



# THE GROWTH SUSCEPTIBILITY TEST OF *SERRATIA MARCESCENS* IN THE PRESENCE OF CRUDE *CAPSICUM ANNUM*

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## Abstract

This study was carried out in order to isolate *Serratia marcescens* (*S.marcescens*) from bovine mastitis and study the effect extract of crude *Capsicum Annum* against *S. marcescens* in vitro. Collection was done for cows from Baghdad and revealed 150 samples for cases of mastitic milk from the period of December 2018 to December 2019. Cultured by selective and differential media then Gram stain was done. After purification of cultured bacteria, biochemical tests occurred and confirmed by API 20 E System and the kit of RapID™ One System. It was clear from the results that 6 (5%) samples out of 150 samples were positive for the target bacteria *Serratia marcescens*. Well diffusion technique was used to detect the antibacterial activity. Results presented antibacterial action of *Capsicum Annum* extract against *S.marcescens*, then showed antibacterial activity of *Capsicum Annum* extract with different concentrations against *Serratia marcescens* isolates. The highest inhibition zone for the bacteria *S.marcescens* at 10mg/ml towards 100mg/ml concentrations ranged from 16±0.57 to 33.3±0.3.

**Key words :** *Capsicum Annum*, antibacterial activity, *Serratia marcescens*, bovine mastitis.

## Introduction

*Serratia* species are one of gram negative family, the enterobacteriaceae, and the ethnic group Klebsielleae, it is opportunistic bacteria which widespread environmentally (Donnenberg, *et al.*, 2010). One of the most important pathogenic species of this bacteria is *Serratia marcescens* (*S. marcescens*), that rare reports gave description for diseases from infections with other spp such as *S.odorifera*, *S.plymuthica*, *S.liquefaciens*, *S. rubidaea* and *S. fonticola* (Mahlen. 2011 and Carrero *et al.*, 1995). Some of *S.marcescens* strains have the ability of producing “prodigiosin” pigment which ranges from deep red color to light pink color depending on the age of the coloniesæ generally *Serratia* growth could occurs in different circumstances and diverse environments, such as the tracts of different digestive systems for various animals, soil and water; at the contrary, *S.marcescens* has slow and weak growing on starchy materials like foodstuffs, that the pigmented colonies could give misguidance while reading the result by appearing as drops of blood (Grohskopf. *et al* 2001 and Ursua 1996). Pathogenic bacteria or mycotic

pathogens seem to be the most infectious agents causing mastitis that about 140 microorganisms were isolated from the mammary glands (Hauber *et al.*, 2017). Moreover, these pathogens in cattle are divided into 2 groups classified as major and minor pathogens, referring to those that cause clinical and subclinical mastitis subsequently (Mekonnen, *et al.*, 2017). The major phase of cow’s infection with mastitis appeared to be during the dry period (Oliveira *et al.*, 2015) while the minor one includes the lactation period (Kahn, 2005), it is worth to mention that specific species of *Serratia* can act as causative agents for producing mastitis as, *S. rubidaea*, *S. liquefaciens* and *S. marcescens*. (Mahlen, 2011 and Radostits *et al.*, 2007). Other side of view believe that mastitis caused by *Serratia* outbreak suppose to be occurred due to microbial growth in teat dip cup or/and in the bedding (Hogeveen, *et al.*, 2011 and Schukken. *et al.*, 2012). Furthermore, teat ends damage or inappropriate hygiene might be accepted reasons for elevating the mastitis inquiry (Vida *et al.*, 2019), at the time that *Serratia* is believed to be easily transmitted environmental microbe by the milking machine (Guarín, 2017). Serological typing can decide whether the detected strain of *Serratia* is a single or different strain, from contagious infectious transmission

