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IMPACT OF DIFFERENT CONCENTRATIONS OF TANNIC ACID ON VARIOUS BACTERIA ISOLATED FROM BEEF MEAT

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Abstract

This investigation evaluated the inhibitory impacts of various tannic acid levels on the growth of bacteria spp. that are isolated from beef in order to discover the likely preventive mechanisms in studying the antibacterial influence of tannic acid. A total of 20 samples of raw beef, five sample each, were taken from each store in Baghdad. These samples were transported to the lab for microbiological analysis directly. The inhibitory zone of tannic acid against the Gram-positive and Gram-negative bacteria was measured. Also, tannic acid was added to the motile media to detect the motility. These bacterial isolates were identified by VITEK system. The VITEK findings showed that the fewest total bacterial counts and appeared to be coliform-free. Gram-negative rods made up the majority of the total isolates from the various types of meat, whereas Gram-positive cocci were the most type of bacteria. Lactobacillus spp. and methylobacterium spp. (a genus of Hyphomicrobiales) were the most common bacteria found in fresh meat. Consequently, this study showed that various tannic acid concentrations might perform some antibacterial activities through a range of mechanisms, such as inhibition, rather than through a particular mechanism of action.

Keywords: Tannic acid, inhibition zone, bacterial motility, Vitek 2 compact

Introduction

The capacity of tannic acid to snare oxidative stress and form molecules has made it more and more crucial as a biopolymer. However, a thorough analysis of tannic acid's blood safety has not been carried out. Some naturally occurring polyphenols produced from botanicals, such as tannic acid, have found increased usage in pharmaceutics as a result of the quick advancements in biotechnology. The polyhydroxy benzene complex tannic acid, also known chemically as 3,4,5-trihydroxy benzoic acid, is commonly present in the leaves and stems of plants, including fruits, persimmons, bananas, teas, pomegranates, and oak leaves. Tannic acid possesses potent physicochemical properties [1, 2].

Tannic acid can attach to gastrointestinal cells, which could disrupt the metabolism; however, it has an antioxidant impact. Additionally, it might stimulate platelet aggregation in the bloodstream and bacteriostasis. It has a strong free radical-trapping capability on polyphenols due to the presence of phenolic hydroxyl groups [3]. Iron-phenol complexes have gained wider acceptance as polymers subsequently. Tannic acid, for instance, may build metal-polyphenol systems by combining with several metal-ions via coordination bonds and being employed as a biodegradable

model medicine; therefore, tannic acid has indeed been proven to have an interfering impact on growth because it decreases enzyme activity and damages cellular membranes, metal-ions, and minerals [4].

It has been demonstrated that the phenolic chemical tannic acid, which is both naturally occurring and synthesized, is a potent inhibitor against both bacteria and viruses [5]. According to several reports, tannic acid found in plant materials possesses inherent anti-inflammatory, antibacterial, antiviral, and antifungal properties [6, 7, 8, 9].

Consumption of unsafe, contaminated meat leads to food-borne diseases, which cause considerable morbidity and mortality in consumers. Staphylococcus aureus is a well-known opportunistic foodborne pathogen and is involved in numerous nosocomial and community-associated (CA) outbreaks worldwide [10]. A high percentage of food poisoning each year is caused by several pathogens including Staphylococcus aureus, Salmonella spp., Clostridium perfringens, Campylobacter spp., Listeria monocytogenes, Serratia spp. and pathogenic E. coli. The initial level of post-productive contamination as well as the numerous intrinsic and extrinsic parameters of a product determines its microbiological stability and consequently affect the safety of consumers causing serious long-term health effect of foodborne hazards [11, 12].

However, there have been few data and evidential studies on the impact of tannic acid on pathogenic bacteria isolated from beef compared with antibiotic sensitivities. Thus, this study assessed the inhibitory effects of different concentrations of tannic acid on the growth of many bacterial types that are isolated from the beef to identify the probable pathways of prevention in examining the antimicrobial impact of tannic acid.

Materials and Methods

Collecting samples

Four distinct areas of Baghdad/AlKarkh were used to gather a total of 20 samples of raw beef meat; moreover, five samples were individually taken from each store in these areas. About 100 grams of sterile, dry, and clean polythene bags containing samples of meat were used to transport them to the lab for microbiological analysis. Once there, they were processed within hours of collection by being cut into small pieces with a sterile blade and cultured in 250 mL of sterile nutrient broth (Oxoid, Basingstoke, UK) for 24 hours under aerobic conditions at 37°C [13]. The beef samples were clinically tested during the trial between December 2021 and January 2022 to identify bacterial isolates in raw meat. The collecting bags were shaken vigorously and the sample was collected.

