Received: 15 April 2023, Accepted: 01 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 8, 1593-1599

Occurrence and Molecular Characterization of Methicillin-resistant *Staphylococcus aureus* in Marketable Milk and Soft Cheese: Effect of Curcumin and Ginger Nanoparticles on its Survival

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Abstract

This study was designed to record the occurrence of *Staphylococcus aureus* with special reference to the methicillin resistance isolate as well as some virulence factors in milk and soft cheese and the antibacterial effect of curcumin and ginger nanoparticles. The samples were collected over a period of December 2021 to April 2022 in different localities in Assiut Government, Egypt. The samples were subjected to microbiological and molecular analysis. Curcumin and ginger nanoparticles (NPs) were prepared by the ultra-sonication method and were characterized by XRD, FTIR, and TEM. From 100 samples of marketable milk and soft cheese (50 for each one), the results showed that 78 samples (78%) were positive for *Staphylococcus aureus*. Moreover, the percentages of *mecA* gene were 21.06%. Also, the isolated strains were carried enterotoxins gene (*sea*) and alpha hemolysis gene (hlα) with percentages of 3.51 and 2.43%, respectively. With the assistance of the well diffusion method, curcumin and ginger NPs showed antibacterial activity against methicillin-resistant *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was obtained by the micro broth dilution method which indicated that the lowest concentrations of curcumin and ginger NPs that inhibited the growth of *Staphylococcus aureus* were 25 mg/ml and 50 mg/ml, respectively. In addition, the results revealed that curcumin NPs have antibacterial activity higher than that of ginger NPs against MRSA.

KEYWORDS
Staph. aureus, MRSA, *sea*, *hla*, curcumin NP, Ginger NP.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the pathogenic bacteria which can cause a wide range of diseases to both humans and animal (Turner *et al*., 2019). MRSA was first reported in 1961 in the United Kingdom where they discovered that type of Staph aureus resistant to all types of β-lactam class antibiotic agents including carbapenems and cephalosporin. This resistance is due to the mobile genetic element, known as staphylococcal chromosomal cassette mec (SCCmec) which carrying *mecA* encodes the protein (penicillin-binding protein 2A (PBP2A), a transpeptidase that helps form the bacterial cell wall. PBP2A has a lower affinity for beta-lactam antibiotics such as methicillin and penicillin than DD-transpeptidase does, so it does not bind to the ring-like structure of penicillin-like antibiotics. This enables transpeptidase activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis. The bacteria can then replicate as normal (Doyle *et al*., 2012; Petinaki and Spiliopoulou., 2012)MRSA cause severe diseases such as bloodstream infections, pneumonia, bone, bacteremia, infective endocarditis, skin and soft tissue infections osteomyelitis, septic arthritis, prosthetic device infections, toxic shock syndrome, meningitis, and foodborne pathogen. Many researchers found MRSA in foodstuffs of animals such as milk and dairy milk products (Algammal *et al*., 2020). Milk is a suitable medium for bacterial development and a means of bacterial transmission such as MRSA when milk or milk products are consumed by humans, so this is a public health hazard. MRSA strains have been identified in a wide variety of dairy products such as cheese, yogurt, butter, ice cream, and pasteurized milk. This MRSA strain also possibly carries the Staphylococcal enterotoxin gene which could potentially cause food poisoning (Zimmerli *et al*., 2009). Therefore, the treatment of this bacteria type using safe drugs extracted from medical herbs such as curcumin and ginger is still a bit challenging.

Curcumin which derived from *Curcuma longa*, while 6-gingerol and 6-shagaol, the major bioactive of Ginger rhizome (*Zingiber officinale*), are types of herbs that showed an effective anti-cancer, anti-inflammatory, anti-diabetes, and they can be promising anti-bacterial activity (Saberi-Karimian *et al*., 2019). Curcumin (diferuloylmethane) has a yellow color and ginger can be red, yellow, or white in color (Hettiarachchi *et al*., 2021) . Curcumin and Ginger have low to no toxicity at active doses and with oral doses as high as 8-12 g per day. However, their uses showed some problems such as low water solubility, poor absorption, fast metabolism, quick systemic elimination, low bioavailability, poor pharmacokinetics, low stability, and low penetration targeting efficacy. These problems decrease their activity against bacteria and virulence genes (Shariati *et al*., 2019). To solve these problems, converting curcumin and ginger into nanoparticles or loading on nanocarriers, which allow them to soluble in water and give high effect against bacteria.

