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Research Article

Processing and Some Phsico-Chemical Properties of White Cheese Made From Camel Milk Using *Capparis Decidua* Fruits Extract As A Coagulant

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Abstract

This study aimed to investigate the processing and some phsico-chemical properties of white cheese made from camel's milk using *Capparis decidue* fruits extract as a coagulant. The fruits extraction was prepared by weighting 20 grams of the crushed dry fruits that shacked with 100 ml distilled water before soaking in water for 24 hours (4°C). The camel milk was heated to 62ºC and then cooled for 5 minutes to 45ºC in an ice water. The extracted fruits of *C. deciduas* were added (3% and 5%). After cheese coagulant was formed, the cheeses were salted with NaCl salt (1% w/v) and stored for 5 weeks into small plastic cups in refrigerator (4°C). The chemical composition was conducted every week. Data were analyzed using statistic 8 programs using the LSD test. The cheese yield using 3% and 5% extract revealed 10.85% and 14.57%, respectively. The data showed significant ($P<0.05$) higher acidity (1.24% vs. 0.64%) in the cheese made using 5% fruits extract compared to that made using 3% fruits extract, respectively. Also, highly ($P<0.001$) significant pH (4.88 vs. 4.81) was found in the cheese made using 5% extract compared to 3% extract, while there was no- significant ($P>0.05$) variation in the protein and fat content. Also, lower total bacterial count was found for the cheese that was produced using 5% *Capparise decidue* compared to those made using 3%. This study showed that the fruits extract of *Capparis decidue* at 5% can be used as a coagulant for camel milk with good yield and acceptable shelf life (5 weeks). This is a promising result, which needs further research investigation for the possibility of the further uses and application of *Capparis decidue* as a coagulant.

Key words: camel milk; *Capparis decidue*; cheese yield; plant coagulant phsico-chemical properties

Introduction

Cheese making from camel milk is more complicated than that of cow, sheep or goat milk, this is mainly due to its low level of total solids, unique composition of casein with a lower amount of kappa casein [1]. Also, camel milk is not sensitive to coagulation with bovine chymosin, most likely due to the major variations between the primary k-casein structures compared to other species [2]. The low content of κ-casein and the large size of camel milk casein micelles compared with those of cow milk prolong the rennet-induced coagulation of camel milk [3]. Camel milk cheese is also characterized by having fragile curd that need longer time to be formed [4]. Camel milk cheeses has smooth and continuous casein network, thinner aggregate strands and smaller pore spaces in comparison to bovine milk cheeses [5].

The technical difficulties of camel's milk coagulation might be due to the functional and coagulation properties of the camel's milk proteins [6]. Also, camel milk cheese was described as having fragile and weak coagulum in addition to the poor yield [7, 8]. The potentiality of using gastric enzymes extracted from camel to coagulate camel's milk was described [9, 10]. Also, camel milk cheese can be prepared by coagulating milk with the use of starter culture [11]. The usefulness of Camifloc enzyme and calcium chloride in making cheese from camel milk was described [4]. It was indicated that addition of lemon juice to camel milk could offer a suitable means for cheaply producing coagulated milk products such as cheese in pastoralist areas [12]. A mixture of *Withania coagulans* (*W. coagulans*) and camel chymosin was found to produce better quality camel and bovine milk cheeses than chymosin alone [13].

Capparis decidua L. (Tundub) is belonging to the Capparaceae family, which is one of the important medicinal plants of Sudan. The plant and its parts are widely used by traditional healers and tribal people for curing variety of ailments [14]. Moreover, *Capparis decidua*, in Sudan has been widely used in folk medicines to cure various illnesses and its biological studies revealed important antimicrobial, anti-oxidative, anti-inflammatory, immunomodulatory and antiviral properties [15]. Recently two novel germacranolides isolated from *Capparis decidua* (Forsk.) have been proved to be potential drug candidates for curing different diseases, e.g., tumors, through CA II inhibitory mechanism [16].

The fruits of *Capparis deciduas* are small, globular, glabrous, fleshy berry, beaked at the apex, resembling a cherry in shape and size [17]. Fresh berries are green, which turn pink on ripening and blackish on drying, moreover numerous seeds are embedded in the pulp of the fruit [17]. *Capparis deciduas* fruits are rich source of the major food components and the important electrolytic minerals [14].

