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Inhibitory Effects of Pomegranate Peel Extract against *Staphylococcus aureus* Causing Animals Mastitis in Sohag Governorate

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ABSTRACT

Mastitis in dairy herds is of considerable significance, among various etiological agents, *Staphylococcus aureus* is the most predominant cause of subclinical mastitis in dairy animals. Increased trends in antimicrobial-resistant bacteria and failure of antibiotics lead to increased incentives to look for alternative control measures such as herbal extracts and their active compounds to be applied in manage and control of causative bacterial diseases. This study was designed to determine the antimicrobial efficacy of pomegranate peel extracts against *S. aureus* isolated from mastitis. Analysis of its content of active ingredients was taken into account using GC-Mass. The disk diffusion assay was used to estimate the antibacterial effects in the *S. aureus* strains. The GC-MS results showed that pomegranate peel extract contained 40 components, 14 of which have documented antibacterial activity including phenolics (gallic acid, caffeic acid, benzoic acid, cinnamic acid) and flavonoid compounds. Five of which exhibited resistance to beta-lactams. Pomegranate peel extract was able to inhibit the growth of *S. aureus* at a concentration of 3.125 mg/ml. Scanning electron microscopy indicated the pomegranate peel extracts disrupted the bacterial cell wall causing cell deformations that would decrease their pathogenicity and could control the disease efficiently. Pomegranate peel extracts represent a promising alternative treatment for mastitis in dairy animals.

1. INTRODUCTION

For dairy herds, mastitis is a considerable and high-costing disease, it causes 70- 80% of total losses. The most predominant pathogens incriminated with animal mastitis are *Staphylococcus, Streptococcus, Escherichia, Mycoplasma* species and *Prototheca ciferrii* [1-3]. *Staphylococcus aureus* (*S. aureus)* is known to be the main causative agent of animal mastitis, is common inhabitant in teat canals, or udder skin, infected udders, and transmitted during milking [4]. Using antibiotics to control animal mastitis may cause harmful milk residues and increase the risk of generating antibiotic-resistant bacteria. Antibiotic-resistant *S. aureus* strains become a critical global health problem. Antibiotic-resistant *S. aureus* can be transmitted to humans through milk, causing serious infections. In 2019, antibiotic-resistant *S. aureus* caused 1.27 million deaths [5]. β-lactam group of antibiotics includes penicillin, methicillin, oxacillin, cephalosporins, carbapenems, and nafcillin [6]. *S. aureus* produces β-lactamase enzyme, that is mainly encoded by the *blaZ* gene causing hydrolysis of the beta-lactams [7]. The global attention to controlling microbial resistance is directed at the synthesis of new drugs, or the development of natural ones. Natural antimicrobials offer efficient and appropriate antimicrobial drugs to humans and animals [8].

Pomegranate or *Punica granatum Linn.* (*P. granatum L.*) belongs to the *Lythraceae* family, is a native tree in North Africa and the Mediterranean [9]. The estimated world's pomegranate annual production was 1.5 million tons [10]. Pomegranate is famous for its medicinal potential [11, 12]. The Pomegranate peels constitute the major fraction (30-50%) of the fruit weight, are non-edible, and are mostly discarded [13]. The Pomegranate peel extract (PPE) is rich in natural compounds such as minerals, organic acids, alkaloids, polyphenols, and phenolic compounds. The phenolic compounds include bioactive phenolic compounds such as ellagitannins, tannins, and anthocyanin, including ellagic acid and punicalagin (2,3-hexahydroxydiphenoylgallagyl-D-glucose). Polyphenols include flavonoids (anthocyanins and catechins) and tannins (ellagitannins and ellagic acid derivatives: punicalagin, punicalin, and pedunculagin) [14, 15]. Additionally, there are flavones, flavanones, and some anthocyanins such as cyanidins and delphinidins [16]. Other phenolic compounds include gallic acid, gallotannic acid, and tannic acid. The alkaloid content consists of isopelletierine, pelletierine, methypelletierine, and pseudo-pelletierine. The extract also contains sugar and calcium oxalate [17].

