ABSTRACT

In this research, antimicrobial activity of Salvia leriifolia leaf extract was investigated on Staphylococcus aureus growth with different concentrations (5,000, 10,000, 15,000 and 20,000 mg/L) in hamburger. Purified S. aureus was inoculated to hamburger and then stored at –12°C. Samples were subjected to microbiological analyses (total viable count and numbers of S. aureus) at different time intervals (15, 30 and 45 days). Results showed that total viable count and the number of S. aureus in all samples with different concentrations of extract declined during storage. This effect was significant at day 15 and 30 for S. aureus and total viable count, respectively. The highest concentration of S. leriifolia extract caused maximum reduction in S. aureus population and total viable count. These data indicated that S. leriifolia extract can exhibit antimicrobial activity against S. aureus, so it can be considered as an alternative natural preservative in such food products.

PRACTICAL APPLICATIONS

As Salvia leriifolia has natural antimicrobial properties and is growing in many regions in Iran increasingly, thus, the extract of some parts of this plant is applicable in food products. On the other hand, the importance of Staphylococcus aureus in meat products as hamburger has been proven previously due to manual handling or post-processing contamination. With regard to the above mentioned, usage of S. leriifolia is considered essential in meat products.
as a natural antimicrobial agent to prevent food poisoning and spoilage. Perhaps, *S. leriifolia* extract has a strong antimicrobial influence against many known pathogens in food products, but it needs to be performed in researches about the effect of this extract on sensory properties and total acceptance in near future.

**INTRODUCTION**

Meat and meat products are important components in people’s diets of developed countries. Their consumption is affected by various factors (Jiménez-Colmenero *et al.* 2001). Health-enhancing functional foods, prepared mostly by meat, are becoming more and more common. Frozen meat products such as hamburger are recognized as safe foodstuff. Outbreaks of foodborne disease are caused by recontamination with pathogens during post-processing (Reji and Den Aantrekker 2004). *Staphylococcus aureus* is one of the most important pathogens that cause staphylococcal food poisoning in meat products and contamination being generally associated with highly manual-handled food (Rasooli 1999). Staphylococcal food poisoning is a major public health risk all over the world (Hall 1997). Since the discovery of antimicrobial drugs, control of bacterial infections has been possible, but there are bacteria that are resistant to antibiotics (Essawi and Srour 2000). In addition, customers are often concerned about the food safety and potential impact of artificial additives on health. Because of undesirable side effects of synthetic preservatives and existence of some antibiotics-resistant bacteria, a survey about new antimicrobial agents derived from plants and natural resources has been remarkably progressed (Mitscher *et al.* 1987; Reische *et al.* 1998). Studies on antimicrobial properties of different plant parts and their extracts have been performed including: garlic, onion, cinnamon, nutmeg, curry, mustard, black pepper, thyme, oregano, sage, rosemary, Jamaican pepper, aniseed, basal, paprika, turmeric, bay, cardamom, cassia, Cayenne pepper, celery, chives, clover, coriander, dill, ginger, savory, marjoram and sumac, marigold, spearmint, basil and quyssum (Zaika and Kissenger 1981; Aktug and Karapinar 1986; El-Khateib and Abd El-Rahman 1987; Deans and Svodoba 1989; Farag *et al.* 1989; Karapinar 1990; Aureli *et al.* 1992; Ting and Deibel 1992; Hefnawy *et al.* 1993; Pandit and Shelef 1994; Holt and Almonte 1995; Sivropoulou *et al.* 1996; Ceylan *et al.* 1998; Hao *et al.* 1998, Arora and Kaur 1999; Nasar-Abbas and Kadir Halkman 2004; Soliman and Badeea 2002).

Seventeen of 58 species of the genus *Salvia* (*Lamiaceae*) are found in Iran, as local *Salvia leriifolia* Benth. is an everlasting herbaceous plant that grows solely in south and tropical regions of Khorasan and Semnan.
provinces, Iran. This plant was introduced in *Florica Iranica* in 1982 and has different vernacular names such as Nowroozak and Jobleh (Rechinger 1982).