Therefore, this work aimed to investigate the MRSA in marketable milk and commercial soft cheese in Assiut city, Egypt, and to examine the inhibitory effect of synthesize curcumin and ginger nanoparticles by ultrasonication technique in water.

ISSN: 2090-6277/2090-6269/

MATERIALS AND METHODS

Ethical Approval

This research was conducted according to guidelines of the Molecular Biology Research and Studies Institute, Assiut University, Egypt (IORG0010947-MB-21-28-A).

Sample Collection

The samples were collected from December 2021 to April 2022, a total of 100 samples of marketable milk and soft cheese included 50 samples of each from cows and buffalos were collected from different areas in Assiut government, Egypt. The samples were collected in sterilized falcon tube, transported in an icebox to the laboratory of Molecular Biology Research and Studies Institute, Assiut University, Egypt.

Isolation and Identification of Staphylococcus aureus

Staph. aureus was isolated by inoculating each sample in tryptic soy broth (Oxoid, England). 10 ml or g of samples was homogenized in 90 ml of sterile enrichment broth and incubated for 24 h at 37°C. A loopful of inoculum from the enrichment was spread on mannitol salt agar (HiMedia, India) and incubated for 48 h at 37 °C, the color changed from red to yellow refers to the growth of Staph aureus. The pure culture was streaked on nutrient agar (Oxoid, England) and incubated for 24 h at 37°C (Haque *et al*., (2018).

DNA extraction

Extraction of DNA from suspected strains and the standard strain was grown on brain heart broth (Himedia, India) and incubate for 24 h at 37°C, for the extraction of DNA by veterinary DNA/RNA extraction kit (INTRON Biotechnology, South Korea). DNA was extracted and stored at -20°C.

Polymerase chain reaction

The PCR amplification was performed in a thermal cycler (Veriti™ Thermal Cycler, Applied Biosystems, USA) by various primers (metabion, Germany) to determine the presence and absence of the pathogenic gene. The sequence of primers cited in Table 1. PCR reactions were performed in final volume 25 µl and included 12.5 µl of 2× PCR master mix (Red Master, Cosmo, England), 150 ng of DNA template (Spectrophotometer, Gene Quant 1300, England), 1 µl of each primer, and the volume was adjusted to 25 µl by Nuclease-free water. The PCR condition: 95°C for 5 min of initial denaturation; 40 cycles of 95°C for 45 sec of denaturation, annealing according to TM of each primer, 1 min for 72°C of extension; and final extension 72°C for 10 min.

Gel electrophoresis

PCR products were run in 1% agarose gel in TBE (pH = 8.3) and amplified using a 110-volt power supply for 1 h, 120 Amp (VWR-300) was stained using ethidium bromide and the image visualized under UV illumination (UV Band-Elutor 91, Biometra) and documentation system (Viber Loumat Kaiser-Viberloument).
Qualitative phytochemical analysis for Curcuma longa and Zingiber officinale

Collection of herbs and processing

The herbs were collected from a local market in Assiut City, then the plants were processed and analyzed. The roots were washed with tap water, distilled water, and dried in an oven at 70°C until complete drying. The roots were then grinded using a sterile electric blender and kept in a glass bottle faraway from sunlight and moisture. The chemical test of the plant samples using standard methods was done on an aqueous extract from each plant and powder of samples (Sotiropoulou *et al*., 2020).

Preparation of aqueous extract

The powder herbs (5 g) were soaked in a beaker containing 100 ml distilled water, stirred using a magnetic stirrer and heated to 45°C for 6 h. Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The water extract was kept in the refrigerator (Krishnaiah *et al*., 2009).

Test of Flavonoids

A few drops of 1% NH_3 were added to the aqueous extract until the observation of yellow color which refers to the presence of the flavonoid (Krishnaiah *et al*., 2009).

Test for alkaloids

The crude extract was mixed with 2 ml of 1% HCl and heated. Mayer's and Wagner's reagents were then added to the mixture. The appearance of the turbidity of the solution was taken as evidence for the presence of alkaloids (Krishnaiah *et al*., 2009).

Test for saponin

About 2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then the formation of emulsions was observed (Krishnaiah *et al*., 2009).

Table 1. The sequence of 16s rRNA for *Staph. aureus* and their virulence genes.

