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Research Article

Assessment of various concentrations of tannin extracts on pathogenic bacteria isolated from beef compared to antibiotic sensitivities

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Abstract: In this study, the inhibitory effect of tannin extracts was compared with some common antibiotics against bacteria isolated from beef meat. 80 samples of local meat were collected randomly from the Baghdad governorate to determine their microbial load. Natural materials such as tannin are used as an anti-bacterial agent and an alternative to industrial additives. The results of the bacterial isolate showed the presence of 124 isolates of beef meat, which were classified into 9 types, including *E. coli* (86.25%), *Salmonella typhimurium* (7.5%), *E. coli*O157:H7 (6.25%), *Staphylococcus aureus* (13.75%), *Listeria monocytogenes* (1.25%), *Klebsiella*spp., *Proteus* spp. (8.75%), *Pseudomonas auregenosia* (18.75%) and *Bacillus* spp. (3.75%). For the results of antibiotics, most of the isolates showed strong resistance to antibiotics; while some of the isolates showed low sensitivity to antibiotics compared to tannin extracts, which gave higher inhibitory effects.

Keywords: Tannin extracts, Pathogenic bacteria, Beef meat, Antibiotics sensitivity.
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Introduction

The tannin extract of Pomegranate (*Punica granatum* L.) is rich in proanthocyanidins with powerful scavenging activity against free radicals. This extract is used in the beverage industry to improve sensory attributes, stabilize color, and act as a redox buffer. Their compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit (Dakheel et al. 2021). The structural nature of tannins consists of two or three phenolic units on a phenyl ring in a relatively sized polymer. Subsequently, as tannin chemical composition, two categories were known hydrolysable and condensed tannins (Papuc et al. 2017). According to studies, utilizing a commercialized tannin composition may be employed as an oxidative agent for many animal products, particularly chicken, seafood, and beef (Ljubojevic et al. 2013; Al-Hijazeen et al. 2016). Tannins are often categorized as hydrolysable or condensed which are water-soluble chemicals precipitating alkaloids, fibers, and other materials (Okuda 2011). Contrary to condensed tannins, which are often made from flavan-3-ols, hydrolysable tannins are composed of alcohol and hydroxyl groups that have been polymerized by phenolic or hexahydroxydiphenic acid (Koleckar et al. 2008).

People and food producers both adhere to the pattern of using environmental antimicrobials to preserve meat (Manyi-Loh et al. 2018). This application is anticipated to progressively rise shortly due to the growing consumer desire for lightly packaged foods, ideally those with natural ingredients (Okocha et al. 2018). Fresh and slightly

processed meats are being consumed more often, and as a result, new ecological pathways for microbial development have arisen (Schirone et al. 2022). Natural additives that display an efficient inhibitory action against a broad variety of undesired bacteria in foods are becoming more popular as a result of these worries and the rising demand for natural produce (Batiha et al. 2021).

Antibiotics have been essential in improving feed efficiency, preventing disease, and supplementing feed at low dosages to increase livestock production's profitability (Chattopadhyay 2014). Antibiotic use in feed is a typical and proper technique in the animal sector that has assisted to intensify cattle output in the contemporary era. However, some worry about using those might result in resistant pathogens and pose a hazard to human safety as animal husbandry becomes more intensive (Seal et al. 2013). This study was carried out to demonstrate presumptively the different concentrations of tannin extracts and their antimicrobial activities on various bacterial species that are isolated from beef meats sold in local markets of Iraq.

Materials and Methods

Sample collection: In total, 80 samples of the local raw beef meat were randomly collected in a sterile sealed polythene bag, from the different local markets in Baghdad province and then sent to the microbiology laboratory for isolation and identification of bacteria. All samples were stored at -20°C until analysis.

