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Effect of *Xanthium Strumarium* Extract on Some Virulence Factor of *Proteus Mirabilis* Isolated from Patients in Ramadi Hospital

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ABSTRACT

Background Due to the increase in urinary tract infections and their spread, which requires continued research, especially *P. merabilis* bacteria, which we highlighted in our study of the phenotypic and molecular aspects of its virulence factors and studying the effect of different concentrations of plant extract on some virulence factors.

Methodology 40 samples were collected from the reviewing and inpatients in Ramadi General Teaching Hospital, Women's and Children's Teaching Hospital, and the Industrial College Unit in the Anbar Health Department in Ramadi, for both genders and for all ages for the period from 1/7/2019 to 5/3/2020.

Result shows the effect of this plant extract on reducing the efficacy of the protease enzyme produced by the bacteria there were significant significant differences between the treatments and the control. The concentration of 90% of the plant extract gave the best result in reducing the effectiveness of this enzyme compared to the control and the rest of the treatments 30% and 60%

Keywords: Xanthium strumarium extract, virulence factor of proteus mirabilis

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INTRODUCTION

P. mirabilis is characterized by being short, Gram-negative bacilli, P. mirabilis ranging in diameter between 0.3 (-1.0) m and length (0.6–6.0 m), mobile and non-forming of blackboards and an optional antenna Facultative anaerobic [1,2]. Fishy oder. [3,4] have Fimbriaes and Flagellates that are non-capsule (Non-capsule) have the ability to produce hydrogen sulfide gas (H2S) when grown on Kligler iron agar and give Phenyl. Pyruvic acid when grown on a medium containing Phenylalanine based on the enzyme Phenylalanine deaminase, positive for catalase and methyl red (Methyl red) and negative for oxidase [5,6]. And it is not fermented for lactose sugar when grown on maconkey medium, and it is fermented for each of the sugar's sucrose, glucose and lactose [7,8] Species of the Proteus genus, including P. mirabilis, have several potential pathways for human infection. These may include hospital-sourced pathways such as hospital food and equipment, intravenous fluids, human contact through contaminated skin surfaces, long-term catheters which are a major site of colonization and infection of P. mirabilis [9,10]. The bacterial species P. mirabilis is found in the human intestine as part of the normal flora, but when it is found outside its natural habitat, it causes infection, and P. mirabilis is widespread in hospitals and care facilities and is responsible for causing infections [11,12] Mirabilis has virulence factors and enzymes, including the protease enzyme that breaks down proteins and Urease, which degrades urea to NH2 (and 3) CO. It also contains toxins such as the hemolytic toxin Hemolysin and Proteus toxin agglutinin [13,14]. Also, a virulence agent that P. mirabilis is involved in adhesins, flagella and colonization [15]. The infection with P. mirabilis is endogenous [4]

METHODOLOGY

Samples collection

40 samples were collected from the reviewing and inpatients in Ramadi General Teaching Hospital, Women's and Children's Teaching Hospital, and the Industrial College Unit in the Anbar Health Department in Ramadi, for both genders and for all ages for the period from 1/7/2019 to 5/3/2020

Isolation and Identification of isolated bacteria

The bacterial isolates were diagnosed by growing them on blood agar and MacConky agar, and then by conducting biochemical tests based on the following bases.

Cultural characteristics

The phenotypic characteristics of P. mirabilis were initially depended on and its colonies were diagnosed, including colony shape, size and color. The focus was on the colonies that were characterized by the phenomenon of female swarming on the medium of solid blood agar. Growing colonies and their cultivation characteristics [7].

Extraction of *Xanthium strumarium*

The aqueous extraction method was adopted to extract this plant according to the method [15]

Biofilm formation test (Micro-titration plate method (MTP)) (quantitative method)

This test was used to detect the ability of P. mirabilis bacteria to form a biofilm by growing the bacterial isolates on the center of infusion of the heart and brain and incubated for a period of (24 hours) at a temperature of 37 ° C (0), after which (200 µl) was added to the pits of titration plates, i.e. by three replicates and the refined The fourth negative control (which was prepared in the same way except for containing the bacterial isolate) and then incubated for a period of (24 hours) at a temperature of (37 ° C) after which it was washed with a physiological saline solution in order to get rid of the remnants of bacteria attached to the bottom and on the sides, then add (200 µl) of alcohol Methanol at a concentration of (99%) for (15 minutes), then add the

dye of Crystal Violet, diluted at a concentration of (1%) for a period of (5) minutes [5]

RESULT

Table 1. The effect of *Xanthium strumarium* extract on the activity of the protease enzyme