Manufacturing of camel dairy products such as cheese using the same technology as for dairy products from bovine milk can result in processing difficulties and products of inferior quality [18]. This because the colloidal structure of camel milk is different from that of cow milk as it has larger casein micelles and smaller fat globules. This suggested the need for introduction of different processing methods and technologies for camel milk processing [19].

Production of cheese from camel milk has been challenging due to the lack of coagulants that can specifically cleave camel milk κ casein [13]. Recently there has been a steady increase in using plant enzymes in addition to the use of chymosin in the cheese industry because they are readily accessible and have simple extraction and refining processes [20]. This study was, therefore, conducted to assess the coagulating properties and some of the phsico-chemical properties of soft white cheese processed from camel milk using two levels of crude extract of *Capparis decidua* extract (3% and 5%).

Materials and Methods

Source of milk, *C. decidua* and salt

Fresh camels' milk was obtained from Camel Research Center, University of Khartoum, and the *C. decidua* fruits were collected from Al-jayli, Khartoum State, Sudan during November 2016. Salt (commercial grade) was obtained from the local market.

Preparation of *Capparis decidua* extract

Twenty grams of the crushed dry fruits were shaken with 100 ml distilled water and soaked in water for 24 hours (4ºC).

Cheese processing

The cheese was made in the Department of Dairy Production, Faculty of Animal Production University of Khartoum. The milk (5.5 liters) was first filtered using clean white cheese cloth, and heated to 62°C then cooled for 5 minutes in an ice water. The extracted fruits of *C. decidua* in water were added at a rate of 3% and 5% (w/v). After coagulation (during 48 hours), the curds were placed into clean cheese cloths and left to drain overnight. Next day, the cheese was salted with commercial NaCl salt (1% w/v) and kept for 5 weeks (4ºC) in the small plastic cups. Some technological and the phsico-chemical properties were assessed.

Analysis of cheese

Coagulation time, cheese yield, the phsico-chemical properties and total bacterial count were estimated at the laboratory of Department of Dairy Production, Faculty of Animal Production-University of Khartoum.

Cheese yield

Cheese yield were calculated as the weight of cheese divided by weight of milk expressed as a percentage.

Physicochemical analysis

The pH values were measured using a pH-meter (Hanna Instrument-model 98107) after calibration of the instrument. Titratable acidity was determined according to the methods of the AOAC [21]. The fat content was determined by Gerber's method and the protein content was determined by the Kjeldahl method [21].

Microbial examination

The camel cheese samples were examined for total bacterial count. Sterilization of equipment; Petri dishes, test tubes, flasks, pipettes and bottles; were sterilized in an oven at 170 °C for two hours. However, the media, mixer, distilled water, and tips were sterilized by autoclaving for 15 minutes at 121°C [22]. Nutrient agar medium was used for determining the total bacterial count; it was obtained in a powdered form and prepared according to the manufacturer's instructions.
Preparation of cheese samples was done by adding 1 gram of cheese into 9 ml of distilled water and blended for two minutes. Then ten folds serial dilution 10^{-1} to 10^{5} were prepared [22]. The plates inoculated in duplicate and left for two minutes to dry, then were incubated at 32°C for 48 hours. The plates were examined and colonies were counted using manual colony counter.

Statistical analysis

Data were analyzed using statistic 8 program. The analysis was carried out by LSD test.

Results

Cheese yield

The yield of cheese was 10.85 for 3% and 14.57 for 5% of water extracted *C. decidua* (Table 1).

Cheese properties	*C. deciduas* (3%)	*C. deciduas* (5%)
Coagulation time	48 hours	24 hours
cheese yield	10.85%	14.57%
pH	4.7	4.7

Table 1: *Coagulation time, cheese yield of camel milk using C. deciduas extract*

Coagulation time of camel cheese

The coagulation time of cheese was found as 24 hours for 5% and 48 hours using 3% of *Capparis decidua* fruits extracts, the pH was 4.7 (acid coagulations) as shown in Table 1.

The effect of the of *C. deciduas* concentration on the fat content of camel milk cheese

The means of fat content for cheeses made from camel's milk using 3% and 5% *Capparise decidue* were 11.30% and 12.50%, respectively (Table 2). There was non-significant (P>0.05) difference in fat content between cheese made from camel milk coagulated with 3% and 5% *Capparise decidue*. However there were high significant (P<0.001) differences in the fat content during the storage period (Table 3).

