

Antimicrobial effected of red dragon(*Hylocereus polyrhizus*) on bacterial isolated from frozen meat .

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Abstract :

The present study pointed to estimated the validation of red dragon fruit (*Hylocereus polyrhizus*) extracts against three microbial pathogens (*Staph. E.coli* and *Salmonella*) isolated from frozen meat samples.

One hundred twenty frozen meat samples were aseptically collected from local markets of Baghdad ,familiar bacteriological mechanism were used to isolate *Staph. aureus*, *E. coli* and *Salmonella spp.* *Ecoli* 35(29.1%) is high percentage,*Staph .aureus* 21(17.5%) and *Salmonella* 14(11.6%) isolates .The antibacterial efficiency of red dragon fruit (*Hylocereus polyrhizus*) extracts was determined by the agar well diffusion method to investigate the minimum inhibitory concentration (MIC) at different concentration started with 20, 10, 5, 2.5 and 1.25 mg/ml. The highest antibacterial activity between 20 mg/mL and 5 mg/mL except in *Salmonella spp.* the value of inhibition was between 20 mg/mL and 10 mg/ml.The highest zone of inhibition of *Staph. Auras* (27 mm) While (22mm ,17mm)of *E.coli* and *Salmonella* respectively at 20 mg/mL. (21,22,12 mm) inhibition zone against *Staph. aureus*, *E. coli* and *Salmonella spp.* respectively at 10 mg /ml and (15, 10) mm against *Staph. Aureus* and *E. coli* at 5 mg/ml. Antimicrobial disc revealed all bacteria resistance to Bactircin, Amoxicillin clavulanic acid and Carbencillin disc, while sensitive to Tetracycline, and intermediate zone of inhibition to Ceftriaxon.

Key words : red dragon(*Hylocereus polyrhizus*), frozen meat ,antimicrobial, *Staph* , *E.coli* ,*Salmonella*

Introduction :

Meat is renowned as a scope intermediate for pervasion food-borne diseases due to its high water action , high protein content, and approximately neutral pH, which create suitable state for the growth of bacteria, contaminated meat depends on number of bacteria , physicochemical properties , time/temperature of storage of meat (Mc Guinness *et al.* ,2009).

Generally , contamination occurs because of inappropriate sanitary conditions and handling in slaughterhouses, the ability of bacteria across contamination by forming the biofilm, in addition to condition before slaughter as feeding and housing including contaminations from skin and feces by contents of digestion system (Doulgeraki *et al.*,2012).

One of the most common food poisoning is *Staphylococcal* and is of major concern in public health programs worldwide (Hennekinne *et al.*,2012). Food poisoning by *Staphylococcal* due to its produced enterotoxins, this enterotoxin characteristic by heating resistance (heat stable), acid-resistant, and resistant to proteolytic enzymes effect as pepsin and trypsin (Jablonski and Bohach 1997).

The normal flora in the intestine of humans and warm-blooded animals is *Escherichia coli* from Family Enterobacteriaceae causes highly foodborne disease. Undercooked ground meat products, raw milk, and contaminated raw vegetables the most contamination in human (Castro *et al.* ,2017)

Salmonellosis are major problem to causes Food borne diseases represent a for health in developed and developing countries, Over two thousand serotypes of *Salmonella* can cause food poisoning in worldwide as *S. typhimurium*, *S. enteritidis*, the most frequently isolated⁶. Risk of salmonellosis represented by the ingested bacteria and multiplying in the intestine of the host and invading the Gastro Intestinal tracts more than to release toxins (Birmingham *et al.*,2006) .

Red Dragon fruit (*Hylocereus polyrhizus*) is a nutritious fruit cultivated throughout Asian countries particularly. The fruit have special shape and majestic color with mouthwatering taste in addition to have vital nutritional ingredients as carotene, calcium, fiber, vitamin B, vitamin C, and phosphorous (Kirti *et al.*,2020). Red Dragon fruit peel extract contain many the phytochemical compounds :Flavonoids, phenols, hydroquinones, and saponins. Also Steroids and triterpenoids composition found in peel therefore could stop the growth of bacteria (Ichsan *et*

al., 2018) .Red dragon fruit peel extract is also proved to be able to inhibit the growth of *Salmonella pullorum* and *Staphylococcus aureus*(Arief *et al.*,2015).

The current investigation was conducted to estimate the bacterial load of Meat in local Iraqi markets and estimated antimicrobial activities of the red dragon fruits extracts were associated with high phytochemical compounds and total phenols contained in the extracts.

Material and methods :

Area of study :

The study was conducted in Baghdad Province, Iraq. was done in the Bacteriology Laboratory, Department of zoonosis unite , Faculty of Veterinary Medicine, University of Baghdad.