The therapeutic and curing properties of this plant were popular among the natives. The leaves have been used for folk medicinal purposes. This medicinal and useful species is a member of *Labiateae* family that has been observed, reported and used in folklore remedies because of its important pharmacological effects (Hosseinzadeh *et al.* 2007). Researches showed that shoots and leaves of this plant had essence and antibiotic properties. The plant is reported to have a wide range of biological activities, such as antibacterial and bacteriostatic effects. Although a number of reports have mentioned the antimicrobial properties of the medicinal plants, little is known about the antibacterial activity of *Salvia leriifolia*. The antimicrobial effects of *Salvia leriifolia* leaves in laboratory media have been well documented (Modarres 2007). No study has been conducted to investigate the feasibility of using *Salvia leriifolia* leaves extracts as potential antimicrobial agents for the preservation of chilled meat and meat products.

Further studies on antimicrobial effects of this plant can introduce new natural preservative which can be used in food industry. The objective of this study was to examine antimicrobial activity of *Salvia leriifolia* leaves extract powder on *S. aureus* in hamburger. In the present study, *in vitro* antibacterial activity of *Salvia leriifolia* extract against *S. aureus* growth in hamburger was evaluated.

**MATERIALS AND METHODS**

**Materials**

**Collection of Plant Material.** *Salvia leriifolia* leaves in flowering stage were collected randomly from Kuh-sorkh area located southwest of Neishaboor, Khorasan Province, Iran in spring 2008. The plant material was washed under running tap water, air dried immediately at room temperature, then homogenized to fine powder and stored in airtight bottles. Voucher specimens were identified by the Herbarium of Botany Department, Ferdowsi University of Mashhad.

**Preparation of Methanol Extracts and Powder.** Air-dried leaves were ground and then separately extracted overnight with methanol in the ratio of 1:20 w/v kept in a shaking incubator (Faraz Teb Tajhiz, Iran) at room temperature (solvent extraction method). This solvent was chosen on the basis of
its degree of polarity. The obtained methanol extract was filtered through Whatman #1 filter paper. This procedure with fresh solvent was repeated for sediment of the previous stage under the same condition. Then the filtered extract of both stages were mixed and then bleached using active carbon (1 g active carbon : 5 g leaf powder) for 15 min in a shaking incubator at room temperature and then filtered through a Whatman #1 filter paper to yield a light brown filtrate. The extract was then concentrated in vacuo below 45°C in a rotary evaporator (Heidolph vv1, Persia, Tehran, Iran). After that, it was dried in an incubator until the constant weight, then stored for about 24 h in a refrigerator and filtered to remove the precipitates. The combined filtrate and the precipitates were evaporated to near dryness in vacuo below 40°C (Duh et al. 1992 and Wu et al. 1982). The distillates were later powdered and then transferred into a sample bottle and stored in airtight bottles until needed for analysis.

Microorganism, Bacterial Strains and Culture Media. Vial of lyophilized S. aureus culture ATCC-29737 was prepared from Persian Type Culture Collection, Iran. Vial of lyophilized S. aureus was opened under sterile condition as recommended by the manufacturer. Seed culture was inoculated in trypton soy broth and trypton soy agar and incubated at 37°C for 24 h in triplicate. Then stock culture was prepared from seed culture for further use.

Preparation of Microbial Suspension of 0.5 McFarland. Bacterial strain was inoculated in slant nutrient agar and incubated at 37°C for 24 h. Then colonies were washed from the slant nutrient agar with normal saline solution, and microbial suspension was diluted with the above-mentioned solution so that the absorption of suspension became equal to that of 0.5 McFarland solution at 530 nm wavelength. In other words, this suspension contains $1.5 \times 10^8$ cfu/g. For the final concentration of S. aureus equal to $5 \times 10^5$ cfu/g, 4 mL was required from above suspension.