Target genes	Primers	Sequence	Product size (bp)	Annealing Temp	Reference
16s rRNA for staph	*16s rRNA-F* *16s rRNA-R*	GTAGGTGGCAAGCGTTATCC CGCACATCAGCGTCAG	229	55°C	Ewida and Al-Hosary (2020)
mecA	*mecA-F* *mecA-R*	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147	57°C	Badua *et al*. (2020)
seA	*seA-F* *seA-R*	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCC	102	54°C	Johnson *et al*. (1991)
Hla	*Hla-F* *Hla -R* *Hla -R*	CTGATTACTATCCAAGAAATTCGATTG CTTTCCAGCCTACTTTTTTATCAGT CTTTCCAGCCTACTTTTTTATCAGT	209	56°C	Li *et al*. (2018)

Test for phenols and tannins

The crude extract was mixed with 2 ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins (Krishnaiah *et al*., 2009).

Quantitative phytochemical analysis

Quantity of Saponins

20 g of dried samples were weighed and transferred into a 200 mL conical flask and then 100 mL of 20% C_2H_5OH was added to the conical and heated with continuous stirring over a thermoblock at about 55°C. The solution was filtrated and then re-extracted with another 20% C_2H_5OH. The extracts were reduced to 40 ml by overheating to 90°C. In the separator funnel, the extract was placed and 20 ml of $(CH_3CH_2)_2O$ and shaken vigorously, two layers had appeared. $(CH_3CH_2)_2O$ layer was discarded and the aqueous layer was recovered and the purification process was repeated. 60 ml of n-C4H9OH was added and the combined n-C_4H_9OH was washed then extracted with 10 ml of 5% NaCl. Evaporation of the solution by heating and the samples were dried in an oven to a constant weight (Krishnaiah *et al*., 2009).

Quantity of alkaloids

The dried samples (5 g) were soaked in a beaker containing 200 ml of 10% CH_3CO_2H in C_2H_5OH covered and stood for 4 h. The mixture was then filtrated, and the aqueous extract was heated to 1/4 of the original volume. A concentrated NH_4OH solution was added until the precipitate is completely formed, filtrated the precipitate and washed with diluted NH_4OH. The residue (alkaloid) was dried and weighed (Krishnaiah *et al*., 2009).

Quantity of tannins

The powder sample (5 g) was weighted and stirred with 50 ml distilled water for 1 h and then filtered off. To 5 ml of the filtrate, 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)6.3H_2O$) was added in a test tube and the absorbance was measured by spectrophotometer at 395 nm within 10 min.

Quantity of flavonoids and phenols

The folin-ciocalteu method was used to determine the total flavonoids and phenols by following these steps. 0.1 g of herb powder was dissolved in 1 mL of deionized water. To the latter solution, a mixture of 2.8 mL of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3) and 0.1 ml of 50% folin-ciocalteu reagent was added. The mixture was incubated at room temperature for 30 min, and its absorbance was measured at 750 nm (Wabaidur *et al*., 2020).

Synthesis of curcumin and ginger nanoparticles (NPs)

Stock solutions from curcumin or ginger were prepared by dissolving 10 mg of curcumin or ginger powders in 1 mL DMSO and were added to 50 ml boiling water with a flow rate of 0.2 ml/min and sonicated for 30 min. The ultrasonication with a power of 100 W and frequency of 30 KHz was used. After the sonication process, the mixture solution was stirred at 500 rpm for 30 min until an orange-colored precipitate was obtained. The supernatant was discarded, and the synthesized nanocurcumin or nano-ginger was obtained by filtration and drying processes and stored for further studies (Shariati, *et al*., 2019; Hettiarachchi *et al*., 2021).

Samples characterization techniques

The crystallinity and structural properties of curcumin and ginger NPs were characterized by an X-ray diffractometer (PW 2103, Philips, Netherlands, with CuK radiation, λ = 1·5406 Å). The diffraction data were collected in the 2θ range from 4 to 80° at a 5°/min scan rate.

Curcumin and ginger NPs were characterized by FTIR (Nicolet spectrophotometer, model 6700), mixing dried powder of nanoparticles with KBr. Spectra were taken in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. The data of FTIR reveal information about functional groups which are present in the curcumin and ginger NPs.

Transmission electron microscopy (TEM, JEOL Model JSM-5400 LV (Joel, Tokyo, Japan)) was used to assess the morphological characterization of the curcumin and ginger NPs.