Frozen Meat sample collection :

One hundred twenty frozen meatsamples were collected from local markets in Baghdad city ,the samples were packed, identified,transferred in ice-box and immediatelyprocessed at zoonosis laboratory –veterinary medicine collage .

Preparation of samples :

The samples were cut with a sterile knife, 25 g of each samples were added to 225ml buffered peptone water 0.1% (Bakhtiary *et al.* ,2016).Thesamples were then homogenized for 2 minutes.Ten fold serial dilutions were prepared forfurther analysis.

***Staphylococcus* isolation :**

One ml of sample suspension added to Mannitol salt platesagar then incubated at 37°C for 24 hours. Gram stain and Biochemical tests was done to identified *S. auras* as catalase activity and coagulase tests.(Bannerman ,2003) Gram stain, catalase enzyme, coagulase and other common biochemical tests were used to confirm the identity of *S. aureus*.

***E.coli* isolation:**

One ml of samples suspension added to MaCconky plates agar and Eosin methylene blue agar incubated at 37°C for 24 hours. Gram stainand Biochemical tests was done to identified *E.coli* by using The API-20E test kit for the identification of enteric bacteria(MacFaddin ,2000).

***Salmonella* isolation:**

One ml of samples suspension added to 10 ml Tetrathionate broth incubated at 37°C for 24 hours then inoculated the enrichment broth on Xylose Lysine Desoxycholate (XLD) agar and Salmonella-Shigella (S.S) agar incubated at 37°C for 24 hours. Gram stain and Biochemical tests was done to identified *Salmonella* by using The API-20E test kit for the identification of enteric bacteria (Koneman *et al.*,2005).

Preparation of red dragon fruit extract :

Dragon fruit was obtained from the popular Malaysia market ,kept cold . then washing with cleaned water and cut the red dragon fruit into small pieces (2mm), dried for 4 days , use the blender until its became powder then weighed .

Soak the dry fruit in 96%ethanol Sterilization as ratio 7.5 times the weight of the powder , filter the maceration by using filter paper. Then dry it by using a rotary evaporator at 45-50 °C.kept the extract at 4°C until further uses. making extract concentration of (200, 150, 100 50, and 25 mg/ml) (Rahayu *et al.*, 2019).

Preparation of microorganism and inoculums :

organisms were culturing on brain heart infusion agar overnight, then used for the preparation of bacterial suspensions diluting overnight cultures in saline and adjusted to 0.5 McFarland turbidity standards to approximately 10⁸ CFU ml for each bacterium.

Agar well diffusion method for antibacterial activity :

Mueller Hinton agar (MHA, Oxoid) , media were prepared according to the instructions of manufacturers company.The Petri plates cultures by bacterial tested inoculated (0.2 ml each) using the sterilized swabs then a sterilized stainless steel borer was used to formed five wells (6 mm diameter), the red dargon extract was dissolved in sterile distilled water at concentrations of (20, 10, 5, 2.5 and 1.25 mg/ml),then 100 µl of each concentration of the plant extract were filled each well . The Petri plates were incubated at 37°c for 24 hours in an incubator and zone of inhibition(mm) was observed and measured using a scale around the extracts (Lino and Deogracios,2006).

Antimicrobial Susceptibility Testing:

The teste done according to the Kirby-Bauer method by diffusion of antibiotics on the surface of Muller-Hinton agar. the preparation of bacterial suspensions diluting overnight cultures in saline and adjusted to 0.5 McFarland turbidity standards to approximately 10⁸ CFU ml for each bacterium then inoculated Muller-Hinton agar with bacterial suspension by sterile swab (0.2 ml each) with dry the plates for ten minutes .Put the disk with 15 mm from the edge, measured the inhibition zone using a scale around the antibiotic disc(Bauer *et al.* ,1966).

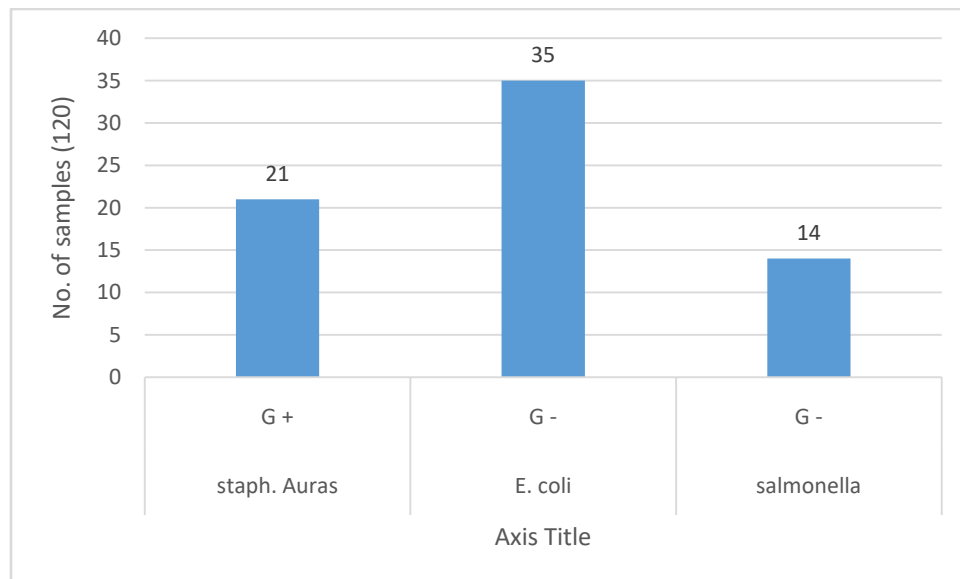
Result and discussion :