Isolation and identification of bacteria: In a polyethylene sac, 250g of raw meat was packed, mixed well in the stomacher, and inoculated into peptone broth with (25gr/225ml) and incubated at 37°C for 24-48hrs. After incubation, about 0.1ml of the inoculated broth was sub-cultured onto plates of Nutrient agar, Blood agar, Eosin methylene blue (EMB), MacConkey agar, Salmonella-Shigella Agar (SS) agar, Sorbitol- MacConkey agar with cefixime telluride, and CIN agar. Microorganisms were identified and characterized through a direct microscopic examination using Gram stain and macroscopic examination of colonies on their specific media. Biochemical examinations were performed using the VITEK^2-system. Finally, the samples were frozen at 70°C in BHI broth containing 20% glycerol for the susceptibility test.

Preparation of tannin extract: According to Dakheel et al. (2021), the technique of pomegranate extract formation was used. The concentrations of 200 and 100mg/ml (w/v) for each sample were achieved by individually dissolving 100 and 50g of dried pomegranate extract in 500ml of sterile water bath. The processed plants were subjected to a water extract for 3 hours at 40°C with the solution being spun at 70ppm. The extract samples were purified by column chromatography on Sephadex LH-20 following the methods of Dakheel et al. (2020). Afterward, these extracts were analyzed for tannin concentration and composition by high-performance liquid chromatography/mass spectrometry. The extractions were frozen, lyophilized, and stored at -20°C for in vitro experiments.

Antibacterial analyses by agar diffusion method: The antibacterial activity of the prepared tannin extract was studied against Gram-positive and negative bacteria and was determined by the agar well-diffusion technique using Mueller Hinton agar media. Bacterial culture (0.5µl) was transferred into a plate using a cotton stick and spread with a sterile glass rod and made holes with 6mm diameter for each concentration added into the well. Then plates were incubated at 37°C for 24h and then the antibacterial activity was determined by measuring the zone of inhibition around each well (Enemor et al. 2015).

Antibiotic susceptibility test: The Kirby-Bauer disc diffusion method on Muller Hinton agar media was used to determine the resistant isolates. The inhibition zone was measured and categorized as resistant. The isolated species were tested against 6 routine and practical antibiotics. Based on the diameter of the clear zone surrounding disks concerning the antibiotic standard table, the susceptible and resistant were Clindamycin (CD),

Table 1. The percentage of bacterial isolates from local beef meat samples.

Isolated bacteria	No. of positive isolates	Percentage of isolates
E. coli	69	86.25%
Salmonella typhimurium	6	7.5%
Proteus sp.	7	8.75%
E. coli O_{157} H:$_7$	5	6.25%
Klebsiella spp.	7	8.75%
Pseudomonas auregenosia	15	18.75%
Staphylococcus aureus	11	13.75%
Listeria monocytogenes	1	1.25%
Bacillus spp.	3	3.75%

Table 2. The inhibition zone of different tannin concentrations (at 5%) for various pathogenic bacteria using the well diffusion method (mm).

Isolated bacteria	(1) Hydrolasable tannin - 95%	(2) Condensed tannin - 98%	(3) Hydrolasable tannin - 88%	(4) Condensed tannin - 88%
E. coli	21	23	23	23
Pseudomonas	19	20	19	18
E. coli O_{157} H:$_7$	22	19	19	20
Salmonella	19	20	19	19
Listeria	17	18	20	20
Proteus spp.	18	18	17	17
Bacillus spp.	18	18	15	19
Klebsiella spp.	20	19	15	19
Staphylococcus aureus	15	20	18	-

2μg/disc; Amikacin (AK), 30μg/disc; Doxycycline (DXT), 30 μg/disc; Erythromycin (E), 15μg/disc; Tetracycline (T), 30 μg/disc; and Trimethoprim (1.25 μg/disc) + Sulfamethoxazole (TS), 23.75 μg/disc (Altalhi et al. 2010).