PPE was reported to exhibit antimicrobial activity against Gram-positive bacteria (e.g. *S. aureus, Listeria monocytogenes, Clostridia, Bacillus subtilis,* and *Bacillus cereus),* and Gram-negative bacteria (e.g. *Salmonella, Escherichia coli, Enterobacter aerogenes Pseudomonas aeruginosa, Vibrio parahaemolyticus,* and *Yersinia enterocolitica)* [18, 19]. The phenolic and the polyphenolic contents are responsible for the antimicrobial activities [20, 21, 22]. The ellagic acid and punicalagins may form a complex with the bacterial cell and cause death, or inhibit protein activities [11]. The antimicrobial effect of the phenolic components is correlated to the number and position of the hydroxyl groups that change the polarity [18]. The pomegranate peel extract was evaluated against β-lactamase-producing bacteria using agar diffusion assay [13]. The current study

evaluated the antibacterial efficacy of pomegranate peel extract against *S. aureus* causing animal mastitis.

2. MATERIAL and METHODS

2.1 Bacterial strains

Ten S. aureus isolates (which cause mastitis in cows and buffaloes) were obtained from the Animal Health Research Institute at Sohag Governorate. S. aureus was re-cultured on Baird-Parker agar medium (Oxoid, CM0257) and incubated for 24 h at 37o C [23]. Utilizing the procedure outlined in Bergey's Manual, the biochemical bacterial characteristics were assessed [24, 25]. The ethical approval number MB- 21- 32-R. We acknowledge that the current study is an in vitro study that does not involve the use of animals or human subjects.

2.2 Preparation of pomegranate peel extract (PPE)

Extract preparation was performed following Hassoon et al. [26] method. Fresh pomegranate fruits were purchased from the market, and peels were manually removed, washed, and dehydrated under indirect sunlight for 5 days. Later, the dried peels were powdered using an electric grinder till converted to fine powder. 20 g of powder was weighted and melted in 200 ml of deionized water the mixture was heated until boiling then cooled, and filtered several times before being centrifuged at 4°C at 4000 rpm for 15 min. The extract was collected in a 250 ml volumetric bottle, and stored at 4°C until used. Diverse concentrations (100, 50, 25, 12.5, 6.25, 3.125%, …, etc) were prepared using two-fold serial dilution using sterile distilled water, and they were filtered through 0.45-µm filters before use.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) of PPE

The PPE was analyzed using Thermo Scientific TRACE 1300 GC coupled with ISQ 7000 MS single quadruple mass spectrometry equipped with the split-split less (S/SL) front method injector attached to an HP-5 fused silica column and interfaced to the flame-ionization detector (FID). 5 µl extract was injected, and carrier gas was Helium (0.5 ml/min). The temperature of the detector were adjusted at 280°C, the injector at 250°C, while the column was linearly programmed from 50°C- 280°C at 5°C/min [22].

2.4 Antibacterial effects of PPE

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PPE. The effect of the extract with concentrations of 100, 50, 25, 12.5, and 6.25 % on the bacterial growth was achieved using agar well diffusion. Inoculation of the pathogenic bacteria was done by a sterile cotton swab on the surface of cooled and dry Muller-Hinaton agar (MHA) plates using bacterial suspension which contains 5×108 cells/ml. Four wells of 5 mm diameter were made at least 1.5cm from the edge of the plate. PPE concentrations of 100, 50, 25, 12.5, 6.25, and 3.125%, 0.1 ml distilled water was used as control were poured into the wells and were allowed to dry for a few minutes before incubating the plates for 24 h at 37oC. After the injection, dishes were left at room temperature for 20-30 minutes, and incubated at 37°C for 24 h. Extract effectiveness was determined by measuring the diameter in mm for the inhibition zone around each well and the rate of the 3 replicates was calculated. PPE concentrations were incubated with

the tested organism for 24 h, then 0.1 ml from each dilution was evenly spread on the surface of Baird-Parker agar plates and incubated for 24 h at 37oC, the concentration that showed no growth, or growth of colonies less than 3-5 colonies was considered the MBC [27, 28].

