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EFFECT OF MEAT EXTRACTS OF UROMASTYX ON THE GROWTH OF SOME PATHOGENIC BACTERIA

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A B S T R A C T

Three Egyptian Uromastyxes were collected from different localities of Egyptian desert. After slaughtering of the lizards, their meat was collected and meat extracts were prepared. Meat extract of Uromastyx were screened for antibacterial activities against bacteria causing wound infection as Staphylococcus aureus, Streptococcus pyogen, E.coli and Pseudomonas aerguinosa. These isolates were tested for susceptibility test using agar disc diffusion test which revealed the development of multi drug resistance. The agar gel diffusion method was used to assay the antibacterial activities of meat extract against tested isolates. The results showed the meat extracts inhibit the growth of bacterial isolates with a mean of inhibition zone ranged from 12.125±3.4 to 13.375±2.9. Staphylococcus aureus showed minimal inhibition concentration at dilution 1/160, followed by Ps.aeruginosa at dilution 1/320, while E.coli and Streptococcus pyogen at dilution 1/640 and 1/1280 respectively.

KEY WORDS: Antibacterial Activity, E.coli, inhibition zone, Staph.aureus, Streptococcus pyogens

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1. I N T R O D U C T I O N

Abscesses are circumscribed collections of purulent material found in several species of animals in a variety of locations. This purulent inflammation is usually caused by one of four pyogenic bacteria such as corynebacterium, pseudomonas, streptococcus and Staphylococcus species. Also Staphylococcus species, Streptococcus species, *Escherichia coli*, and Pseudomonas species may cause inflammation and infection of the medullary cavity, cortex, and periosteum of bone. Animals may have lameness, pain, abscessation at the wound site. Uromastyx aegyptius microlepis (Spiny-tail Lizards) belongs to the family Agamidae. It's a solid yellowish color with a usual size of 40 cm of which almost half of this consists of the tail. Males are little larger than females but generally it is best to differentiate between male and female by the presence of prefemoral pores in males. Daub population is distributed throughout Arabia, Southern Iraq, Jordan and Syria [3, 13]. An adult Daub may weigh up to 2 kg. This reptile is a diurnal one and becomes active during the warm season in temperatures ranging from 39 to 41°C [19]. It hibernates during winter in its burrows for a period of 2 to 5 month. Uromastyx is herbivorous but occasionally eat insect especially when they young, feeds on a large variety of plant species as well as on some insects such as grasshoppers and beetles as well as its meat is regarded as a delicacy [2,4]. Studies were applied on the habitat and biology of this wild animal as a prerequisite for future preservatory efforts. The objective of the present in vitro study was to assess the antimicrobial efficacy of meat extract on *Staphylococus aureus*,

Streptococcus pyogen, *E.coli* and *Pseudomonas aeruginosa* as bacteria causing wound infection.

2. MATERIALS AND METHODS

2.1. Preparation of bacterial suspension:

Staphylococcus aureus, Streptococcus pyogen, E.coli and Pseudomonas aeruginosa were obtained from bacteriology department Animal Health Research Institute, Dokki. Each microorganism was grown overnight in brain-heart infusion (BHI) broth, adjusted to a 0.5 turbidity reading on the McFarland scale (1.5×10^8 bacteria/mL) according to British society for antimicrobial chemotherapy [5]. All strains are field strains collected from abscesses of different animals.

2.2. Antibacterial disc used:

Fourteen discs of antibacterial agents were used (Ampicillin 5µg, Amikacin 30µg, Penicillin 10u, Gentamicin 10µg, Ciprofloxacin 2.5µg, tetracycline 10µg, Polymyxin B 300u, Neomycin 30µg, Cefoxitin 30µg, Erythromycin 5µg, Cefotaxime 5µg, Colstin 10µg, *Enrofloxacin* 2.5µg and Streptomycin 10µg).

*2.3. Agar disc diffusion test plate method**:** according to BSAC [5] and measuring zones:*

Plates were inoculated by the adjusted suspension within 15 min to by dipping a sterile cotton-wool swab into the suspension and the excess was removed by turning the swab against the side of the container. The inoculum was spread evenly over the entire surface of the plate by swabbing in three directions. The plate was allowed to dry before applying discs. Discs were firmly applied to the surface of an agar plate which had previously been dried and incubated at 35-37°C in air for 18-20 hours. Diameters of zones of inhibition were measured in (mm) (edge should be taken as the point of inhibition as judged by the naked eye)

2.4. Meat Extraction

Three male Dhub (Uromastyx aegyptius) inhabited in dry habitats were captured from Egyptian desert, brought directly to the laboratory. The animals were sacrificed by decapitation then the blood was collected in clean centrifuge tubes and serum was prepared after clotting by centrifugation at 3000 rpm for 20 min. The sacrificed animals were immediately dissected on a cold plate to remove the tissues studied in the present investigation. Fresh Dhub meat samples (hind limb, fore limb and mid tail) were rapidly weighed (an average of 32 g) and cut into small pieces then homogenized using an homogenizer operating at maximum speed for 1 min in a buffer containing 0.1 M phosphate buffer (pH 6.5) in a ratio of 1:3 (w/v homogenizer in a buffer containing 0.1 M phosphate buffer). All homogenates were centrifuged at 10,000 xg for 45 min. The supernatant fractions were separated according to Kareru et al. [12].