Microbiological Sampling and Hamburger Inoculation and Treatment Application. Hamburger samples were obtained from one of the factories in Mashhad and were delivered to laboratory under sterile conditions. Then, these samples were cultured on Baird-Parker Agar (BPA) and plate count agar (PCA) and immediately, 1,200 g hamburger was mixed with 4 mL microbial suspension containing S. aureus from a portion of this mixture (200 g) that was chosen as a control. The remaining portion of the mixture (1,000 g) was divided by four and each portion was mixed with a different concentration of the antimicrobial agent according to Table 1. Finally, sterile
plastic containers (60 mL) were filled with 20 g of hamburger, closed hermetically and stored at −12C.

**Microorganisms in Hamburger Samples and Determination of Inhibitory Concentrations and Antibacterial Activity Assay.** Microbial viable counts were determined after 15, 30 and 45 days of storage at −12C by plating serial dilutions of hamburger homogenate. Hamburger samples of 20 g were homogenized in a stomacher (Lab blender 400, Seward Medical, London, UK) for 2 min in 180 mL of ringer solution; microbial counts were done in duplicate on two samples. Total counts were determined using agar (PCA; Difco Laboratories, Detroit, MI) incubated at 30C for 2 days. *S. aureus* was determined by the spread plate method using BPA (Merck, Rahway, NJ) supplemented with egg yolk telluride emulsion (50 mL/L, Merck). The plates were incubated at 37C for 24 h (Baird and Lee 1995). Colonies with typical *S. aureus* morphology (i.e., circular, black, convex and with or without light halo on BPA) were counted, subjected to Gram staining and examined microscopically to confirm coccus morphology. Isolated colonies on BPA were cultured as streak method on Monitol Salt Agar and then these colonies were subjected to confirmatory tests including coagulase and peroxidase tests. All confirmed counts were converted and reported as log cfu/g. The antibacterial activity of *Salvia leriifolia* leaves extract was assessed by decreasing in log cfu/g of the test culture during storage.

**Statistical Analysis**

The experimental design was completely randomized and data from the *S. aureus* and total viable count were analyzed separately. Data were analyzed using analysis of variance. A significant difference was used at 0.05 probability level and differences between treatments were tested using the least significant difference test. All statistical analyses of data were performed using MSTATC software. Figures were plotted by Excel software (v1.4, Dynamics Corporation, Fort Worth, TX).
RESULT AND DISCUSSION

Results showed that the addition of leaf extract powder was effective in decreasing the \textit{S. aureus} and total viable count decreased at all concentrations, as shown in Table 2. Regarding the effect of extract on \textit{Staphylococcus} counts, numbers of \textit{S. aureus} increased in control treatment up to 15th day and after that decreased on the 45th day. The initial increasing trend during the first 15 days could be due to the presence of fat, protein, water and salt contents in processed foods like hamburger that they improve microbial resistance (Snyder 1997). Another reason for the increase in the number of \textit{Staphylococcus} may be due to the resistance of this bacterium against freezing (Douglas 2004). In the control treatment, the reason for the decreasing trend from the 15th until the 45th day is unknown. The effect of extract level on \textit{S. aureus} counts was not significant in T1, T2 and T3 treatments during the 45-day storage. The highest concentration of extract (T4 treatment) showed the decreasing trend during all storage time.

