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Microbial growth inhibition of Rosa damascena petal extract toward the isolated Staphylococcus aureus from Iranian traditional cheese

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ABSTRACT

Staphylococcus aureus is a ubiquitous organism commonly isolated from bulk raw milk suffering from mastitis that resulted as a major concern for the safety of traditionally produced cheeses. Preservative materials usage can prevent the growth of S. aureus during cheese making. Rosa damascena petals extract have antibacterial and antioxidant properties activities against. The aim of this study is to investigate the effect of bacteriostatic extract of R. damascena petals on growth of S. aureus isolated from Iranian traditional cheese. In this investigation, the extraction of R. damascena petals was produced after soaking in ethanol and distillation under vacuum evaporator rotary. The concentrations of extraction were prepared in 4000, 2000, 1000, and 500 ppm and the bacteriostatic effects of extract were measured with calculating the diameter of inhibition zone in microbial culture medium. Results showed that the maximum inhibition affect was the concentration 4000 ppm. It was concluded that the extraction of R. damascena petals can be useful as a natural bacteriostatic additive for the control of S. aureus in traditional cheese.

1. Intoduction

Staphylococcus aureus is a Gram-positive pathogen typical mesophilic, grow at temperature 7–48 °C and optimum at 37 °C which produced heat resistance enterotoxin. It is a common pathogen in human and warm blooded animal that isolated from skin, mucous membranes, raw milk and meat animal. Raw cow's milk may contaminate with S. aureus and the presence of it in cheese made from raw milk can be a health hazard for consumer (1,2,3).

Usually, human and domestic animals are sources for food contamination to S. aureus. So it is expected that all products of animal origin, or foodstuffs that are directly manipulated by human and distributed, to be contaminated (4). Probability of high incidence of toxicity and gastroenteritis will be if animals milk with mastitis to be consumed or used for cheese production (2). Food poisoning caused by Staphylococcus enterotoxin widely throughout the world that cause gastroenteritis are common. There have been outbreaks by milk products such as cheese, dried milk, and chocolate. Appearance of acute effects of staphylococcal enterotoxigenic strains after a short incubation period (1–7 h) and could cause abdominal pain, nausea, vomiting, and diarrhea (5). Consumption of cheese made from raw milk has been outbreak (6,7). Some dairy technologies have been changed after food poisoning outbreaks, for example, in Stilton cheese, where a wide intoxication of staphylococcal origin was registered in 1989 and then, the technology shifted from raw to pasteurized milk (8), but also traditional raw milk cheeses consumption are worldly widespread. Traditional cheese is a milk product that is consumed in rural, tribal and urban areas of Iran and it is made from milk of sheep, goat, and cow. For getting the better flavored cheese, sometimes milk does not receive heat or it can be less than heat pasteurization. When the cheese cloth was made, the

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whey exits through cotton filter and solid cottage cheese is produced. Then, the clot is divided into smaller pieces by knife. A part of cottage cheese may consume as fresh and the rest may add salt on it as a preservative and it is used for a long time. Some producer added plant essential oils or extract which have flavoring or preservative effects.

Constituent components of plant essential oils and extract have antibacterial effects. Many use them for controlling the growth of pathogenic bacteria in food sources that are used as food preservatives. Food stuff international organizations have a new approach to a health food and used natural food preservative instead of chemicals preservative. Because of the possible adverse effects of high doses of natural food preservative on taste, smell and color of food not met with great interest, but the rose plant is compatible with popular tastes.

Rosa damascena is one of the most important Rosa species for extract and essential oil production (9). Rosa petal tea is a caffeine-free source with the highest antioxidant property (10). There are some studies about the anti-HIV, antibacterial and antioxidant activities of R. damascena essential oil (11,12,13). R. damascena plants in Iranian traditional medicine are medicinal plants and contain compounds such as citronellol, geraniol, nerol, linalool, which are antibacterial (14). Due to its wide application in Persian folk medicine, its known background and because it grows in many Provence's in Iran specially in Kashan city and as a result it can be obtained very cheap it may be a good available natural source of antibacterial and a good preservative for food stuff in order to prevent spoil of food consignments and also improving the quality of food products. The aim of this study is determination of microbial growth inhibition of R. damascena petal extract towards the isolated S. aureus from Iranian traditional cheese.