2.5. Dilution of meat extract:

Two fold dilution of meat extract were applied to be tested for antibacterial efficacy (1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640). In order to suggest methodologies for screening the natural products antimicrobial activity, two different qualitative methods were evaluated as follows: agar diffusion test and the determination of minimum inhibitory concentration (MIC).

2.6. Agar gel diffusion test plate method; Ouchterlony *`s technique* [12]:

The bacterial suspension was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled

sterile Mueller-Hinton agar plates, two wells of about 6.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 ml) of the extract of each dilution were then introduced into the wells in the plates.
A control well was in the center with 0.1ml of saline. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur according to Esimone et al. [8]. The systems were incubated for 24h at 36ºC ± 1ºC for 18-20h, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm according to Abayomi [1] and BSAC [5].

2.7. Maximum Inhibitory Concentration (MIC) and Interpretation of MIC:

The MIC of the meat extracts was determined according to the macro broth dilution technique according to BSAC [5] and CLSI [6]. After the meat extracts has been diluted, a volume of the standardized inoculums equal to the volume of the diluted meat extracts is added to each dilution vessel, bringing the microbial concentration to approximately 500,000 cells per milliliter. The inoculated, serially diluted meat extracts is incubated at an appropriate temperature at37ºCfor the test organism for a pre-set period, usually 18 hours. The more consistent the incubation period, the more reproducible the test results after incubation; the series of dilution vessels is observed for microbial growth, usually indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial. The minimal bactericidal concentration (MBC) or the minimum lethal concentration (MLC) of an antibacterial which is defined as the maximum dilution of the product that will kill a test organism can be determined by subculturing last clear MIC tube onto growth medium and examining for bacterial growth.

3. RESULTS

The inhibition zone of different antibacterial agent against different organism was illustrated in table (1), where most of tested microorganisms are resistant to most of antibacterial agents. Pseudomonas aeruginosa was resistance to ampicillin, cefoxitin, cefotaxime, ciprofloxacin, colstin, neomycin and penicillin G and sensitive to enrofloxacin, tetracycline erythromacin, gentamicin and Streptomycin, E.coli was resistance to ampicillin, neomycin, penicillin, ciprofloxacin and cefoxitin and sensitive to amikacin, cefotaxime colistin, enrofloxacin, tetracycline erythromacin, gentamicin and Streptomycin, Staphylococcus aureus was resistant to amikacin, ampicillin, cefotaxime, cefoxtin, colstin, neomycin and penicillin and sensitive for ciprofloxacin, enrofloxacin, erythromacin, gentamicin, neomycin, streptomycin and tetracycline.
Streptococcus species were resistant to amikacin, colstin, gentamycin, neomycin, pencillin and cefoxitin and sensitive for cefotaxime, enrofloxacin, erythromacin, streptomycin and tetracycline.

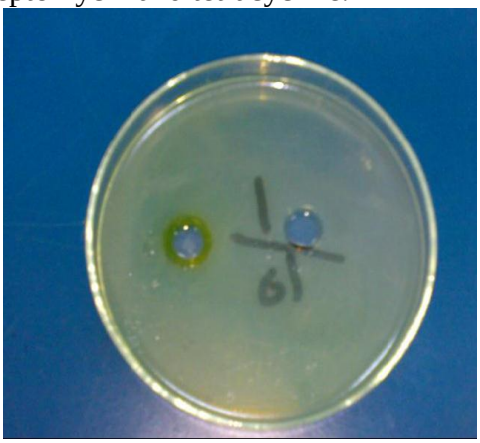

Fig. 1 Represent disc diffusion method: Inhibition zone of meat extract (1/10) against *Staph aureus*

Table 1 Zone inhibition/mm of different antibacterial agents among the different isolates

Antibiotic disc	Disc potency	Zone of inhibition/mm of different isolates			
		Ps. aeruginosa	*E.coli*	*Staph.aureus*	*Strept pyogen.*
Amikacin	30µg	13	19	0	0
Ampicillin	5µg	9	13	9	14
Cefotaxime	5µg	18	30	11	20
Cefoxitin	30µg	19	20	12	18
Ciprofloxacin	2.5µg	10	26	25	22
Colstin	10µg	0	13	0	0
Enrofloxacin	2.5µg	30	33	28	29
Erythromycin	5µg	10	13	10	10
Gentamicin	10µg	15	17	15	11
Neomycin	30µg	0	12	10	10
Penicillin G	10u	11	0	11	14
Polymyxin B	300u	11	0	0	10
Streptomycin	10µg	9	11	12	13
tetracycline	10µg	10	25	14	10