Antibacterial effect of curcumin and ginger NPs

The antibacterial activities of curcumin and ginger NPs were carried out by using well diffusion method. In this method, 15 ml of sterilized media was poured into perti plates and let solidify. Then, 100 µl of inoculum bacterial suspension was added and spread on the agar (Oxiod, England) and left to dry for 5 min then the wells in the gel was made, 100 µl of NPs was added, and incubated for 24 h at 37°C (Wang *et al*., 2020).

Determination of the Minimum inhibitory concentrations (MICs)

Microbroth dilution methods were used to determine the MICs, which is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Different concentrations of nano-curcumin and nano-ginger were prepared in distilled water (500, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.2 mg/ml). The culture of bacteria was adjusted with Pbs saline to obtain the final concentration of 5×10^5 CFU/ml. 100 µl of NPS was mixed with 100 µl of brain heart broth (Oxiod, England) and 20 µl of bacterial suspension and the optical density was measured by using an ELISA reader (sunrise Absorbance Reader, TECAN) at 600 nm. Then, the mixture was incubated (shaking incubator, N-Biotek(NB-205)) for 24 h at 37°C and the optical density measurement was repeated. In this method, the positive control sample that contained the broth with a bacterial suspension only without the plant nanoparticles, while the negative control sample that contain only the broth with distilled water (Sanusi *et al*., 2019; Shivaee *et al*., 2021).

Statistical analysis

Manipulation of data and descriptive statistical analysis were done using Microsoft Excel (Microsoft Office, Microsoft).

RESULTS

Results of phytochemical analysis

The most beneficial antioxidant was studied because it plays an important role to attack human diseases. The qualitative analysis of the phenolic compounds, flavonoids, alkaloids, saponins and tannins for curcumin and ginger were high and moderate amounts in phenols concentration of both herbs, respectively,

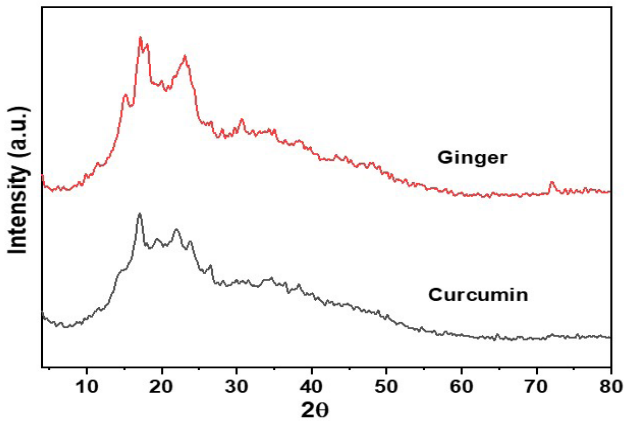

Fig. 1. XRD patterns of curcumin and ginger NPs.

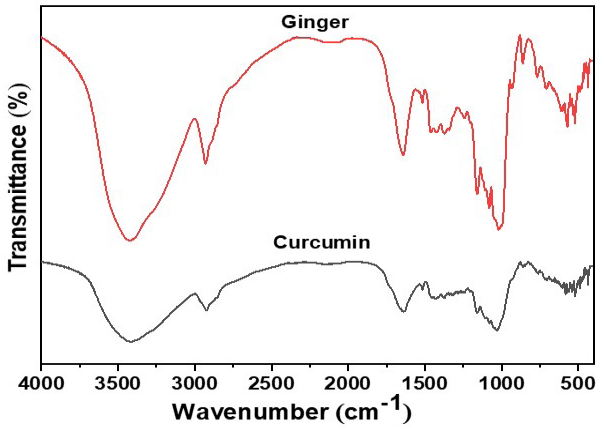

Fig. 2. FTIR spectra of curcumin and ginger NPs.