Processing of the samples using culture media

The development of a diverse spectrum of non-fibrous organisms is supported by the general-purpose medium known as nutrient agar. Because it promotes the development of several kinds of bacteria and fungi and includes many of the nutrients required for bacterial growth, nutritional agar is widely used.

Before biochemical or serological testing, nutrient agar can also be employed for quality control and purity verification. Beta-hemolysis, which was carried out following normal techniques by

streaking bacteria together with S. aureus on blood agar plates containing 5% blood and noting zones of hemolysis, was used to identify colonies produced on sheep blood agar plates.
Gram-negative bacteria can only be grown on the selective media (EMB agar), not Gram-positive bacteria. The different gram-negative bacteria and enteric bacteria also referred to as coliforms and fecal coliforms may be isolated and distinguished using EMB agar.

The motility

The motility media were made using Milli-Q and sterile water. To inoeulate into the motility experiments, the Bacteria samples were cultured in Luria-Bertani (LB) broth and agar. Swim was LB broth with 0.3% w/v agar added as supplements. To obtain the concentration that is required for this study, tannic acid was added to the motility media. Before usage, the solution and the tube were allowed to dry completely. Although this study found that the same results were obtained after drying the motility tubes for an hour or overnight, this method of motility tube preparation was said to produce the most reliable results [14]. Investigated were bacteria that had developed in the presence of tannins. Agar at a concentration of 0.5% (w/v) and the recommended tannin solutions were added to the samples.

Vitek 2 compact system

The samples were collected from cattle beef in Baghdad Governorate on the side of Al-Karkh and the beef samples were obtained from the Forelimb of cattle Sources (pieces of meat from butcher/ Forelimb of calf) that were frozen overnight. Areas from which meat samples were collected from Al-Jihad, Al-Amiriya, Al-Dora and Al-Sidiyah.
All media were prepared according to their manufacturer's instructions after they were heated on the burner to dissolve the constituents completely, then autoclaved, distributed into sterile Petri dishes or tubes, and left to solidify at room temperature. The Petri dishes were incubated at 37 ° C for 24 hours, to ensure sterilisation. Then, contaminated media were neglected. Some of these media need special additives. After that, they were ready to use for the isolation of bacteria or kept at 4 ° C. The confirmed samples were sent to Identification of isolates using the VITEK 2 system at a central health laboratory automated system VITEK – 2 Compact system (Biomerieux, France) was utilized to confirm the defined specimens obtained earlier manually [15].

Results and discussion

The inhibition zone

The inhibitory zone of tannic acid against the Gram-positive and Gram-negative bacteria was measured in the current study at varied diameter ranges of (22, 25, 30 and 35) cm. Significant variations existed in each bacterial group's antibacterial activity (Figure 1/ A & B).
Furtheremore, the ability of tannic acid to combat Gram-positive and Gram-negative isolates from meat samples has been demonstrated. The alterations in bacterial growth showed that tannic acid had an inhibitory effect on both groups overnight (Figure 2/ A, B & C). According to this research, the interaction between tannic acid and bacterial cell walls caused changes in bacterial growth. Regardless of the availability of bactericidal drugs, the ability of Gram-positive and Gram-negative bacteria to build biofilm has presented a problem to the treatment of these bacterial diseases; as a

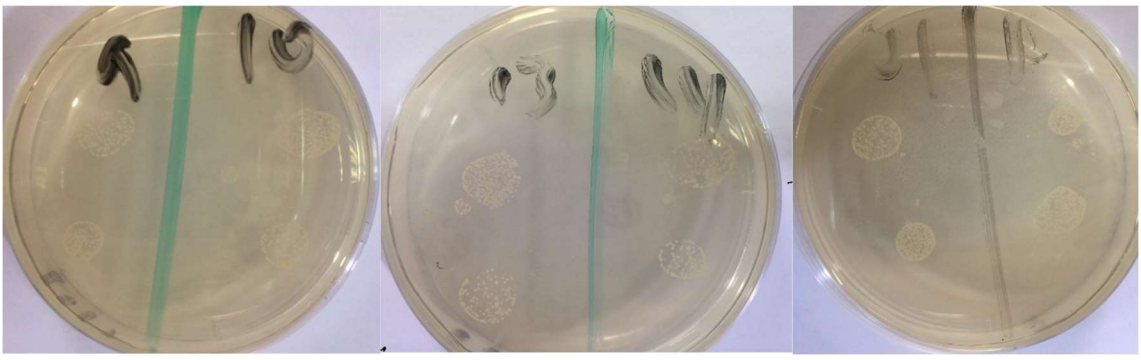
result, a study has found that tannic acid possesses antibacterial activity [16]. As a result, it has demonstrated strong action, especially against certain bacterial species using growth method on agar plates.