Results

The results showed the existence of 124 bacterial isolates, many of them contained mixed infection classified into 9 types, including *E. coli* (86.25%), *Salmonella typhimurium* (7.5%), *E. coli* O_{157}:H_7 (6.25%), *Staphylococcus aureus* (13.75%), *Listeria monocytogenes* (1.25%), *Klebsiella* spp., *Proteus* spp. (8.75%), *Pseudomonas auregenosia* (18.75%) and *Bacillus* spp. (3.75%) (Table 1). Regarding tannin concentrations (5%), higher results were obtained for the inhibitory zone in 5% condensed tannin as 98% than hydrolysed tannin at 95%. Similar to hydrolyzed tannin at 88% showed better results than condensed tannin at that same concentration i.e. the inhibition zone of condensed tannin at 88% was better than hydrolyzed tannin at 95% (Table 2; Fig. 1). Table 3 and Figure 2 show the inhibitory zone in tannin extract concentrations at 10% for various pathogenic bacteria isolated from beef. The inhibition zone of hydrolyzed tannin at 88% had higher results than condensed tannin at 88%. The inhibition zone of 10% condensed tannin at 98% revealed higher results than hydrolyzed tannin at 95%. Condensed tannin at 98% offered better results than condensed tannin at 88%, although the inhibition zone of hydrolyzed tannin at 88% gave

Fig.1. *The inhibition zone of different tannin extracts (5%) against bacteria; (1) HT 95%, (2) CT 98%, (3) HT 88%, and (4) CT 88%.*

Table 3. *The inhibition zone of different tannin concentrations (at 10%) for various pathogenic bacteria using the well diffusion method (mm).*

Isolated bacteria	*(1) Hydrolysable tannin – 95%*	*(2) Condensed tannin - 98%*	*(3) Hydrolysable tannin - 88%*	*(4) Condensed tannin*
E. coli	*25*	*24*	*35*	*32*
Pseudomonas auregenosia	*20*	*30*	*30*	*30*
E. coli O_{157} $H:_{7}$	*25*	*26*	*35*	*25*
Salmonella typhimurium	*22*	*25*	*41*	*24*
Listeria monocytogenes	*20*	*26*	*35*	*25*
Proteus spp.	*23*	*18*	*22*	*25*
Bacillus spp.	*20*	*25*	*43*	*30*
Klebsiella spp.	*50*	*50*	*38*	*40*
Staphylococcus aureus	*30*	*24*	*40*	*23*

Fig.2. *The inhibition zone of different tannin extracts (10%) against bacteria; (1) HT 95%, (2) CT 98%, (3) HT 88%, and (4) CT 88%.*

Table 4. The sensitivity results of bacterial isolates from beef to antibiotics.

Antibiotics \ Bacteria	Staph.	*E. coli* $O_{157}H_7$	*E. coli*	Salm.	Klebs.	Prote.	Lister.	Pseudo.	Bacill.
Clindamycin	R	R	R	R	R	R	R	R	R
Amikacin	23	15	20	21	22	20	23	7	17
Doxycycline	R	R	R	13	15	8	R	R	R
Erythromycin	R	R	R	10	7	R	R	R	R
Tetracycline	R	R	R	15	15	11	8	5	R
Trimethoprim, Sulfamethoxazole	10	R	R	30	R	R	8	R	R