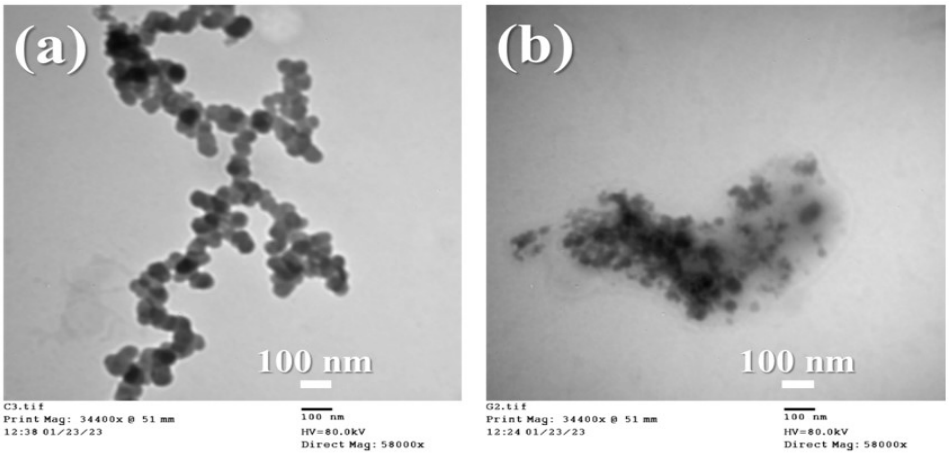

Fig. 3. TEM images of (a) curcumin, and (b) ginger NPs.

while the other components in both plants were in low amounts. Moreover, the quantitative analysis *Curcuma longa* and *Zingiber officinale* are cited in Table 3.

Nanoparticles characterization

The XRD, FTIR, and TEM images of curcumin and ginger NPs are displayed in Fig. 1, 2, and 3, respectively.

The antibacterial activities of curcumin and ginger NPs

The inhibitory effect of curcumin and ginger NPs against MRSA showed that 29±1.1 and 18.3±1.5 mm, respectively. In addition, MIC of both plants was 12.5 and 25 mg/ml, respectively.

DISCUSSION

Out of 100 samples of milk and soft cheese 78 samples were positive for *Staph. aureus* with a percentage of 84 and 72%, respectively, which was lower than that recorded by Haque *et al.* (2018). While, it was higher than that recorded by Shekhan *et al.*, (2011); Abd El Halem. (2019) and Sadek and Koriem (2020). The high percentage of *Staph. aureus* may be due to the contamination of milk from animal, milk handlers and the equipment used in the milking process. The percentage of MRSA obtained from the examined milk and soft cheese were 21 and 6%, respectively, these results nearly agreed with that obtained by Haque *et al.* (2018), while, the obtained results were lower than (Al-Ashmawy *et al.*, 2016). The percentages of sea and *hla* genes were reported as 3.51 and 2.43%, respectively, which agreed with that found by Kou *et al.* (2021).

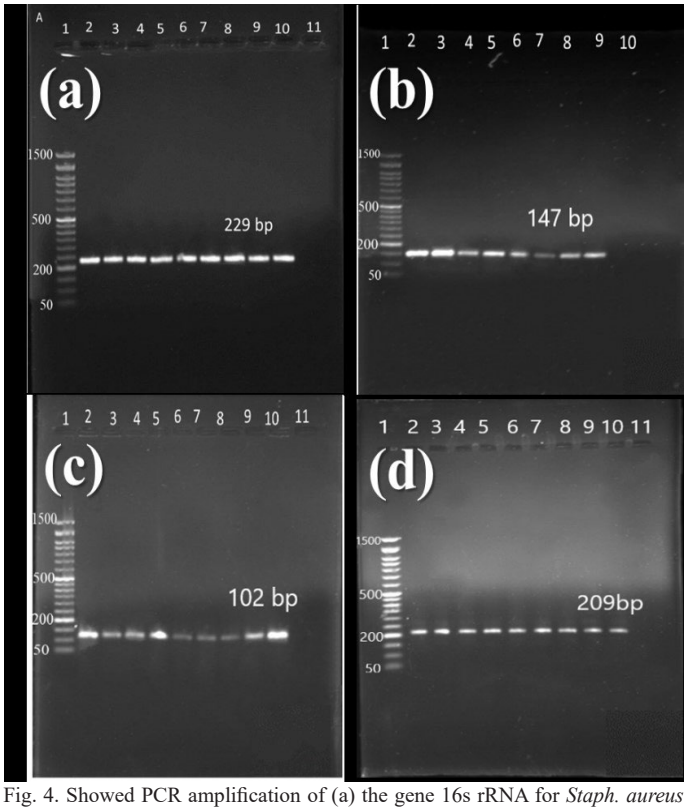

Fig. 4. Showed PCR amplification of (a) the gene 16s rRNA for *Staph. aureus* (229 bp), (b) the *mecA* gene in MRSA (147 bp), (c) the *sea* gene in MRSA (102 bp), (d) the *hla* gene in MRSA (209 bp) which lane (1) ladder and lane (2-10) +ve for each gene and lane (11) –ve controls.