Additionally, its activity was effective over isolates that were Gram-positive and Gram-negative and resistant to most antimicrobial drugs. This suggested that tannic acid would work well for the colonization and infection of multi-drug resistant of those bacteria [17]. Inhibiting cell wall growth is a possible approach to Gram-positive. This idea was corroborated by the results of the lysozyme to kill bacteria by hydrolyzing cell membranes in the cell walls, and tannic acid may amplify this impact. Moreover, tannic acid could inhibit Gram-positive from stopping microbial activity compared to Gram-negative [18]. This variation could be influenced by several bacterial strains employed in the experiment or the calibre of the tannic acid.

The findings from [19] also supported a distinct bactericidal mechanism whereby a tannic acid prevented a crucial enzyme of the synthetic compositions in bacteria. These findings suggested that there may be many antimicrobial pathways; nevertheless, more research is required. The most significant species of bacteria for in vitro studies trials to develop the bacterial growth on animal tissues [20].

A **B**

Figure 1: The inhibition zone of antibacterial properties on the plates: **A)** Gram-positive bacteria, on different concentrations of tannic acids (95%, 90% & 88%), including positive and negative control; **B)** Gram-negative bacteria, on different concentrations of T A (95%, 90% & 88%), including positive and negative control.



A **B** **C**

Figure 2: Effect of tannic acids (TA) on total bacterial count using the dilution methods. **A)** total bacterial growth with 95% TA. **B)** total bacterial growth with 90% TA. **C)** total bacterial growth with 88% TA.

The motility

The effects of tannic acid (high concentrations 95%) on both Gram-positive and Gram-nagative of bacterial motility of surface associated twitching motilities were examined in the inhibitory zone of tests. These bacterial species were able to engage in all three modes of motility under control circumstances without the presence of tannic acid (Figure 3). Interestingly, even after tubes were incubated for up to 48 hours, varying concentrations of tannic acid totally prevented the bacterial motility. On the other hand, the low concentration (88%) showed less obstruct of the motilities (Figure 4). However, this investigation found a significant difference at the various time points. Compared to when these compounds were absent (control), there was less motility when they were present. Additionally, high concentration of tannic acid, compared to low concentration of tannic acid, which is resulted in reduced the motilities at the 72h with both Gram-positive and Gram-nagative bacteria.

This study confirmed that the interaction of tannic acid with bacterial motility altered pathogen activities. This research matched to a study, which has also been discovered that tannic acid to have anti-motile properties despite the availability of bactericidal medications, the propensity of Gram-positive and Gram-negative bacteria to form microbial cells has complicated the treatment of various bacterial infections [16].

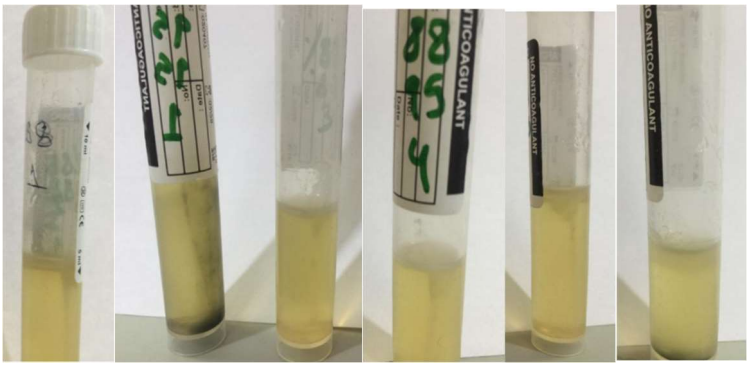


Figure 3: Effect of tannic acids (TA) of different concentrations (95% & 88%) on the motility of Gram-nagative bacteria.