2.5. Scanning Electron Microscope (SEM)

Discs of bacterial culture were subjected to the PPE and fixed in a phosphate buffer solution (50 mM, pH 7.2) containing 2% (w/v) glutaraldehyde, then maintained at room temperature. The fixed bacterial discs were washed three times in phosphate buffer (30 min each) before being post-fixed in osmium tetroxide solution (1% (w/v)) in the same buffer for 2 days at 4 °C. Samples were washed in cacodylate buffer three times for thirteen minutes each and then dehydrated using an ascending series of ethanol 30,50,70,90 for 2 hours, 100% for two days, and then to amyl acetate for two days. Critical point, liquid carbon dioxide was used in drying the samples. Each sample was stuck on metallic blocks 'using silver paint. Samples were evenly gold-coated at a thickness of 15nm using the gold sputter coating apparatus, and examined using a JEOL JSM 5400 LV scanning electron microscope 15-25 kv and photographed.

2.6. Statistical Analysis

The Statistical Program for Social Science (SPSS), version v.26 computer software. SPSS Inc. Chicago, USA, was used to calculate the means and standard deviations (SD)

3. RESULTS

Staphylococci colonies on Baird Parker agar medium (BPA) appeared as black colonies surrounded by a white halo zone (**Fig. 1a**). Microscopically, *S. aureus* appeared as a Gram-positive coccus with grape-like clusters (**Fig. 1b**). *Staphylococci* fermented mannitol in the mannitol salt agar medium (MSA), golden-yellow colonies on a yellow background, differentiated from pink colonies and pink background for saprophytic *Staphylococci* (**Fig. 1c**). *Staphylococci* showed a positive reaction for oxidase, catalase, and coagulase tests, as well as β hemolysis production on sheep blood agar (**Table 1**).

Table 1. Morphological and biochemical characterization of *S. aureus* isolated from ten animal mastitis samples (n=10)

Sample	Biochemical reactions	Occurrence (out of 10)	%
Colonies on BPA*	black colonies surrounded by clear halo zone	**10**	**100**
Gram-staining	Gram +ve	**10**	**100**
Colonies on MSA**	Yellow colonies	**8**	**80**
Catalase test	+	**7**	**70**
Oxidase test	+	**7**	**70**
Microscopic morphology	Cocci or spherical	**7**	**50**
Arrangement	arranged in clusters like a bunch of grapes	**5**	**50**
Slide coagulase	+	**5**	**50**
Tube coagulase	+	**5**	**50**
Mannitol fermentation	+	**5**	**50**
β hemolysis	+	**5**	**50**

*BPA: Baird-Parker Agar, **MSA: Mannitol Salt Agar.

3.1. Gas Chromatography-Mass Spectrometry analysis of PPE

The various components of PPE were qualitatively analyzed using GC–MS as shown in Table 2. The major components of pomegranate peel extract, selected based on high incidence (Area %), are classified into their respective chemical groups: Alkaloids (2-Pyrazoline, 5-ethyl-1,4-dimethyl; Aconitine), Amines (Isobutylamine), Hydrocarbons (Cyclohexane derivatives), Fatty acid esters (Tetradecanoic acid, 2-hydroxy-, methyl ester; Methyl 2-hydroxydodecanoate; 9,12-Octadecadienoic acid (Z, Z)-, phenylmethyl ester), Fatty acids (Oleic Acid), Diols (1,2-Decanediol), Nitrogen-containing compounds (6-Nitroundec-5-en) as cleared in Table (2).

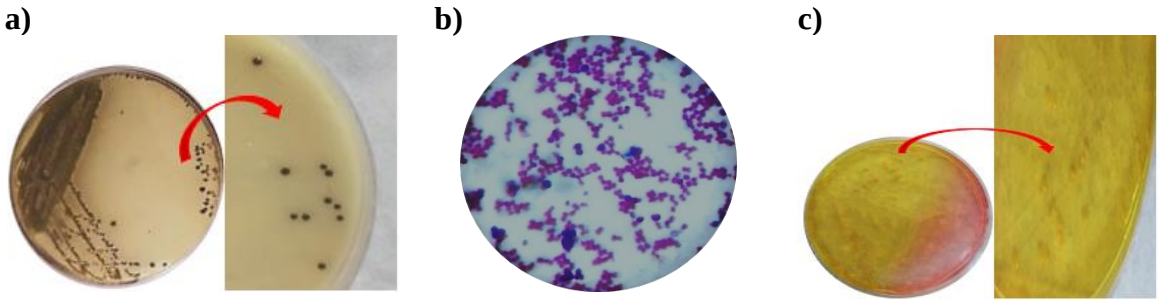

Figure 1. a) *Staphylococci* colonies on BPA, black colonies surrounded with white halo zone. **b)** microscopic morphology of *S. aureus:* appear as Gram +ve cocci, grape-like clusters. **c)** *Staphylococci* colonies on MSA, golden-yellow colonies on a yellow background, differentiated to pink colonies with a pink background for saprophytic *Staphylococci*.