The presence of these virulence genes has a high risk to human health because it causes several diseases. The *mecA* gene was found in the bacteria cells, which allows it to be resistant to

Table 2. Occurrence of *Staph. aureus* and its virulence genes in the examined samples.

Samples	No of the examined samples	*16s rRNA*		*mecA*		*seA*		*hla*	
		No.	%	No.	%	No.	%	No.	%
Marketable milk	50	42	84	21	42	10	20	7	14
Soft cheese	50	36	72	6	12	3	6	2	4

Table 3. Quantitative analysis of the antioxidant compounds that are present in *Curcuma longa* and ginger.

Component	*Curcuma longa*	*Zingiber officinale*
Alkaloids (mg/g)	2.9±0.7	1.2±0.85
Saponins (%)	1.1±0.05	1.52±0.1
Phenols (mg/g DW)	23.9±0.45	16.475±1.05
Flavonoids (mg/g DW)	5.72±0.41	3.93±0.06
Tannins (%)	1.04±0.03	3.3±0.32

values are presented as mean±SD of 3 replicates.

antibiotics such as methicillin and other beta lactam antibiotic. (Kitai *et al*., 2005) stated that the source of food contamination by MRSA is mainly of human origin as food handlers and not due to animal infection. *Staph. aureus* produces enterotoxins (SEs) that cause food-borne diseases. Because its toxins are short, water-soluble, extracellular protein and heat stable, which make killing it by boiling is very difficult. Also, its resistance to many environmental conditions such as freezing, low pH and drying, so the treatment of this toxin needs a high effort (Grispoldi *et al*., 2019). SEA is the most common toxin in staphylococcus-related food poisoning (Pinchuk *et al*., 2010). Alpha-hemolysin a cytotoxic protein that had the ability to lyse erythrocytes and other eukaryotic cells. Also secreted in nontoxic soluble forms and it was important in experimental endocarditis and pneumonia (Wang *et al*., 2020).

The phytochemical analysis showed that *Curcuma longa* and *Zingiber officinale* have different types of antioxidants with different amounts. The amounts of phenolic and flavonoids compounds in ginger in their rhizomes are 16.47 and 3.93 mg/g dw, respectively and these results agreed with that obtained by (Ghasemzadeh *et al*., 2010). The amount of tannins was found to be 3.3 mg/g, which was higher than 0.02 mg/100g which was recorded by Ogbuewu *et al.* (2014). While the obtained amount of saponins was 1.52 mg/g which was higher than 4.01mg/100g, found by Ogbuewu *et al.* (2014). The amount of alkaloids was obtained equal 1.52 mg/g which was lower than the percentage 6.52%, which was recorded by Kela *et al.* (2021).

On the other hand, the amounts of phenolic and flavonoids compounds in *Curcuma longa* were 23.9 and 5.72 mg/g dw, respectively. The obtained flavonoid is higher than that recorded by Hayat and Sabri (2016), they found it had range from 2.2 to 3.11 mg quercetin/g dry plant. Moreover, the obtained amount of phenols agree with that recorded by Djikeng *et al.* (2018). The percentage of tannins obtained was 1.04%, which agree with the percentage that recorded by Mughal. (2019) (1.08%). While, the amounts of alkaloids and saponins were found to be 2.9 and 1.1%, receptively, which were higher than that recorded (0.76 and 0.45%, respectively) by Ikpeama *et al.* (2014).

The amounts of these phytochemicals play an important role in increasing the effectiveness of herbs. For example, the presence of total phenolic compounds such as phenols in high amounts in curcumin and ginger have a role as an antioxidant, anti-inflammatory, antiapoptosis, cardiovascular protection, play roles in the regulation and adjusting the levels of diabetes in blood, inhibition of angiogenesis and cell proliferation activities, anti-cancer and maintaining normal blood pressure. Alkaloids belonging to a beta-carboline group possess antimicrobial, anti-HIV and antiparasitic activities (Patel *et al*., 2012). Tannins are water-soluble polyphenols, tannins have a role also as anti-cancer, virucides, antioxidant, antimicrobial, wound healing and antidiarrheals. Saponins are amphiphilic molecules consisting of carbohydrate and triterpenoid, the presence of saponins with alkaloids help in hypertension treatment also saponins are known as anti-microbial, anti-cancer and decrease cholesterol in the blood (Kela *et al*., 2021).