Descriptive								
activity of protease enzymes U/ml								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
30%	5	9.80	.837	.374	8.76	10.84	9	11
60%	5	7.40	.894	.400	6.29	8.51	6	8
90%	5	3.40	.548	.245	2.72	4.08	3	4
control	5	17.80	.837	.374	16.76	18.84	17	19
Total	20	9.60	5.443	1.217	7.05	12.15	3	19

It is a plant species from the genus Xanthium of the compound family, and this genus includes between three and several plant species, according to different botanists It is a herbaceous plant around, with a strong stem and densely branching, with a height of between 30 - 120 cm, covered with hair, and has a fragrant smell (smell not desirable), yellowish green color, stems strong, branched. Research indicates xanthatin has anti-cellular properties, and thus has potential Its effect on reducing the proliferation of cancer cells the fruit extract has antiseptic and anti-fungal properties of some strains of bacteria and fungi, and references indicate its anti-spasmodic, hypoglycemic properties the decoction of the leaves and the fruits are popularly used for its analgesic, astringent, diuretic properties, and in the therapy of mucositis For the nose. The leaf soak is used topically to get rid of some kinds of parasites, and as an astringent in the cure of wounds. Table 1 indicates the impact of this plant extract on decreasing the efficacy of the protease enzyme produced by way of the microorganism there had been sizable variations between the redress and the control. The attention of 90% of the plant extract gave the satisfactory end result in lowering the effectiveness of this enzyme in contrast to the manage and the relaxation of the remedies 30% and 60%

Table 2. ANOVA table of effect of *Xanthium strumarium* extract on the activity of the protease enzyme

ANOVA					
activity of proteas enzymes U/ml					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	552.800	3	184.267	294.827	.000
Within Groups	10.000	16	.625		
Total	562.800	19			

Table 2, analysis table of variance, shows that there are significant differences between the treatments and the control, where the treatment of plant extract 90% gave the highest significant difference in reducing the activity of the protease enzyme, as the mean of this treatment reached 3.4, followed by a concentration of 60%, where the mean of this treatment reached 7.4 As for the last treatment, 30% had a mean of 9, compared to control, where the mean was 17

Table 3. The effect of *Xanthium strumarium* extract on the production of biofilms

Descriptive								
production of biofilm								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
30%	5	19.20	.837	.374	18.16	20.24	18	20
60%	5	13.60	1.140	.510	12.18	15.02	12	15
90%	5	8.60	1.140	.510	7.18	10.02	7	10
control	5	27.40	1.517	.678	25.52	29.28	25	29
Total	20	17.20	7.245	1.620	13.81	20.59	7	29

The biofilm is a collection of microbiology and its adhesion to the surface of host cells in an aqueous environment surrounded by extracellular polymers, which are multiple sugars, as the membrane participates in infection, antibiotic resistance, and germ's adhesion to surfaces, which is one of the first steps in the formation of Biofilm. P. mirabilis bacteria form a biofilm that causes serious problems in catheterized patients and about (93.75%) of P. mirabilis isolates showed the ability to form biofilm produces P. mirabilis biofilm. Crystalline as a result of urease activity in the presence of other urinary components such as ions. Crystalline biofilms form in the urinary environment and ultimately prevent urine flow through the catheter. Table 3 shows the effect of the plant extract on the formation of the bacterial biofilm, where the effect of the extract varied according to its concentration, as it gave the best result at a concentration of 90% of the extract in reducing the production of the biofilm, reaching 8.6% compared to the control, where its concentration reached 25%

Table 4. ANOVA table of effect of *Xanthium strumarium* extract on the production of biofilms

ANOVA					
production of biofilm					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	974.800	3	324.933	232.095	.000
Within Groups	22.400	16	1.400		
Total	997.200	19			

Table 4 shows the analysis of variance of the impact of the plant extract on the production of the biofilm, as there had been huge variations between all redress and control, as nicely as there had been good sized variations between each treatment. The awareness of 90% of the plant extract gave the pleasant full-size distinction in contrast to the relaxation of the remedies and manipulate in decreasing biofilm production. These biofilms structure on the catheter surfaces lead to issues such as the trade in the pH by way of the impact of urease, which breaks down urea in the urine, and then raises the ammonia manufacturing pH, permitting the minerals that are deposited on the catheter's biofilm to structure what is acknowledged as a mineral catheter that inhibits urine float and leads to extreme scientific complications, in general, antibiotic cure and everyday catheter modifications are ineffective in treating P. mirabilis infections, particularly catheter obstruction and kidney infections). The formation of biofilms helps shield the pathogen from host protection mechanisms and enhances its resistance to antimicrobial drugs.

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