Treatment / Chemical Composition	*Capparise decidue* concentration (3%)	*Capparise decidue* concentration (5%)	SE	L.S
Protein (%)	13.19[a]	13.35[a]	0.45	N. S
Fat (%)	11.30[a]	12.50 [a]	0.71	N. S
Acidity (%)	0.64 [b]	1.24 [a]	0.61	*

[a, b]: Values in the same row bearing different superscript letters are significantly different (P<0.05)
L.S = Significant level * = significant (P<0.05) *** = significant (P<0.001) SE = stander error

Table 2: *The effect of C. decidue coagulant concentrations on the chemical composition of the processed cheese*

Physico-chemical properties	**Storage period**						
	Day 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**SE**	**LS**
Protein (%)	14.9[a]	10.10[c]	16.76[a]	18.09[a]	14.73[b]	0.71	***
Fat (%)	10.25[b]	10.00[b]	10.75[b]	11.75[b]	16.75[a]	1.13	***
Acidity (%)	0.55[a]	0.64[a]	2.22[a]	0.62[a]	0.68[a]	0.96	NS
pH	4.81[d]	4.84[c]	4.85[bc]	4.86[b]	4.88[a]	6.18	**

[a, b,c] : Values in the same row bearing different superscript letters are significantly different (P<0.05)
L.S = Significant level **= significant (P<0.01) *** = significant (P<0.001) SE = stander error

Table 3: *Effect of the storage period on Physico-chemical properties of cheeses made from camel milk using 3% and 5% Capparise decidue*

The effect of the of *C. deciduas* concentration on the protein content of camel milk cheese

The means of protein content for cheeses made from camel's milk using 3% and 5% *Capparise decidue* were 13.19% and 13.35%, respectively (Table 2). There were non-significant (P>0.05) differences in protein content between cheese made from camel milk coagulated with different level of *Capparise decidue* (3% and 5%). However, there were high significant differences (P<0.001) differences in the protein content during the storage period (Table 3).

The effect of the of *C. deciduas* concentration on the titratable acidity of camel milk cheese

The means of acidity for cheeses made from camel's milk with addition 3% and 5% *Capparise decidue* were 0.64% and 1.24%, respectively (Table 2). There was significant (P<0.05) difference in titratable acidity between the cheeses made from camel milk using 3% and 5% *Capparise decidue*. There was also non-significant (P>0.05) difference in the titratable acidity during the storage period (Table 3).

The effect of the of *C. deciduas* concentration on the pH of camel milk cheese

The means of pH for cheeses made from camel's milk with addition of 3% and 5% *Capparise decidue* were 4.81 and 4.88, respectively (Figure 1). There was high significant (P<0.001) difference in the pH between cheeses made from camel milk using 3% and 5% *Capparise decidue* extract. There were also significant (P<0.01) differences in the pH during the storage period (Table 3).