The XRD patterns of curcumin and ginger NPs are shown in Fig. 1. The XRD pattern of curcumin showed reflection at 20 values of 17°, 19.3°, 21.9°, 23.8°, 26.5°, and 28.1°. While the XRD profile of ginger NPs exhibited diffraction peaks at 20 values of 15.2°, 17.1°, 18°, 19.9°, 23.1°, 26.5°, 28°, 30.6°, and 72°. The observed peak at 2θ ~ 23° may be attributed to the β-sheet structure of the protein (Zhao *et al*., 2015). The average crystallite sizes of curcumin and ginger NPs were calculated based on the Scherrer equation (Said *et al*., 2014; Ali *et al*., 2022), and found be 13.2 nm, and 12.4 nm, respectively.

The FTIR spectra of curcumin and ginger NPs are displayed in Fig. 2. The spectrum of curcumin showed bands that assigned as follow: 3418 cm^{-1} (Phenolic hydroxyl groups), 2924 cm^{-1} (Aromatic C-H streching), 1640 cm^{-1} (C=C stretching), 1515 cm^{-1} (υ (C=O), δ (CCC) and δ (CC=O) [4, 5]), 1429 cm^{-1} (C=H), 1373 cm^{-1} (Alkene CH_2 scissoring (Gunasekaran *et al*., 2008), 1160 cm^{-1} (C–H stretching), 1031 cm^{-1} (C–N stretching), 860 cm^{-1} (CH in plane bending), 760 cm^{-1} (CH out of plane bending), 542 cm^{-1} (Aromatic C-C-C out of plane bending), and 437 cm^{-1} (Aromatic C-C out of plane bending). while, The spectrum of ginger showed bands that characterized as follow: 3424 cm^{-1} (O–H stretching vibration), 2930 cm^{-1} (Methylene C–H asymmetric stretching), 1644 cm^{-1} (C=C stretching), 1516 cm^{-1} (Aromatic skeletal stretching (Amponsah *et al*., 2022), 1414 cm^{-1} (Aromatic skeletal combined with C–H in-plane deforming and stretching (Zhao, *et al*., 2015), 1371 cm^{-1} (C–N stretching), 1158 cm^{-1} (υ (C=O), υ (C-O-C)), 1081 cm^{-1} (υ (C-O-C)), 1020 cm^{-1} (Amino acid, δ (C–H) , υ (C-C) (Trp) (Zhao, *et al*., 2015) , 861 cm^{-1} (υ (C-H)), 768 cm^{-1} (υs (pyr fold (Zhao, *et al*., 2015) + υ (H)), 570 cm-1 (δ (C–Br) (Hussein *et al*., 2017), 532 cm^{-1} (δ (pyr rot (Zhao, *et al*., 2015), and 437 cm^{-1} (δ (pyr rot)).

The morphology of curcumin and ginger NPs was studied by TEM, and the obtained images are shown in Fig. 3 (a, b). The TEM image of curcumin displays spherical particles with an average diameter of ~ 72.5 nm, which arranged in bunches-like structure. While ginger showed spherical particles with an average diameter of ~ 50 nm with some agglomerated particles.

Nano-curcumin and nano-ginger had effects against MRSA with MICs equal to 25 mg/ml for ginger and this result agree was that recorded by Sanusi *et al.* (2019). They used gingers which were collected from different zones to evaluate their activities against different bacteria species. On the other side, nano-curcumin showed higher activity against MRSA compared to ginger the inhibition zone and MICs data. The obtained activity of nano-curcumin on MRSA was similar to that obtained by Hettiarachchi *et al.* (2022). Teow *et al*., 2016 carried out that the inhibitory effect of curcumin on *Staph. aureus* mainly due to the damage occur on the bacterial membrane.

CONCLUSION

The hygienic qualities of examined marketable milk and soft cheese are poor, in addition, the isolates of *Staph. aureus* carry many virulence genes such as *mecA*, *sea* and *hla* genes which can cause many human infections. Moreover, the curcumin and ginger nanoparticles show effective antibacterial activity against MRSA.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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