Table 2. The major components of Pomegranate peels (*Punica granatum L.)* extract according to GC-MS analysis

	RT	Compound Name	Molecular Formula	Molecular Weight	Area %	Related Group
1	25.19	2-Pyrazoline,n 5-ethyl-1,4-dimethyl	$C_7H_{14}N_2$	126	1.17	Alkaloid
2	57.69	Isobutylamine	$C_4H_{11}N$	73	1.09	Amine
3	58.89	Aconitine	$C_{34}H_{47}NO_{11}$	645	1.04	Alkaloid
4	60.30	Cyclohexane, 1,3,5-trimethyl-2-octadecyl4	$C_{27}H_{54}$	378	1.32	Hydrocarbon
5	72.86	Tetradecanoic acid, 2-hydroxy-, methyl ester	$C_{15}H_{30}O_3$	258	1.59	Fatty acid ester
6	73.17	Cyclohexane, 1,4-dimethyl-2-octadecyl	$C_{26}H_{52}$	364	1.47	Hydrocarbon
7	73.34	1,2-Decanediol	$C_{10}H_{22}O_2$	174	2.40	Diol
8	73.87	Cyclohexane, 1,3,5-trimethyl-2-octadecyl	$C_{27}H_{54}$	378	1.01	Hydrocarbon
9	73.97	Cyclohexane, 1,4-dimethyl-2-octadecyl	$C_{26}H_{52}$	364	1.14	Hydrocarbon
10	74.85	Cyclohexane, 1,4-dimethyl-2-octadecyl	$C_{26}H_{52}$	364	1.33	Hydrocarbon
11	75.13	Oleic Acid	$C_{18}H_{34}O_2$	282	1.9	Fatty acid
12	76.09	Methyl 2-hydroxydodecanoate	$C_{13}H_{26}O_3$	230	1.16	Fatty acid ester
13	77.22	9,12-Octadecadienoic acid (Z, Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	1.09	Fatty acid ester
14	77.45	6-Nitroundec-5-ene	$C_{11}H_{21}NO_2$	199	1.47	N- compound

3.2. Antibacterial effects of PPE compared with antibiotics

The antibacterial properties of PPE were tested against five selected isolates of *S. aureus.* As shown in Table 3, PPE produced inhibitory zones (22-25 mm; with a mean of 23±2 mm) against *S. aureus* strain 1 (st1) isolated from mastitis milk, which were comparable

to those of some antibiotics such as trimethoprim, gentamycin, ofloxacin, chloramphenicol, ciprofloxacin were 22, 23,25, 26, and 24 mm in diameter, respectively. And higher than those produced by penicillin; floxacillin, polymyxin-B, vancomycin, and erythromycin showed zones of 4, 14, 15, 16, and 12 mm in diameter respectively. St 2 was inhibited by antibiotics such as trimethoprim, gentamycin, chloramphenicol, and vancomycin were 22, 23,20, 30, and 22 mm in diameter, respectively. And higher than those produced by penicillin; floxacillin, polymyxin-B, and erythromycin, and ciprofloxacin showed zones of 2, 15, 15, 18, and 14 mm in diameter respectively. While st3, trimethoprim, gentamycin, ofloxacin, chloramphenicol, and vancomycin were 20, 23,20, 30, and 22 mm in diameter, respectively. And higher than those produced by penicillin; ofloxacin, floxacillin, polymyxin-B, erythromycin, and ciprofloxacin showed zones of 3, 12, 15, 16, 14, and 15 mm in diameter respectively. Inhibitory zones of trimethoprim, ofloxacin, floxacillin, chloramphenicol, vancomycin, erythromycin, and ciprofloxacin were 25, 23,24, 28, 24, 26, and 25 mm in diameter, respectively against st4. And higher than those produced by penicillin, gentamycin, and polymyxin-B e showed zones of 0, 16, and 18 mm in diameter, respectively. Trimethoprim, gentamycin, ofloxacin, chloramphenicol, and ciprofloxacin produced zone against st 5 of 26, 22,25, 26, 30, 22, 28, and 26 mm in diameter, respectively. And higher than those produced by penicillin, and polymyxin-B, showed zones of 0, and 16 mm in diameter, respectively.