2. Materials and methods

2.1 Iranian traditional cheese sample collection

Some samples of Iranian traditional cheese were collected randomly from Tehran markets and they were transferred to laboratory under the refrigeration conditions.

2.2 Isolation of S. aureus

30 g from the middle of cheese sample weighted in the sterile conditions in a becher and add 70 ml cooked meat broth and homogenized on the shaker for 5 min. Samples incubated at 37 °C for 24 h. After this time, serial dilutions were made in sterile 0.1% peptone solution and 0.1 ml of the sample was spread on the surface of plates containing Baird's Parker agar medium. Then, plates were incubated at 37 °C for 48 h for characteristic colonies. Colonies of S. aureus are dark gray to black, shiny, medium-sized colonies, clear halos surrounding colonies, white edge surrounded by a clear zone 2–5. These colonies were selected for examination of microbial growth inhibition of R. damascena petal extract.

2.3 Preparation and extraction of R. damascena petal extract

The petal of R. damascena flowers were obtained from growing region in Kashan city of Iran during the flowering period in May to mid-June, 2012. The petals were dried in a dark place at room temperature. Dried petals were powdered and stored at 4 °C until use. Fifty grams of powder was macerated in 500 ml of ethanol (85%). Extracts were prepared using the cold maceration process for 72 h at room temperature under constant shaking and filtered with Whatman No. 1 filter paper. The liquid was evaporated at a vacuum rotary evaporator and the extract was put in the oven at 45 °C to make dry. Then dry extract was dissolved in 50% dimethyl sulfoxide to a final concentration of 4000 ppm. The obtained sample was stored at 4 °C and further used for antibacterial tests.

2.4 Evaluation of antimicrobial effects

2.4.1 Agar- well diffusion me

For the inoculums turbidity was visually adjusted to 0.5 McFarland turbidity standards (approximately 1.5×10^8 CFU/ml) (15). Four plates selected for using four concentrations of extract (4000, 2000, 1000 and 500 ppm) and on each plate were made three wells (5 mm diameter) with sterile Pasteur pipettes. 50 µl of each extract solution were added in each well. Dimethyl sulfoxide (DMSO) (50% concentration) was used as a negative control, then inoculums were swabbed on the surface of Mueller Hinton agar plates three times, by rotating the plates approximately 60° with a sterile cotton swab.

Extract antimicrobial effect was measured by calculating the diameter of the zone of inhibition of bacterial growth and expressed in terms of the average diameter of the zone inhibition in millimeters. Antibiotic (Vancomycin) and DMSO used as controls.

2.4.2 Determination of minimal inhibitory concentration (MIC)

To determine the minimal inhibitory concentration (MIC) extract, two-fold serial dilutions of the extract (4000 ppm) were prepared in tubes with brain and heart infusion broth as diluents. Each dilution was seeded with bacterial suspension equivalent to 0.5 McFarland turbidity standards and incubated for 24 h at 37 °C.

MIC was taken as opacity and transparency regard to bacterial growth, or no growth. Because extract

tarnish the environment, the method used to measure bacterial growth by spectrophotometry, and the experiments are repeated three times for each sample.

2.5 Statistical analysis

The gathered data were entered to the software SPSS version 16, Inc, Chicago, IL, USA and statistical analyses were done for determination of mean and standard deviation of the data. The mean difference was considered as statistically significant at a probability level of $P < 0.05$.

3. Results

Influence of ethanol extract of R. damascena on S. aureus isolated from Iranian traditional cheese is shown in Table 1. According this table, the average inhibition zone diameter extract on S. aureus at a concentration of 4000 ppm was 17.8 mm, with decreasing concentration of the extract, inhibition zone diameter was slaked. The mean difference of 4000 ppm and 500 ppm concentrations was <0.05 and it can be significant at this level.

Table 1. Average microbial growth inhibition zone diameter at different concentrations of Rosa damascena extract.

Extract concentration (ppm)	Mean ± SD (mm)	Min	Max
4000	17.8 ± 0.79*	17.3	18.4
2000	12.7 ± 0.97	12.0	13.3
1000	10.5 ± 0.96	9.7	10.8
500	5.7 ± 1.47*	4.2	6.8

*The mean difference is significant at the 0.05 level.