According to table(1) , figure (1) the percentage of contaminated *Staph. Aureus* *Salmonella* and *E.coli* in frozen meat in current study show that the isolation rates of *Ecoli* 35(29.1%)is high percentage, *staph* 21(17.5%) and *Salmonella* 14(11.6%) isolates conferring to their morphological characterization and biochemical tests,this results agreement with(Abdelraouf *et al.* ,2013) who found 11 (7.3%) samples were positive with *Salmonella* in Gaza strip ,also(Eman *et al.* ,2014) found *Escherichia coli* (40%) and *Staphylococcus aureus* (29%) in butchers shops, with *E.coli* (19%) ,*S.aureus* (28%) and *Klebsiella sp.* (9%) in restaurants,while Other reports recorded that *S.aureus* frequently are present in low numbers on raw meat surface occurs infrequently (Saadia and. Hassanein(2010).The reason of contamination may be to the location of the animal (housing) ,feeding in addition to and the instrument used in slaughter the animals and places of slaughter as well as the butchery itself and the contamination of hands and even the wood used to cut flesh and without sterilization

Table (1): percentage of *Staph. Aureus*, *Salmonella* and *E.coli* isolated from frozen meat :

samples	Bacterial isolated	Gram stain	Percentage
Frozen Meat (120)samples	<i>Staph .auras</i>	G+	21(17.5%)
	<i>E.coli</i>	G-	35 (29.1%)
	<i>Salmonella</i>	G-	14(11.6%)

Figure (1):percentage of *Staph. Aureus*, *Salmonella* and *E.coli* isolated from frozen meat



Morphology and biochemical test :

All staphylococcal isolates from different samples showed the colonies of *Staphylococcus aureus* on the Mannitol salts agar utilization of salt and give yellow color, coagulase positive, oxidase negative, catalase positive, and DNase positive, in addition beta hemolysis test on blood agar (Table: 2) hemolysis (α , γ or β hemolysis) on blood

Table (2): Result of Biochemical reaction of *S.aureus* :

Biochemical reaction of <i>S. aureus</i>	
Slide Coagulase test	+
tube Coagulase test	+
Oxidase test	-
Catalase test	+

All *E.coli* isolates from different samples fermentation the lactose in MacConky agar and give pink in color and give metallic sheen in Eosin methylene blue agar with results of API-20E test kit for the identification of enteric bacteria.

Salmonella isolates give pink colonies with black centers on Xylose Lysine Desoxycholate (XLD) agar, and pale, smooth, rounded black centered colonies on

Salmonella-Shigella (S.S) agar and results of API-20E test kit for the identification of enteric bacteria.

Antimicrobial effect of red dragon fruits :

In our study and according to table (3) the inhibition zone of red dragon fruit (*Hylocereuspolyrhizus*) extract against the growth of *Staph. Aureus*, *Salmonella* and *E.coli* show the biggest drag zone found against *staph .aureus* (27 mm), (22 mm) against *E.coli* and(17mm) against *Salmonella* in 20mg / ml .

while *S . aureus*, *E.coli* and *Salmonella* had 21mm,16 and 12mm respectively in10 mg/ml of the extract concentration .

At 5 mg/ml showed the highest zone of inhibition in *S.aureus*15mm and *E.coli* 10mm while in *Salmonella*not sensitive to the extract . all bacteria were not sensitive to the extract at 2.5mg/ml and 1.25 mg /ml. (fig.2).

Our results revealed the highest zone of inhibition Gram-positive bacteria, *S. aureus*, was extra susceptible to the antibacterial activity of the red dragon fruit peel extract are agreement with(Manihuruka *et al* .,2017) who reported High inhibition zone produced by red dragon fruit against *staph . aureus* and supported by Arief *et al* ., 2015)who analysis that the Gram-positive bacteria were more susceptible to antibacterial activity due to the absence of a lipoprotein wall that capable of preventing antimicrobial compounds.