Table 3. Inhibitory zones produced by PPE compared to those of some antibiotics against *S. aureus* isolated from mastitis milk.

St AB	P	TR	GEN	OF	FO	PB	C	VA	E	CIP	PPE
1	4	22	23	25	14	15	26	16	12	24	26
2	2	22	23	15	20	15	30	22	18	14	25
3	3	20	22	12	15	16	26	25	14	15	24
4	0	25	16	23	24	18	28	24	26	25	23
5	0	26	22	25	26	16	30	22	28	26	22

St: Strain; AB: antibiotic; P=Penicillin; TR= Trimethoprim; GEN= gentamycin; OF= Ofloxacin; FO= Floxacillin; PB= polymyxin-B; C=Chloramphenicol; VA= Vancomycin; E= Erythromycin; CIP=Ciprofloxacin; PPE: Pomegranate peel extract.

3.3 Evaluating the inhibitory effect of PPE against S. aureus

The MICs of PPE against 5 strains of *S. aureus* (**Table 4, and Figure 2**) PPE produced inhibitory zones (22-26 mm; with a mean of 24±2 mm) at 100% concentration, compared to 18-23 mm; with a mean of 20±2 mm) at 50% PPE, 15-19 mm; 17±2 mm in diameter, at 25%, respectively. PPE at 12.5% produced inhibitory zones (12-16 mm; with a mean of 14±2 mm), compared to 10-12 mm; with a mean of 11±1 mm) at 6.25% PPE. The Mics were at 3.125% PPE-produced inhibitory zones (7-8 mm; with a mean of 7.5±0.5 mm).

Table 4. The inhibitory effect of PPE against *S. aureus* isolated from mastitis milk using agar well diffusion assay**.**

Strain / Conc.*(µg/ml)	St1	St2	St3	St4	St5	Mean ±SD
100	26	25	24	23	22	**24±2**
50	23	22	19	18	18	**20±2**
25	19	18	17	15	15	**17±2**
12.5	16	15	14	13	12	**14±2**
6.25	12	10	12	11	10	**11±1**
3.125	8	7	8	8	7	**7.5±0.5**
1.5625	6	8	R	R	R	**7±1**
0.78125	R	R	R	R	R	**R**

***Conc.: Concentrations, St: strain, SD: standard deviation, R: resistant

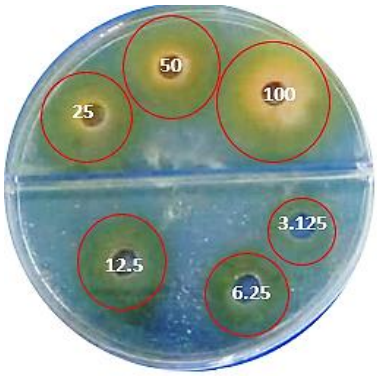

Figure 2. Zone of inhibition produced by PPE at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml.

Scanning Electron Microscopy

Figure (3) showed that PPE destroyed *S. aureus* cells, changes in cell morphology, and a reduction in cell counts.