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or from point source (Teresa *et al.*, 2015). This disease clinical signs might characterized by milk discoloration and flakes (Mari *et al.*, 2019), the tendency of infections varied from chronic to long lasting (months to years); actually, infection develops regardless milk production or production string (Ruegg, 1992). For a long period of time, especially last couple of decades, maintaining human health was the major goal for the researchers and scientists, therefore, they tried hardly to use natural products and plants as therapeutics with valuable effects after intensive studies depending on plant compounds for pharmaceutical purposes according to WHO, the manufacture of sterile medical products covers a wide range of product types (sterile active substance through to finished dosage form) (Sabine, K. 2020), medicinal plants act as awesome source for producing a variety of drugs, that over 80% of the whole population from developed countries completely depending on using traditional medication, which has specific compounds derived from medicinal trees or plants (Ciddi, 2012). Though, safety and efficiency for these plants should be detected properly with more studies (Syta, *et al.*, 2012). World widely, medicinal plants depended as effective antiviral (Muhmud, *et al.*, 2005) antibacterial and antifungal agents due to the safety from harmful side effects and resistance that pathogenic microorganisms could show towards antibiotics (Burna, 2019). Generally, more focusing on extracting biological active materials from plants and herbs that used as medicine, these extracts contributing in curing diseases like urinary tract infection, cervicitis, vaginitis, gastrointestinal disorders (Anjana, 2009) and skin infections such as herpes simplex virus type (1) (Muhmud, *et al.*, 2005).

As a nutritional material, Chili peppers are in use for a large number of populations not only for the strong flavor and aroma, but also to prolong food spoilage in which their huge contents of capsaicin reaches millions of Scoville heat units, this was encouraging enough to examine their antimicrobial and antifungal action. Thus, chili pepper extracts was tested to be determined instead of artificial preservatives in the food industry to resist pathogens (Kim, 2014). *Capsicum* species and its chemical material of Capsaicin were studied in chili peppers, presenting high level of biological activity effecting digestive systems, nervous system, and cardiovascular system; The reason behind that is the content of vitamin C (up to 6 times the concentration of an orange) in the *Capsicum* fruits when analyzed Chemically (Brito, *et al.*, 2009). Since consumer are always in need to the safety along with the good quality foods, there was a demand to use natural origin chemical

preservatives for foods especially with the increasing awareness of the diseases outbreaks and fight bacterial infections with antibiotics has been a longstanding cornerstone of modern medicine (Susanne, 2019). Moreover, food polluter microbial pathogens are facing the problem of the repeated usage of the drugs leading to side effects and resisting drug use issue which in some cases no medication is sufficient to resist the microorganism such as the case of *S. marcescens*, making the need to new cost consuming, more simple, more safe to be used as an effective treatment, hence, using natural nutritional materials would be the solution to avoid using chemical therapeutics like depending on chili peppers to control microorganisms that *Capsicum* genus considered one of the effective antimicrobial and antifungal compounds (Morrine, *et al.*, 2011).

## Materials & Methods

### Milk sampling

Collection of 150 samples was done from the areas of Abu- Ghraib, College of Veterinary Medicine/ University of Baghdad, College of Agriculture/ University of Baghdad, Al-Dora and Al-Radhwanya during the period of December 2018 to December 2019, samples collection occurred from cows suffering from clinical and subclinical signs of mastitis. At the very beginning, tap water was used to wash the udder in order to remove undesired dirt then dry with clean towel, the teat dipped in Iodine solution 1:1000 and left to dry followed by dipping in 70% alcohol then dried, before sample taken one or two streams of milk discarded.

10 ml sterile test tubes were used to transfer milk in cooled container to the lab.

### Preparation of *Capsicum annum* extract

*Capsicum annum* extract from was prepared following the method described by Bacon *et al.*, 2016 (Bacon, *et al.*, 2016), without solvent, extract residues were stored immediately at 4°C until re-use.

### Identification of *Serratia marcescens*

#### Cultural and Microscopical characteristics

Using Nutrient agar to grow the bacteria and examine the colonies shape and color, was followed by other media to grow the suspected isolates, such as MacConkey agar, Luria agar, Xylose lysine deoxycholate agar by streaking method. Raffinose, arabinose, peptone broth and sugar fermentation were similarly used to check identification. Moreover, light microscope was used along with Gram stain to differentiate between Gram negative and/or Gram positive bacteria. Then bacterial culture was purified and biochemical tests were done, like API 20 E System,

RapID™ ONE System kit to confirm the diagnosis of *S. marcescens*.

### Statistical Analysis

SPSS program was helpful to analyze obtained data, while Duncan Multiple Test was the program in use to compare between the means (Duncan, 1955).

## Results and Discussion

### Microscopically, cultural characteristics, biochemical and confirmatory tests

Out of the all 150 milk samples, only 6 isolations showed their positivity to be belong to *Serratia marcescens* bacteria, this representing 4% of the milk samples isolates, thus, our results are in agreement with the results of (DiGuardo, 1977), which revealed that 4(3%) out of 120 cows affected by the same bacteria and causing mastitis.