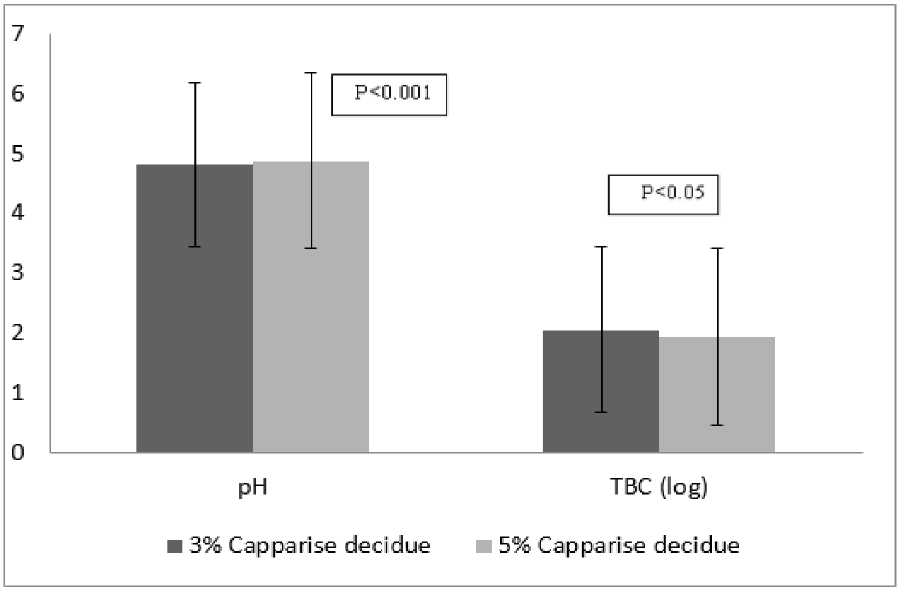

Figure 1: *Variations of the pH and total bacterial count of camel milk cheese processed using two concentrations of Capparise decidue as a coagulant*

Total bacterial count of cheeses made from camel milk using *Capparise decidue*

Means of log total bacterial count (TBC) for cheeses produced from camel milk using *Capparise decidue (*3% and 5%) were 2.06 and 1.93, respectively. Significant (P<0.05) differences in total bacterial count were found (Figure 1). There were also high significant (P<0.001) differences in total bacterial count during the storage period (Figure 2).

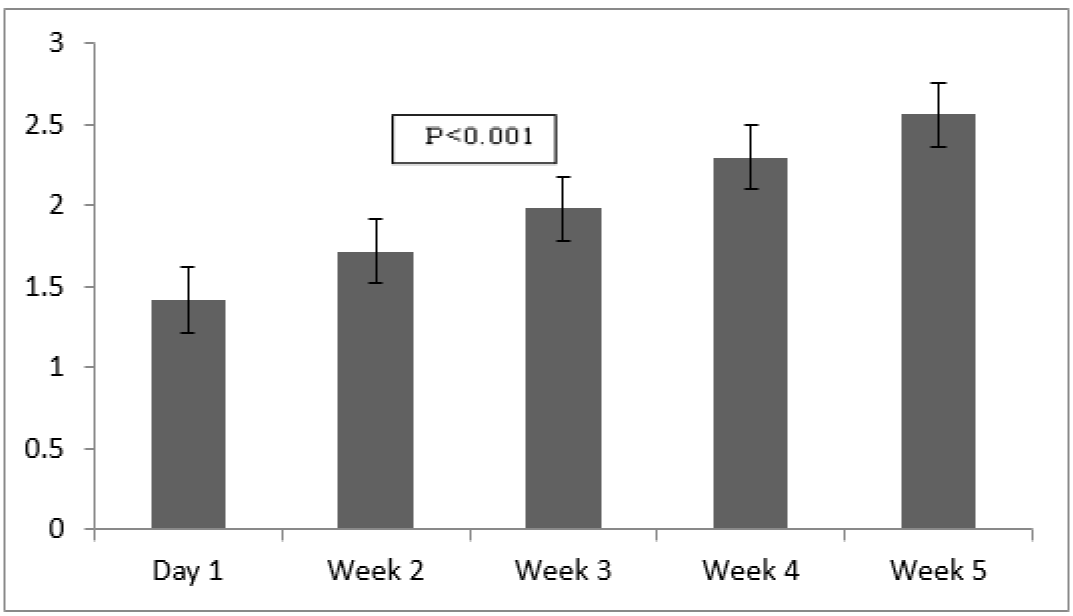

Figure 2: *Effect of the storage period on the total bacterial count of the cheeses made from camel milk using Capparise decidue*

Discussion

The present study has shown that the fruits of *Capparise decidua* are useful in making cheese from camel milk (Table 1). One reason that *Capparis deciduas* fruits are rich sources of calcium indicated the possibility of helping curd formation [14]. Moreover, the flowers and fruits of *C. decidua* are used in diet as vegetable and in pickle production because of their high nutritive ingredients like proteins, carbohydrate, minerals and vitamins [23, 24]. In folk medicine, mixture of equal quantity of fruit powder and sugar is prescribed in rheumatism and they are given in diarrhea in cattle and goats [25]. In Sudan, the fruit of *C. deciduas* is relished by camels and wherever within their reach, by goats and the fruits are also consumed by man [24]. Also, several some previous studies have been performed using plant-derived enzymes for cheese making [13, 26, 27]. These enzymes are present in almost all kinds of plant tissues and it appears to be a general rule that all proteolytic enzymes have the ability to clot the milk under the appropriate conditions [28].