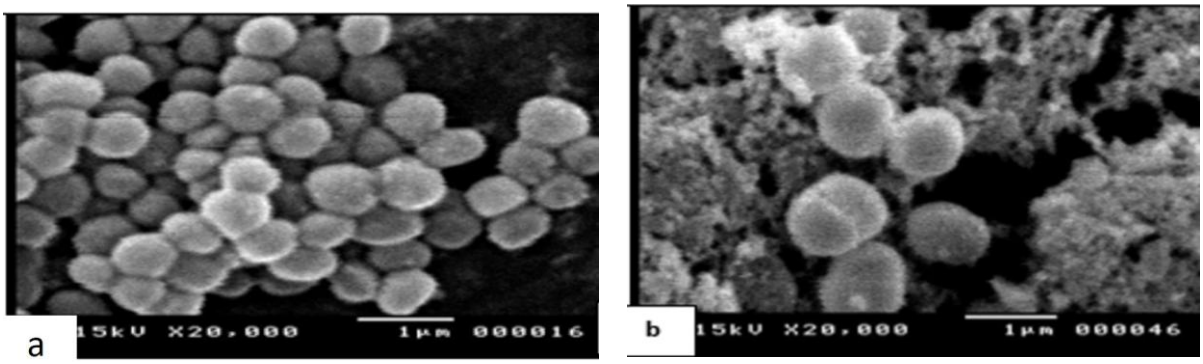

Figure 3. Scanning Electron Microscopy, a) Control S. aureus colonies. b) S.aureus colonies after the addition of PPE.

4. DISCUSSION

Mastitis is a significant disease for dairy herds, primarily caused by pathogens with *Staphylococcus* spp. as a common culprit. Antibiotics are commonly used to treat mastitis, and may lead to the development of antibiotic-resistant bacterial strains and undesired antibiotic residues. Thus, plant extracts are investigated as an alternative treatment [3, 29]. Plants containing various types of active phytochemicals which are helpful components against antibiotic-resistant pathogens in replacement of antibiotics without adverse side effects [30, 31].

Polyphenolic phytocomponents in the PPE have multiple hydroxyl groups on the phenolic rings, hydrolyzable tannins (ellagitannins and gallotannins), condensed tannins (include pro-anthocyanidins), and flavonoids (including flavonols, anthocyanin, and flavanols) [30]. PPE rich in bioactive plant ingredients and showed better antibacterial effect such as gallic acid, phenolic punicalagins, rutin, and flavonols (flavonones, flavones, and anthocyanidins) [32]. The major components of PPE based on high incidence (Area %) are classified into their respective chemical groups Alkaloids (2-Pyrazoline, 5-ethyl-1,4-dimethyl; Aconitine), Amines (Isobutylamine), Hydrocarbons (Cyclohexane derivatives), Fatty acid esters (Tetradecanoic acid, 2-hydroxy-, methyl ester; Methyl 2-hydroxydodecanoate; 9,12-Octadecadienoic acid (Z, Z)-, phenylmethyl ester), Fatty acids (Oleic Acid), Diols (1,2-Decanediol), Nitrogen-containing compounds (6-Nitroundec-5-en). Das *et al.* [32] compared the concentrations of total polyphenols (53.65), flavonoids (51.52), anthocyanins (21.03), and hydrolysable tannins (51.02) were found in water PPE and 62.71, 102.02, 85.60, and 139.63 in the methanolic PPE. Singh *et al.* [33] found α, β punicalagin and ellagic acid as 13.86% and 17.19% in the ethanol/aquas extract, respectively, while, gallic acid was not detected. By mass spectrometry, PPE contained punicalagin and ellagic acid as the chief components [34]. Due to the absence of a standardized extraction methodology, the findings are variable [35]. The appropriate and safe use of PPE is hard to assess [36].

PPE was indicated to be able to inhibit the growth of *Staphylococcus* spp. under studying (inhibition zone diameter 8-24mm compared to the control). The PPE showed zones of inhibitions ranging from 8-24 mm at different concentrations of 3.125- 100% of the extract. Similarly, Khan and Hanee [37] and Ada and Candemir [38] found zone values between 22-24 and 12-22 mm, respectively. Also, Triwahyuni *et al.* [39] reported inhibition zone diameters of 19.19±0.43, 16.85 ± 0.58, 13.84 ± 0.39, and 11.63 ± 0.88 mm at concentrations of 80, 60, 40, and 20%, respectively. Silva et al. [40] revealed that pomegranate peel extract inhibited all *Staphylococcus* with zones of inhibition of 20 mm. A higher result was obtained by Chinsembu [41] an aqueous PPE (500 mg/mL) produced an inhibition zone of 16–32 mm against *S. aureus* ATCC 25,923. A lower inhibition for tested organisms was detected with water PPE in ranges of 0–15 mm [42]. PPE showed 25, 22, and 19mm inhibition zone diameters, and MICs were 3.9, 7.8, and 7.8mg/mL for *S. typhi, S. typhimurium,* and *E. coli*, respectively [34]. The lipopolysaccharide layer and periplasmic space in the cell walls of Gram-negative bacteria caused a lower sensitivity to the PPE. Variations could be related to some specific properties of the organisms or extraction methods [22]. The multi-layered peptidoglycan was shown as the main factor for antimicrobial resistance. On the other hand, pomegranate extract similarly inhibited both Gram-negative and Gram-positive (*S. aureus*) organisms [43].