Table 2 Zone of inhibition/ mm of the meat extract on the bacterial isolates

	Dilution	Inhibition zone in mm			
		Ps. aeurginosa	*E.coli*	*Staph. aureus*	*Strept pyopgen*
Meat extract	1/10	16	16	16	16
	1/20	16	15	16	16
	1/40	16	16	15	16
	1/80	15	16	14	15
	1/160	14	16	11	14
	1/320	8	15	10	12
	1/640	10	9	8	10
	1/1280	9	9	7	8
	1/2560	0	0	0	0
Sum		104	112	97	107
Mean ±SD		13±3.2	14±2.9	12.125±3.4	13.375±2.9

Table 3 Maximum Inhibitory Concentration (MIC) of meat extract against different microorganism

	Dilution	Maximum Inhibitory Concentration*			
		Ps. aeurginosa	*E.coli*	*Staph. aureus*	*Strept .spp*
Meat extract	1/10	+	+	+	+
	1/20	+	+	+	+
	1/40	+	+	+	+
	1/80	+	+	+	+
	1/160	+	+	+	+
	1/320	+	+	-	+
	1/640	-	+	-	+
	1/1280	-	-	-	+
	1/2560	-	-	-	-

4. DISCUSSION

*Pseudom*onas aeruginosa exhibits a high degree of drug resistance [9, 11] as well as Staph. aureus [16, 18]. The current data revealed that E.coli showed resistance to different antibacterial agents (ampicillin, tetracycline polymexin. These results agreed with previous study [14]. Also Streptococcus *pyogen* showed multidrug resistance.The development of antibiotic resistance can be viewed as a global problem in microbial genetic ecology. It is a very complex problem to contemplate, let alone solve, due to the geographic scale, the variety of environmental factors, and the enormous number and diversity of microbial participants. The present study was pointed to find other material as meat extract of Dhub to act as antibacterial agents. Agar gel diffusion and MIC were used to study the antibacterial activity of meat extract of dDhub. The meat extract exhibits antibacterial activity against *Ps. aeruginosa, E.coli, Staph. aureus and Streptococc*us pyogen with a mean of inhibition zone ranged from 12.125±3.4 to 13.375±2.9 using agar gel diffusion technique. The activity of meat extract disappeared at a dilution 1/2640. Maximum Inhibitory Concentration (MIC) showed the antibacterial activity, where the lowest concentration (highest dilution) of test agent preventing appearance of turbidity (growth) is considered to be the minimal / minimum inhibitory concentration (MIC). *Staph.aureus* showed inhibition at dilution 1/160, followed by *Ps.aeruginosa* at dilution 1/320, while *E.coli* and streptococcus pyogen dilution 1/640 and 1/1280 respectively. These results may be to Uromastyx is feeding on different type of the plant dessert. Earlier studies [10, 7] identified 23 plant species which may contain antimicrobial active agents such as alkaloids, resins, and tannins, which inhibit the growth of the bacteria. These established a good support to perform further research on this animal as base for the development of new drugs which can be used as a natural substance against common wound bacterial infections. There is a variation in antibacterial activities meat extract against different microorganism which revealed that concentration of meat extract of duhb play a role on its activities as well as the high resistance of different microorganism. Previous work [17] showed that ESbLs are the derivatives of common b-lactamases (TEM and SHV b-lactamases) that have undergone one or more amino acid substitutions near the active site of the enzyme, thus increasing their affinity and the hydrolytic activity.

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الملخص العربى

اجريت هذه الدراسة بهدف اختبار تاثير مستخلص لحم الضب على نمو البكتريا المسببة لعدوى الجروح. تم تجميع عدد ثلاثة من الضب المصري من مختلف صحراء مرسى علم – البحر الاحمر و عمل مستخلص من اللحم بعد الذبح . تم اختبار تاثير مستخلص لحم الضب على نمو البكتريا المسببة لعدوى الجروح عن طريق اختبار اقراص الجل السابق تحضيره ضد الميكروب العنقودي الذهبي الميكروب السبحي والميكروب القولوني والميكروب الاخضر الصديدي. اظهرت النتائج تثبيط لنمو تلك الميكروبات بمتوسط حيز من عدم نمو قُدر بـ 12,125±3,4 الى 13,375±2,9. كما اظهرت نتائج تثبيط النمو ان الميكروب العنقودى، الميكروب الاخضر الصديدى، والميكروب القولونى، و الميكروب السبحى توقف نموها فى وجود مستخلص لحم الضب حتى فى اقل معدلات تخفيف وصلت الى 160/1، 320/1، 640/1، و1280/1 على الترتيب.

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