The inhibition zone observed in gram negative bacteria *E.coli* with medium zone and less in *Salmonella*according to results are conducted by(Nurmahani *et al.*, 2012)The inhibit the growth occur due to Phenolic compounds in the extracts of pomegranate peel Phenolic compounds may inhibit the growth of bacteria with the ability of damaging the cytoplasmic membrane and proteins as well as inactivated some bacterial enzymes (Choiet *al* .,2011).

Antibacterial activity against both bacteria was also observed by (Tahera *et al* .,2014).

Table (3): The average of inhibition zones of red dragon fruit against *Staph. Aureus*, *Salmonella* and *E.coli*:

Concentration of red dragon	Zone of inhibition (mm diameter)		
	<i>Staph. Aureus</i>	<i>Salmonella</i>	<i>E.coli</i>
1.25mg/ml	-	-	-
2.5 mg/ml	-	-	-
5mg/ml	15	-	10
10mg/ml	21	12	16
20mg/ml	27	17	22

Figure (2): The average of inhibition zones of red dragon fruit against *Staph. Aureus*, *Salmonella* and *E.coli*:

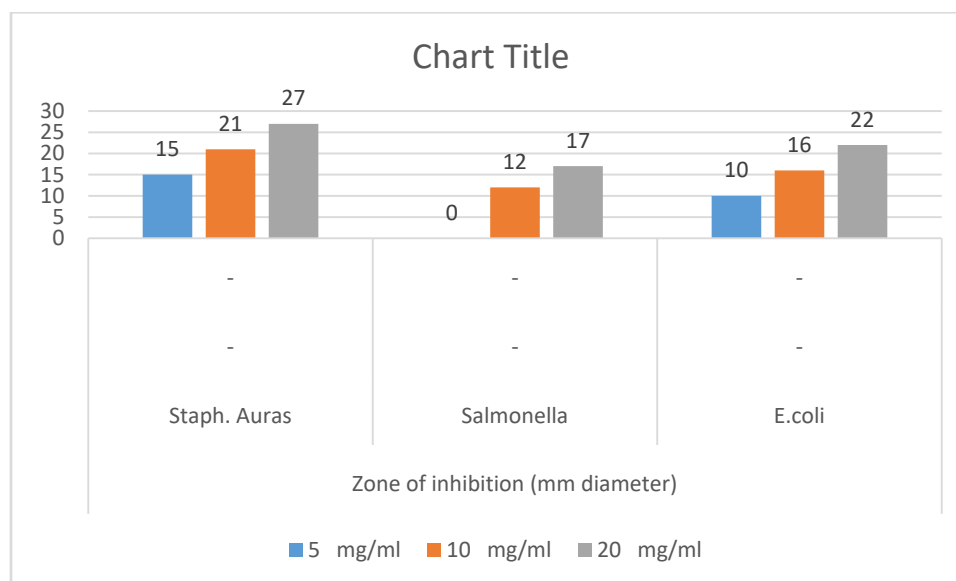


Table (4) revealed different results of microbial against the antimicrobial disc. All bacteria showed resistance to Bactircin, Amoxicillin clavulanic acid and Carbencillin disc against the antimicrobial disc .The study showed that all the isolates were sensitive to Tetracycline, also all isolated intermediate zone of inhibition to Ceftriaxon .

Our results matching with (Eman *et al* .,2014)who showed *S.aureus* isolated from fast food samples more resistance rate to commonly used (Lincomycin ,

Erythromycin and) and *E. coli* low resistance to amoxicillin. Also agreement with (Cooket *al.* ,2009) reported about the antimicrobial resistance in *Campylobacter*, *Salmonella*, and *Escherichia coli* isolated meat from southern Ontario.

In Saudi Arabia (Al-Humam, 2019) showed Antimicrobial susceptibility of 40% of *E. coli* isolates to be ESBL is higher than (Bouchillon *et al.* ,2004) who reported Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* from some European countries .

Table (4): Mean of inhibition zone diameters of antibiotics:

Antibiotic	Ceftriax on (CRO)	Trimeth eprime (TMP)	Genta mycin (CN)	Doxyc yclin	Azithro mycine (AZM)	Carben cillin (Py)	Tetracy cline (TC)	Amoxic illin clavulan ic acid	Bacti rcin(B)
<i>Staph</i>	I	I	S	S	S	R	S	R	R
<i>E. coli</i>	I	R	R	I	I	R	S	R	R
<i>Salmonella</i>	I	S	S	I	S	R	S	R	R

R:resistance , I: intermediated , S:sensitive

Conclusion :

Based on the results of this study its concluded the red dragon fruit extract (*Hylocereus polyrhizus*) have antibacterial activity against *Staph. aureus* *E. coli* and *Salmonella*. The red dragon fruit extract (*Hylocereus polyrhizus*) concenter Natural antibacterial material .

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