In the present study, PPE showed MIC and MBC were 3.125, and 6.25 mg/ml, respectively. Silva et al. [40] obtained a higher MIC value (10 mg/ml) compared to 50 mg/ml for peel and leaf extracts, respectively, without inhibition of any of the strains tested [40]. Ökmen et al. [3] reported that the MIC of 3 different PPE (ethanol, methanol, and water extracts) against 2 reference strains of *S. aureus* was 6.5- 13 mg/ml, also, coagulase-negative strains showed similar MICs. Lower values were obtained by Gil *et al.* [44], and Xu et al. [45] who reported the MIC against *S. aureus* were 0.19, and 0.25 mg/ml. Also, Gosset-Erard *et al.* [19] found that both α and β Punicalagin forms showed MIC of 0.3-1.2 µg/ml. The MIC values could be affected by extract composition, the geographical region, harvesting season, plant age, the growth stage, the method of drying, and extraction, respectively [46].

The polyphenolic compounds (flavonoids, tannins) obtained from the PPE were effectively inhibit bacterial growth [47, 48]. They form complexes with the proteins in the bacterial cell wall [11] as they interact with the sulfhydryl groups of the extracellular protein matrix to inhibit their activities and finally destroy them [47]. Similarly, tannins in the PPE disrupt the cell–microbial adhesion and delay mineral consumption by the bacterial cells [49]. Similarly, the phenolic compounds in the PPE, may have multiple antimicrobial mechanisms, e.g. diminishing the bacterial cell wall, interacting with its composition, and disrupting the cytoplasmic membrane [50], damaging membrane protein, interfering with membrane-integrated enzymes [51], altering fatty acid and phospholipid components, disrupting enzymatic mechanisms for energy production and metabolism, modifying nutrient uptake and electron transport, affecting the DNA and RNA synthesis, and destroying protein translocation and the function of mitochondria [52, 53]. The most potent antibacterial contents in the PPE could be synergistically contributed to oligomeric ellagitannin, together with anthocyanins (pelargonidin-3-galactose and cyanidin-3-glucose) and flavanols (quercetin and myricetin) [54]. Polyphenols in the PPE (gallic acid, punicallins, and ellagic acid) effectively inhibited the growth of pathogenic organisms [55]. Punicalagin had a good anti-staphylococcal effect due to increasing potassium efflux, causing structural cell membrane damage [45]. Although PPE has a complicated chemical composition, its effective antimicrobial effect is well-investigated, the direct reasons for the PPE antimicrobial activities are still not clear, and other antimicrobial constituents still need further studying [21, 56].

CONCLUSION

The optimal management of animal mastitis using ordinary antibiotics is considered expensive for farmers and farm owners. Therefore, global attention is directed towards other alternatives to reduce the of cost using chemical substances by using a biological product as medications and also to face the challenges resulting from the resistance of many pathogens to antibiotics, it was necessary to find alternative, effective and inexpensive solutions at the same time. The present study was conducted to evaluate the antibacterial activity of the PPE against *S. aureus* as a main cause of bovine mastitis. PPE showed promising antibacterial activity against *S. aureus* causing animals mastitis which revealed its feasibility and potential application as biological control agents in the management of diseases affecting animals, especially cow and buffalo. This work provides valuable information about the effect of PPE due to its ingredient and active constituents and effective substances on the bacteria causing animal mastitis disease.

Based on the results shown above, which showed growth inhibition and an effective effect on bacterial cells morphology, it has been suggested that the extract can be used as an antibacterial on the infected areas in animal pens to eliminate harmful pathogens. Further applied studies to determine the treatment doses, safety margins, and its correlation to the degree of infection, without causing a harmful health effects in the treated animals.

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