Optical absorption rate in different concentrations of R. damascena extract was examined by spectrophotometry and the results have been shown in figure 1. Results show that the MIC was taken as the last dilution with no noticeable growth and the lowest optical absorption at spectrophotometry. Dilution of R. damascena extract with 2000 ppm was observed as an expected MIC for S. aureus isolated from Iranian traditional cheese.

4. Discussion

Influence of ethanol extract of R. damascena on S. aureus isolated from Iranian traditional cheese was done at this study. The ethanol extract of Rosa damascene had an antimicrobial activity on S. aureus. The most efficient In vitro results obtained by alcoholic extract of Rosa damascene on S. aureus with 17.8 mm inhibition zone in 4000 ppm. Also, this study indicated that increasing the concentration of the extract caused a decrease in bacterial population, because the optical absorption rate of extract different concentrations by spectrophotometry is decreased in more extract concentration of R. damascena. DMSO 50% did not affect the growth of S. aureus. MIC was determined for the ethanolic extract of 4000 ppm concentration because this concentration showed maximum antibacterial activity than other concentrations.

Untreated milk can be contaminated with pathogens such as S. aureus (1). In recent years, much research in the field of anti-bacterial, anti-viral, anti-fungal Different plants have been carried out (11,16,17). In more research, it has reported that organic extracts effective on Gram-positive bacteria than gram-negative (18). Mirza surveyed the effect of distillation on extraction of important compounds in rose water and conclusion that efficiency water extracted from vacuum pressure distillation was double than normal pressure distillation. Mahmood, et al. review the combinations tetra hydroxy

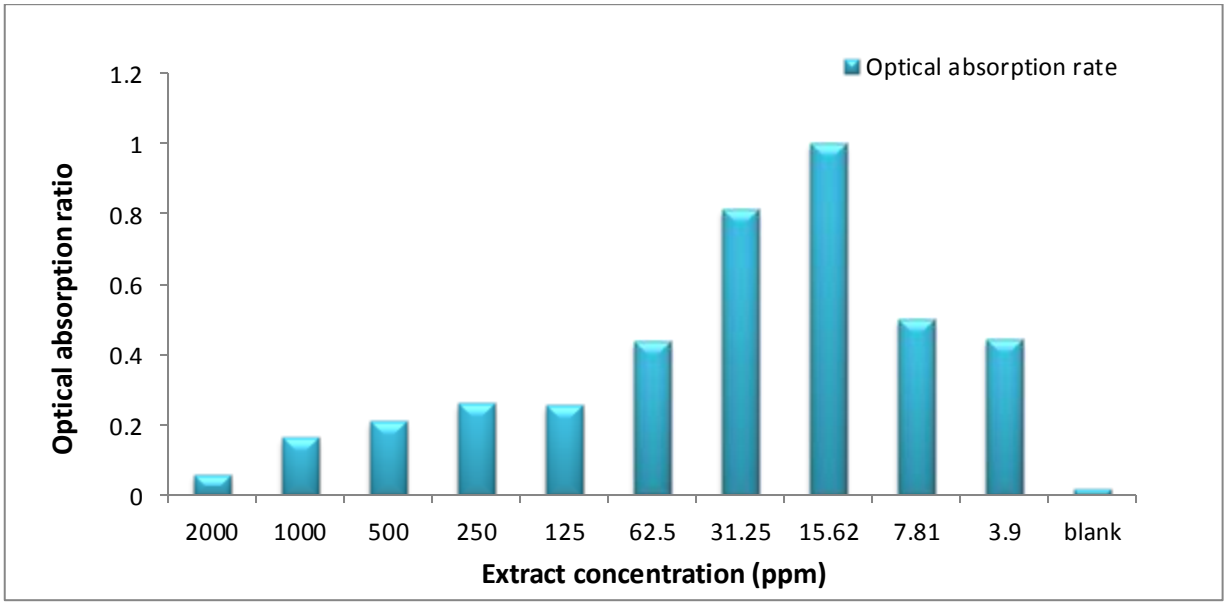

Figure 1. Optical absorption rate in different concentrations of Rosa damascena extract by spectrophotometry

flavenon (Kamfrol-1) Rosa extract and concluded that due to the inhibition of the activity of proteases were anti-virus (HIV).