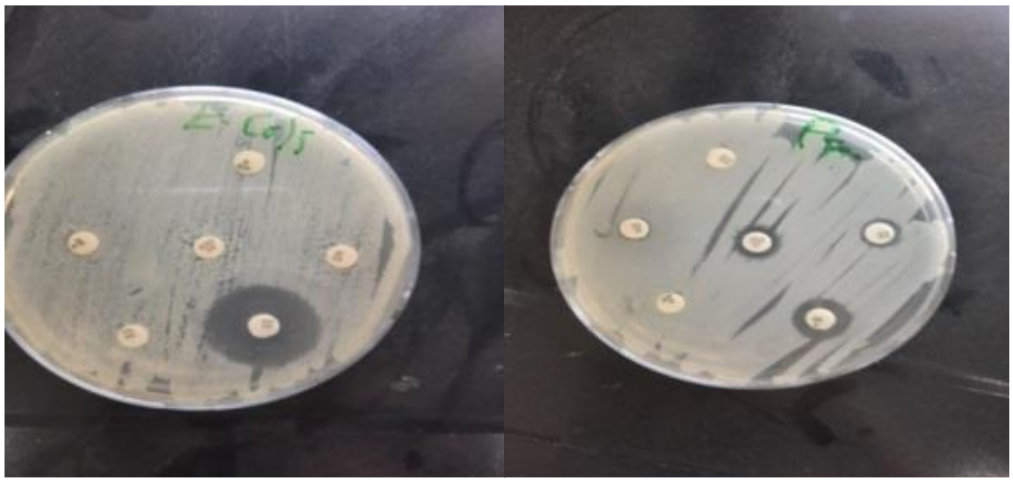

Fig.3. The inhibition zone of different antibiotics against isolated bacteria.

significant results to hydrolyzed tannin at 95%.

The results of isolated bacteria showed a significant inhibition zone of tannin extracts to all concentrations compared with the used antibiotics. The results of the isolated bacteria revealed that tannin extracts significantly inhibited the growth of the bacteria at all concentrations compared to other antibiotics. In the current study, the results of isolated bacteria showed a significant inhibition zone of tannin extracts to all concentrations compared with the antibiotics used. In terms of antibiotic sensitivity, most beef isolates exhibited high resistance to antibiotics Clindamycin (CD), Erythromycin (E), and Trimethoprim, and Sulfamethoxazole (TS); while some of the isolates showed great sensitivity to antibiotics Amikacin (AK), Tetracycline (T) and Doxycycline (DXT) (Table 4, Fig. 3).

Discussion

Numerous investigations have reported the antibacterial activities of pomegranate tannin extract on gram-positive and gram-negative pathogenic microbes. Its flesh meat also has benefits because of the pomegranate's (tannin extracts) antimicrobial properties (Hanani et al. 2019). The microbes present in meat has a great impact on the substance's antibiotic. Although bacterial Gram-types were found in the current study, it is suggested molecular detection of bacteria in future studies; this might greatly extend the storability of meat. Mehdizadeh et al. (2020) also found that compositions of several pomegranate-containing and other plant phenols can prolong the shelf life of meat.

The natural contents of pomegranates, which include a variety of flavonoid polyphenols, are involved in the cell degradation of pathogens. The present study showed that pomegranate extracts are significantly affected the bacteria. The breakdown of the cell walls is the most plausible pathway behind

the antibacterial action of extracts comprising polyphenols (Dilucia et al. 2020). Consequently, the molecular building of tannins, which has several hydroxyl groups on a phenyl ring in a massive polymer, gives them their unique features. According to the polyphenol groups in their structures, tannins were divided into two groups. These two groups were designated hydrolysable tannins and condensed tannins, which affect the ionization channels and the structure of bacterial cell walls (Okuda & Ito 2011).

Since genetic mutual interaction was anticipated in the collected phenotypes with other bacteria in the meat, a cumulated complicated circumstance of resistance was observed. Before being slaughtered, the habitat of the animals may have caused emerging antibiotic resistance, which has led to an increase in the prevalence of emerging combination-resistant variants in the ecological system (Ahmed & Dakheel 2020). Foods regarding antibiotic-resistant are impacted by many stresses from farm to consumer. A process of metabolic changes accumulative resistances to stresses improves the ability to survive in several ways, mainly using biofilm defenses. The sanitary regimes that would be built via knowing the genetic activity of these bacterial operations that exist in the meat naturally or invasion, in terms of antibiotic sensitivity, are millstones among several elements such as the food chain and meat environment. Coucke et al. (2022) argued many techniques to increase the plant-based meat substitutes to reduce agro-environmental influences meat ecosystem. Utilizing polyphenol plants, which are natural substance components, is one antibacterial substitute (Monger et al. 2021).

Conclusion

According to the results, tannins may be effective on the growth of bacteria including *E. coli*, *Salmonella typhimurium*, *E. coli* O_{157}:H_7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas auregenosia* and *Bacillus* spp. in meat. Based on the results, different oncentrations of tannins had effects on bacterial growth, offering a possible substitute for antibiotics. Hygienic meat may be a useful substitute for antibiotics in the industrial sector.

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