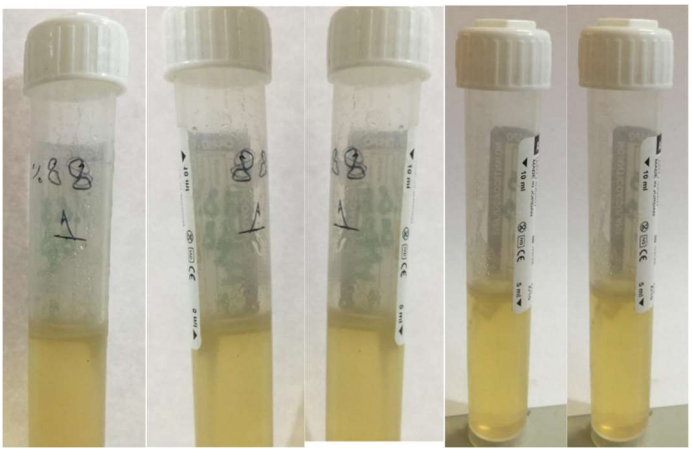
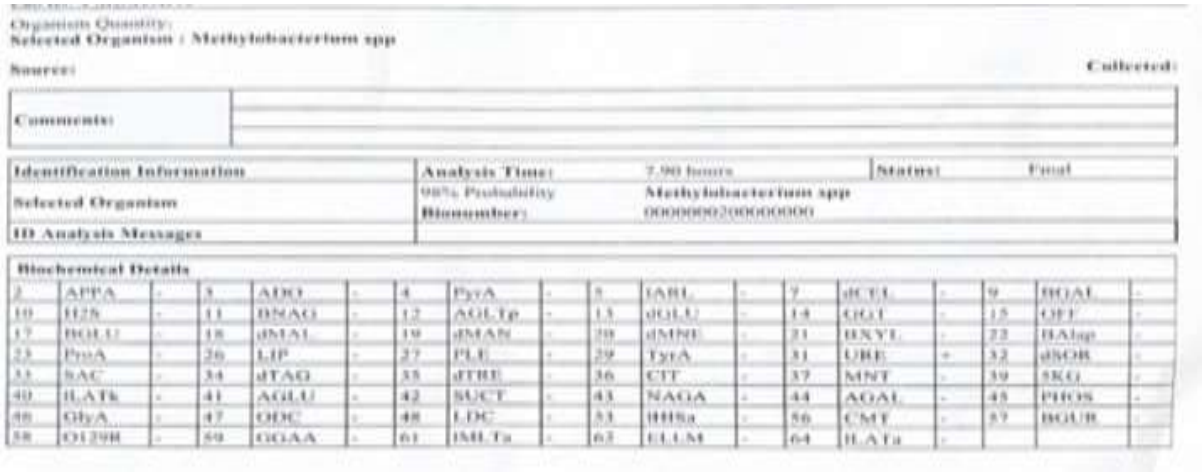


Figure 4: Effect of tannic acids (TA) of different concentrations (95% & 88%) on the motility of Gram-positive bacteria.

The VITEK 2 system

These bacterial isolates were identified by their biological and morphological characteristics. The vitek-2 small system, developed at the Learning Center in Baghdad, Iraq, supported it.

The bacterial isolates were identified by VITEK2 compact. It is a small system made up of an instruments and a software. The 64 wells on the chemical trays can each hold a different test substrate. Different metabolic processes, such as acidity, alkalinization, enzyme hydrolysis, and development when inhibiting chemicals are present, are measured by substrates. According to the VITEK findings, the fresh beef samples had the fewest total bacterial counts and appeared to be coliform-free. Gram-negative rods made up the majority of the total isolates from the various types of meat, whereas Gram-positive cocci were the most type of bacteria. *Lactobacillus* spp., *Staphylococcus* warneri and *methylobacterium* spp. (a genus of Hyphomicrobiales) were the most common bacteria found in fresh meat.



Organism Quantity:
Selected Organism : Methylobacterium spp
Source: Collected:

Comments:	

Identification Information	Analysis Time: 7.90 hours	Status: Final
Selected Organism	98% Probability	Methylobacterium spp
ID Analysis Messages	Bionumber:	0000000200000000

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	HISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	OGAA	-	61	ISILTa	-	63	ELLM	-	64	ILATa	-			

Organism Quantity:
Selected Organism : Staphylococcus warneri
Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	7.82 hours	**Status:**	Final
Selected Organism	90% Probability	**Staphylococcus warneri**		
	Bionumber:	000002003221031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	-	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 5: The results of VITEK2 compact system

The difference might be impacted by the various microbial isolates used in the tannic acid experiment; it also suggested a unique antimicrobial technique whereby a tannic acid stopped microbes from producing an synthetic compositions [20]. These results indicated that there might be numerous antibacterial mechanisms, but further study is still needed. The most important types of bacteria to study in in vitro experiments to develop bacterial growth on organisms [21].

Conclusion:

These findings simultaneously showed that tannic acid may significantly affect bacterial cell walls and disrupt their structure to perform the antimicrobial activity of being able to prevent MRSA from developing a growth curve. However, additional research is still needed to determine the underlying processes of tannic acid impact. Even though this was a trial, the results shed light on the value of tannic acid in the management of Gram-positive and Gram-negative bacterial infections.

Therefore, this research demonstrated that different tannic acid concentrations could exert some antibacterial properties through a variety of mechanisms, such as inhibitory effects, but not through a specific mode of action.

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