There has been much interest in recent years on compounds derived from plants and herbs for their medicinal properties or biological activities (19). Throughout human history, infectious diseases are known to have been treated with herbal remedies (20). In a study, it has been reported that the anti-bacterial and antioxidant activity of extracts of Rosa. Antioxidant and anti-bacterial effects of Aeromonas hydrophila, Bacillus cereus, Enterobacter aeroginosae, Enterococcus faecalis, Escherichia coli, O157:H7, Klebsiella pneumoniae, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas fluorescens, Salmonella entridies, Salmonella typhimurium, S. aureus, Yersinia entroculitica, and Pseudomonas fluorescence was shown. The highest effect was on M. smegmatis and S. entridies (13). Also, Basim and Basim (11) stated the anti-HIV, antibacterial, and antioxidant essential oil rose effects.

Jafarzadeh Khaledi et al. (21), (2010) reported that rosemary essential oil can inhibit the growth of S. aureus in commercial instant soup significantly. Also, Yousefli et al. (22) studied the extract of Antimicrobial effect of Salvia leriifolia leaf extract powder against the growth of S. aureus in hamburger and they announced that S. leriifolia at the highest concentration (20,000 mg/kg) caused maximum reduction compared to other concentrations and extract at the lowest concentration (5000 mg/kg) was less effective in initial S. aureus population and microbial total count. In a study, Mahmoudi et al. (23) demonstrated that 0.03% and 0.015% of Mentha longifolia L. essential oil had the highest inhibitory effect on the growth of S. aureus when it was used in combination with Lactobacillus casei, and its reduction rate at the end of the storage periodin experimental treatments were 1.89 and 2.32 Log more than the control group respectively. In other research, Tabatabaie Yazdi et al. (24) examined the inhibitory effects of extracts of Lamiaceae plants (Thymus vulgaris L., Mentha spp., and Ziziphora tenuir L.) on the population of S. aureus in industrial Doogh samples with response surface method. They reported that the greatest reduction of Staphylococcus population in 24 h is 1.98 Log/ml (24). Mirzaei et al. (25) isolated coagulase positive S. aureus from traditional cheese through using the culturing method and they determined the methicillin resistance to the gene. They announced that had the resistant mec-Agene and 100% of the isolates demonstrated simultaneous resistance to more than three antibiotics (25). In the present study, the extract of R. damascena petal at the highest concentration (4000 ppm) caused maximum inhibitory zone diameter compared to other concentrations of the extract. The lowest concentration of inhibitory zone diameter was observed in 500 mg/kg which can effective in growth and survival of S. aureus. In a research, Gandomi Nasrabadi et al. (26) showed that the MIC of afsantine essential oil against S. aureus is estimated 3000 ppm. The methanolic extract has shown antimicrobial activity against S. aureus is estimated 10 mg/l. The inhibitory zone of alcoholic extract of this plan was 6 mm for S. aureus in disk diffusion method. The lowest inhibitory of alcoholic extract was <10 mg/ml (26). In another study, Gandomi Nasrabadi et al. (27) reported that the antibacterial effect of aqueous and alcoholic extracts from petal of the saffron (Crocus sativus L.) on some food borne bacterial pathogens is different and S. aureus can be a resistant bacterial strain. MIC of saffron extracts was estimated 40 mg/ml against S. aureus bacteria using agar dilution method. The aqua and methanolic extracts showed antimicrobial activity against S. aureus by broth microdilution and MIC 40 mg/ml. The inhibitory zone diameter in disk diffusion method for S. aureus was zero in all of extract concentrations (0–40 mg/ml) (27).

Abbassifar et al. (4) reported that 300 and 150 ppm of Zataria multiflora Boiss extract have the highest inhibitory effect on the growth of S. aureus and when these extract concentrations are used in combination with cheese starter culture, the counts of the pathogen are decreased significantly below its toxic dose in feta cheese. In the present research, the data indicated that R. damascena petal extract can exhibit antimicrobial activity against S. aureus isolated from Iranian traditional cheese.

5. Conclusion

It Can be stated that the Rosa damascene extract have potential of growth inhibitory on S. aureus grown in traditional cheese produced in the lower temperature pasteurization or during preparation and it can be used as an alternative natural preservative in food products. In the continuing and further research it requires more extensive studies of antimicrobial effect resistance plant extracts at different stages of production and manufacture of cheese.

Conflict of Interests

Authors have no conflict of interest.

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