More time is needed to coagulate camel milk using the 3% *C. deciduas* extract (48 hours) compared to that using 5% *C. deciduas* extract (24 hours) as shown in Table 1. This might be because the present study uses the crude whole fruits extract. The antimicrobial and the anti-oxidative, properties of *Capparis decidua* were reported [15]. Hence this study recommended the important of isolating and purification of the active useful proteplytic enzymes for cheese making. Most of the plant proteases have limited use in cheese production due to their high proteolytic nature that resulted in defects flavors and texture in addition to the low cheese yields [29]. On the other hand, camel milk is known to have limiting ability for enzymatic coagulation as it exhibits a rennet-induced coagulation time two to three-fold longer compared with bovine milk [6]. The coagulation of camel milk could be possible only by the action of proteolytic activity of enzymes of plant extract juices rather than on the action of acidity of the juices [30]. Soft cheese can be prepared from camel milk, however the obtained cheese showed weak structures and the coagulation time for camel milk was longer than that for bovine milk, Moreover the specific coagulation behavior of camel milk was attributed to the differences between the composition of the casein fraction in camel milk and that in bovine milk [5].

The yield of cheese from camel milk was higher when using 5% *C. deciduas* extract (14.57%) compared to 3% *C. deciduas* extract (10.85%). The later yield was similar to that mentioned 10.5–11.5% [31]. Also, only 8.70±1.22 cheese yield was obtained when using ginger crude extract compared to 11.4±0.36 when using chymosin [32]. However, the camel milk coagulated by the addition of the starter culture gave higher cheese yield (13.22±4.487%) compared to the cheese prepared by direct acidification (11.70±2.345%) [11]. On the other hand, a yield of 11.3% was obtained using Camifloc enzyme with addition of calcium chloride, against 10.2% without addition of calcium chloride [4]. The low yield of camel milk cheese might be due to the high temperature that affected dry matter intake that reduced total solids in milk, which is the main factor in cheese processing [33]. Moreover, the presence of high concentration of lysozymes and lactoferrins that are mainly affected by the hot seasons and a shortage of feed and water, making cheese from camel milk more difficult and complicated [33]. The reason could also be due to the longer coagulation time and the weak fragile coagulum of camel milk [4, 33]. Also, the production of camel milk soft cheese is very challenging compared to the milk from cow as camel milk has slight size fat globules and meager coagulation property [34]. This behavior is probably due to the large size of casein micelles [35] and most likely due to the major variations between the primary k-casein structures compared to other species [2, 3]. Also, the naturally occurring antimicrobial lactoferrins in camel milk retard microbial starter activities, thus hindering curd formation [36].

In the present study (Table 1) a yield of 14.57% was obtained from camel milk using 5% *C. decidua* extract, which is similar to the conclusion that soft cheese of an acceptable quality and a cheese yield of 14.57% could be produced by adjusting the fat content of camel milk to 1.82%, total solids level to 14% and using rennet powder at a ratio of 1.5 mg (100 g)-1 [30]. It was demonstrated that supplementing camel milk with increasing volumes of lemon juice enhanced cheese yield along with an added shelf life by extending the benefit of higher acidity [12]. However, *W. coagulans* extract protease alone is not sufficient to produce good quality cheese especially camel milk cheese but a mixture of *W. coagulans* and camel chymosin produced better quality camel and bovine milk cheeses than chymosin alone [13].

The fat content of camel's milk cheese obtained in this study revealed 11.3% and 12.5% for 3% and 5% *C. decidua* extract, respectively. This is agreed with 12.90±0.14%, which was obtained when prepared soft unripened cheese using ginger crude extract [32]. However fat content of 13.6±0.20% and 13.4±0.10% were obtained when prepared soft white cheese from camel milk using linear programming technique for two levels of rennet [30]. The data presented previously revealed non-significant increase in the level of fat for camel cheese [4]. Similar to the present study, the fat content showed non-significant (P>0.05) during storage period for camel's milk cheese [8]. The fat content of camel milk cheese decreased during the storage period because the size of fat globules of camel milk is very small and most of it may retain in the whey liquid. This was explained as that the reduction of fat content of camel milk cheese might be due to the smaller size of fat globules and the fragility of the casein micelle network [33]