Results varied at culturing samples showing differed reactions on media morphologically, that at MacConkey agar and after incubation at 37°C for 24hours, *S. marcescens* appeared as red colored colonies due to their ability to ferment lactose sugar and produce pigments as showed in Fig. 1. These results are in agreed with (Quinn, *et al.*, 2004). Microscopically, they appeared as gram negative rods, on other hand, biochemical identification of *S. marcescens* confirmed that the bacteria were Gram negative and gave negative test results for oxidase, Urease and Indole tests; at the time that it gave positive results for Catalase, citrate utilization and DNase tests; Besides, it was lactose non- fermenter, motile, and for the TSI it was: y/y, and, these results were shown in table 1 as (Karleigh, *et al.*, 2016). Confirmation of the diagnosis was depending on API 20 E system and RapID™ONE System

Many workers investigated the antimicrobial activity of *Capsicum annum* fruits, reaching the suggestion that the antimicrobial components are the Capsaicin or other cinnamic acid pathway intermediates (Asmaa, *et al.*, 2017).

Different pathogens show resistance to specific antimicrobial agents depending on their strains and types (Karleigh, *et al.*, 2016). Therefore, trial occurred using

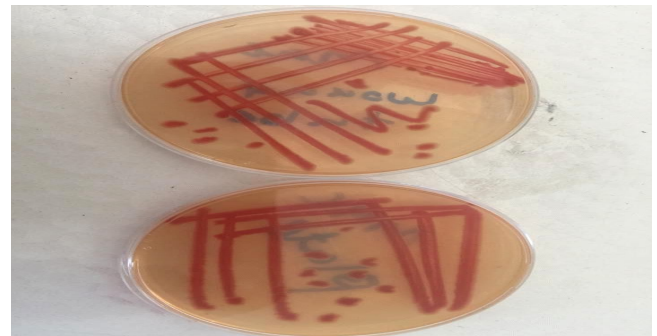
disc diffusion method on Mueller–Hinton Agar agreeing to (Jawetz, *et al.*, 2007)

Sensitivity of *Serratia marcescens* after (24hrs.) of incubation, represented by the inhibitory zone diameters towards different antibacterial concentrations revealed similarity with the findings of (Asmaa, *et al.*, 2017). the results showing high action for the extract of *Capsicum Annum* as an antibacterial agent against specific isolates, as shown in Fig. 2, the Highest inhibition zone is ( $33.33 \pm 0.33$ mm) was recorded against *S. marcescens spp.* respectively using disc diffusion method from extract ascending from low- high concentrations, while proportional increasing for diameters of the inhibition zones with every using of 20, 40, 60 and 80 mg/ml concentrations towards the same tested strain with statistically significance at ( $p < 0.01$ ), on other hand, statistically insignificance appeared between 20 and 40mg/ml concentrations at ( $p < 0.01$ ) as shown in (Table 1).

Lowest inhibition zone ( $16.0 \pm 0.57$ mm) was recorded for *S. marcescens* is towards the tested isolates also from extract at a concentration of 10mg/ml to the highest concentration 100 mg/ml. as shown in Fig. 3.

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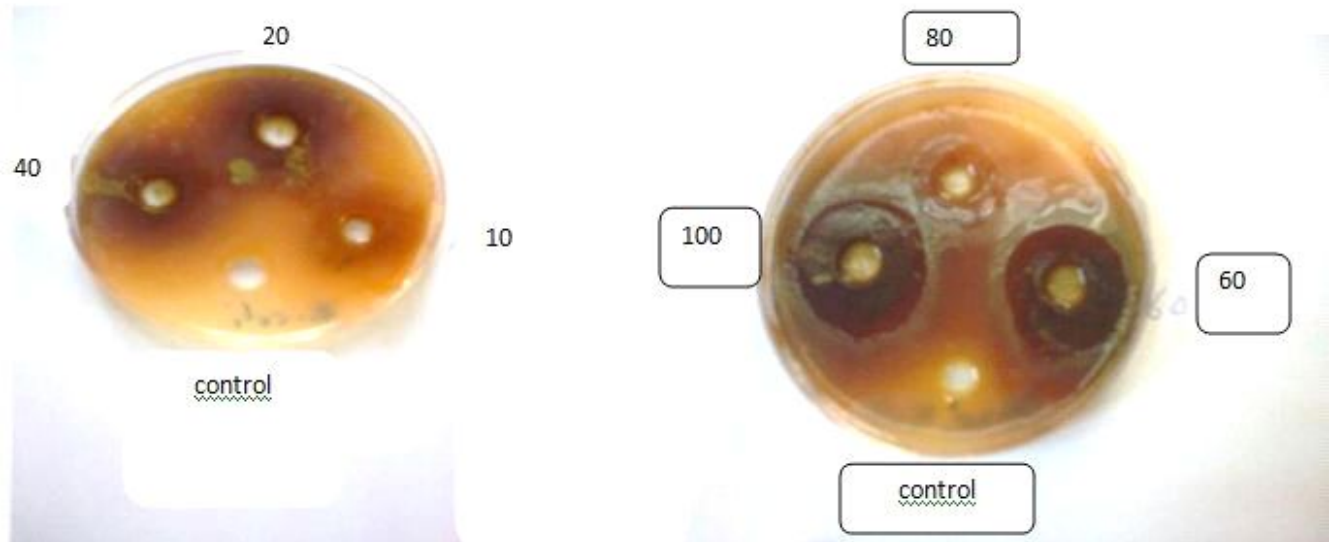


