044	.000
		Deviation	9621.360	2	4810.680	5345.200	.000
	Quadratic Term	Contrast	7449.800	1	7449.800	8277.556	.000
		Deviation	2171.560	1	2171.560	2412.844	.000
	Cubic Term	Contrast	2171.560	1	2171.560	2412.844	.000
Within Groups			14.400	16	.900		
Total			50359.000	19			

ANOVA table of Effect of cappers extract on the activity of protease enzyme from Staphylococcus bacteria, this table shows the values of F and significant degrees for all transactions, where the results showed that there were significant differences for all transactions compared to the control.

Table (3) Effect of Licorice extract on the activity of protease enzyme from Staphylococcus bacteria

Concentration	Protease activity U/ml				
	Frequency	Frequency	Frequency	Frequency	Frequency
30 % of Licorice extract	77	76	71	73	72
60 % of Licorice extract	66	65	61	63	61
90 % of Licorice r extract	44	42	43	44	41
Control	177	176	176	173	172

Table (3) shows the effect of Licorice extract on the activity of protease enzyme. The results showed significant differences for the effect of Licorice plant extract on the effectiveness of protease enzyme compared to the efficacy of control where the effect of using Licorice extract on the low efficacy of this enzyme has given treatment that The concentration of the extract was 90%, the best result compared to the rest of the treatments and control.

Table (4) ANOVA table of Effect of Licorice extract on the activity of protease enzyme from Staphylococcus bacteria

ANOVA

activity of protease (U/ml)

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		51961.350	3	17320.450	3785.891	.000
	Linear Term	Contrast	41330.890	1	41330.890	9034.074	.000
		Deviation	10630.460	2	5315.230	1161.799	.000
Within Groups			73.200	16	4.575		

Total	52034.550	19			

ANOVA table of Effect of Licorice extract on the activity of protease enzyme from Staphylococcus bacteria, This table shows the values of F and significant degrees for all transactions, where the results showed that there were significant differences for all transactions compared to the control.

Table (5) Multiple comparison for Effect of cappers extract on the activity of protease enzyme from Staphylococcus bacteria

Multiple Comparisons

Dependent Variable: activity of protease (U/ml)

LSD

(I) conceteration of capper extract	(J) conceteration of capper extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	30%	97.600*	.600	.000	96.33	98.87
	60%	110.000*	.600	.000	108.73	111.27
	90%	130.400*	.600	.000	129.13	131.67
30%	0	-97.600-*	.600	.000	-98.87-	-96.33-
	60%	12.400*	.600	.000	11.13	13.67
	90%	32.800*	.600	.000	31.53	34.07
60%	0	-110.000-*	.600	.000	-111.27-	-108.73-
	30%	-12.400-*	.600	.000	-13.67-	-11.13-
	90%	20.400*	.600	.000	19.13	21.67
90%	0	-130.400-*	.600	.000	-131.67-	-129.13-
	30%	-32.800-*	.600	.000	-34.07-	-31.53-
	60%	-20.400-*	.600	.000	-21.67-	-19.13-

*. The mean difference is significant at the 0.05 level.

Table (6) Multiple comparison for Effect of Licorice extract on the activity of protease enzyme from Staphylococcus bacteria

Multiple Comparisons

Dependent Variable: activity of protease (U/ml)

LSD

(I) conceteration of Licorice extracts	(J) conceteration of Licorice extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	30%	101.000*	1.353	.000	98.13	103.87
	60%	111.600*	1.353	.000	108.73	114.47
	90%	132.000*	1.353	.000	129.13	134.87
30%	0	-101.000-*	1.353	.000	-103.87-	-98.13-
	60%	10.600*	1.353	.000	7.73	13.47
	90%	31.000*	1.353	.000	28.13	33.87
60%	0	-111.600-*	1.353	.000	-114.47-	-108.73-
	30%	-10.600-*	1.353	.000	-13.47-	-7.73-
	90%	20.400*	1.353	.000	17.53	23.27
90%	0	-132.000-*	1.353	.000	-134.87-	-129.13-
	30%	-31.000-*	1.353	.000	-33.87-	-28.13-
	60%	-20.400-*	1.353	.000	-23.27-	-17.53-

*. The mean difference is significant at the 0.05 level.

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