Table 2 and 3 showed that the protein content of camel's milk cheese revealed 13.19% and 13.35% using 3% and 5% *C. decidua* extract. This is in agreement with the values, which was reported previously (13.35%) for camel milk cheese using ultrafiltration process [37]. However, 29.15±2.62% and 22.15±2.32% were reported for cheese obtained from camel milk by using Camifloc without and with addition of calcium chloride, respectively [4]. Also, significantly higher amount of protein content was found in the cheese prepared by the addition of the starter culture (21.30±0.638%) compared to that prepared by the direct acidification (17.67±1.528%) [11]. Camel milk cheese showed significant (P<0.05) differences during the storage period in the protein content (Table 3). Also, significant (P<0.05) differences were obtained for the content of protein in the fourth day compared to the first 3 days of storage [4].

The protein content decreased after 15 days towards 21 days of storage [38]. The camel milk is reported as useful component of the diet for individuals who show allergic reactions to the protein fraction of cow, ewe or goat milk, as camel milk does not contain beta-lactoglobulin and the content of alpha-casein is much lower than in milk of other herbivores mentioned [39]. A variation among the individual casein was reported for the camel milk compared to other livestock species [40]. It was also confirmed that camel milk contains higher protein (especially casein), while lower in whey milk than human milk [41]. Moreover, compared with bovine milk, camel milk exhibited a lower degree of hydrolysis after reaction with pancreatic enzymes [42]. This will strengthen the capacity of camel milk products as functional foods as was mentioned before [43, 44]. The enzymatic digestibility and antioxidant activity of camel α-lactalbumin were highlighted [45]. Camel milk proteins contained satisfactory balance of essential amino acids. It contains disease-fighting immunoglobulins, which are small in size, allowing penetration of antigens and boosting the effectiveness of the immune system [46].

The acidity of the cheeses was estimated as 0.64% and1.24% for the camel cheese obtained using 3% and 5% *Capparise decidue* fruits extract, respectively (Table 2). Also, the acidity was found as 1.09% when producing cheese from camel milk using different levels of salt [7] and was 0.57±0.06% for soft unripened cheese using chymosin [32]. The lower levels of camel milk acidity might be due to the presence of the antimicrobial agents in the camel milk [47]. Similarly, *C. decidua* has antimicrobial properties [15, 48]. Moreover, the texture of the cheese prepared by using the starter culture was specifically highly preferred in comparison to the cheese, which was obtained by citric acid addition [11]. There was non-significant increase in the level of acidity for camel cheese during the storage (Table 3), as was reported earlier [4]. However, the increase in the acidity of camel cheese during the storage period, probably due to the growth of bacteria in the cheese during storage [49].

The pH of the cheese was found as 4.8 and 4.88 (Figure 1), this result agreed with that reported 4.87±0.04 for the soft unripened cheese using ginger crude extract [32]. However, this finding disagreed with their result when they prepared soft unripened cheese using chymosin; the pH was 5.27±0.02. Similarly, 5.55, 5.93 and 6.00 were reported for camel milk cheese using 0%, 0.5% and 1% salt, respectively [7]. However lower pH value (3.48) after 180 minutes for camel milk gels was obtained, this decline in the pH value of milk gels is related to the added glucono-δ-lactone [35].

The lower TBC reported for the cheese produced from camel milk using 5% *Capparise decidue* compared to those made using 3% *Capparise decidue* (Figure 1) indicated the antimicrobial and antioxiadant properties of *Capparise decidue* [15, 48].

The average means of total bacterial count for the two concentrations of *Capparise decidue* fruit extract were increased during the storage period (Figure 2). The obtained values for the TBC in the present study was lower than that reported in a previous study [7, 49] dealing with camel milk cheese. Similar to the present study, camel milk cheese was significantly (P<0.05) different during the storage period in the total bacterial count [49]. However, the decrease obtained in the TBC is likely due to the effect of high lactic acid in the cheese samples, which suppressed the growth of microorganisms [50]. This was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin and immunoglobulin in camel milk [51]. Also, low levels of TBC were obtained for camel milk cheese coagulated with 5% *Capparise decidua* fruit extract compared to 3% (Figure 1 and 2) indicating the antibacterial properties of *Capparis decidua* as was explained above.

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