**Fig. 1:** Colonies of *Serratia marcescens* on Luria agar showed the ability of bacteria to produce pigment.

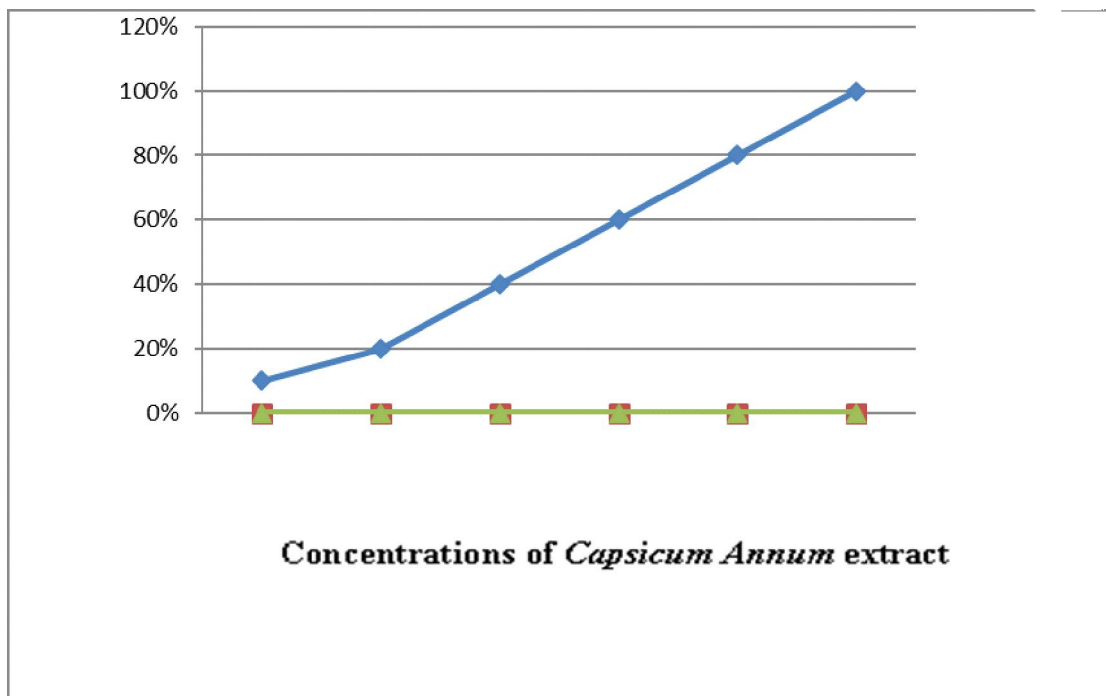
**Table 1:** The mean zone inhibition diameters (mm).

Bacterial Spp.	Concentrations of <i>Capsicum Annum</i> extract					
	10%	20%	40%	60%	80%	100%
<i>S. marcescens</i>	16±0.57Fa	21±0.57Ea	27.3±0.6Da	29±0.57Ca	31.3±0.6Ba	33.3±0.3Aa

Capital letters denoted the significant differences between concentrations at ( $p < 0.01$ ) while small letters denoted the significant differences between isolates at ( $p < 0.01$ ).



**Fig. 2:** Inhibition zones resulted from using different concentrations of *Capsicum Annum* extract against *S. marcescens*.



**Fig. 3:** The relationship between concentration concentrations of *Capsicum Annum* extract (%) and zone inhibition (mm) for *S.marcescens*.

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