Among the treatments, only in T4 treatment was the difference significant between initial count and final count after storage. As shown in Fig. 1, the highest effect and the lowest one are related to T4 and T1, respectively. The most important point in this matter is that the highest influence was seen during the first 15 days for all extract concentrations and the number of \textit{S. aureus} became similar in control and other treatment on 45 days and there was no significant difference between them ($P < 0.05$). Kamanzi Atindehou \textit{et al.} (2002) evaluated the effect of some crude ethanol extracts from some plant species. The 148 tested extracts showed strong activities against the gram-positive cocci (\textit{S. aureus} and \textit{Enterococcus faecalis}). The influence of \textit{Jatropha curcas} on \textit{S. aureus} was described. Habibi \textit{et al.} (2000) determined the structure and antimicrobial activity of a new labdane diterpenoid (8 (17), 12E, 14-Labdatrien-6,19 olide) (compound I) isolated from aerial parts of \textit{S. leriifolia}. The results presented that compound I was active against the gram-positive bacterium \textit{S. aureus}. It has been shown that the methanolic extract of Nowroozak leaves has antioxidant activity. Butein, a chalcone, is the major antioxidant component of Nowroozak leaves extract (Haddad Khodaparast \textit{et al.} 2008). Chalcones are a kind of flavonoid component and their antimicrobial activity has been proved in many research (Modarres 2007; Hosseinzadeh \textit{et al.} 2009). Hosseinzadeh \textit{et al.} (2009) determined the pharmacological and toxicological effects of \textit{S. leriifolia}. The leaf oil of the plant contained beta-pinene (31.5\%), 1.8 cineole (24.7\%) and alpha-pinene (17.5\%). Also, saponins and tannins are found in the different parts of the plant, especially in the plant leaf and root. Alcoholic radix extract of \textit{S. leriifolia} also contained a small amount of alkaloids. Shelef (1983) showed that the gram-positive bacteria were generally more sensitive to plant extracts.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>S. aureus</th>
<th></th>
<th></th>
<th></th>
<th>Total viable count</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
<td>Day</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Control</td>
<td>5.729 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.734 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.314 ± 0.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.606 ± 0.05&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>Control</td>
<td>6.416 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.592 ± 0.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.137 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td>5.729 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.802 ± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.778 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.405 ± 0.03&lt;sup&gt;def&lt;/sup&gt;</td>
<td>T1</td>
<td>6.416 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.528 ± 0.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.974 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>5.729 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.741 ± 0.02&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.582 ± 0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.231 ± 0.54&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>T2</td>
<td>6.416 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.471 ± 0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.594 ± 0.15&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>5.729 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.714 ± 0.34&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.831 ± 0.02&lt;sup&gt;bce&lt;/sup&gt;</td>
<td>5.306 ± 0.7&lt;sup&gt;def&lt;/sup&gt;</td>
<td>T3</td>
<td>6.416 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.477 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.664 ± 0.05&lt;sup&gt;bce&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>5.729 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.622 ± 0.02&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.249 ± 0.25&lt;sup&gt;def&lt;/sup&gt;</td>
<td>4.887 ± 0.08&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>T4</td>
<td>6.416 ± 0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>6.341 ± 0.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.316 ± 0.34&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a–f Change in number of *Staphylococcus aureus* and total viable count during storage in hamburger at control and treatments.
than gram-negative bacteria. His results showed that both plant extract are bactericidal rather than bacteriostatic. Oussalah et al. (2007) reported that *S. aureus* was the most sensitive to over 18 oils tested as compared with *Escherichia coli* O157:H7 and *Salmonella typhimurium* because of a single layer wall. Lambert et al. (2001) also showed similar results about high sensitivity of *S. aureus* to oregano essential oils. In the flowering stage of Nowroozak, similar results were obtained with Modarres’ results; in their study, the maximum inhibitory effect was related to 20,000 mg/L concentration and the most sensitive organism to this essential oil was *S. aureus*. Some scientists reported a loss of antimicrobial activity when applying the plant extracts to foods. The reason for this phenomenon could be caused by a number of factors. For example, Ismaiel and Pierson (1990) and Shelef (1983) observed a protective effect of high fat levels. They mentioned that antimicrobial activity of spices and oils declined in foods as a result of the solubilization of the antimicrobial agents into the food lipid fraction. Some researchers implied that the reason of antibacterial activity of some plant extracts such as *rosemary* and *Origanum vulgare* L. is related to the presence of polyphenols and carvacrol, respectively, and their influence on membrane fluidity. The carvacrol that was the most important compound in *O. vulgare* L. has a hydrophobic nature has resulted in cell death in *S. aureus*. Similar results were seen in *Kale* extracts due to their phenolic acid contents and antibacterial activities against *S. aureus* (Romano et al. (2009); Carneiro de Barros et al. (2009); Ahmet Ayaz et al. (2008). Figure 2 shows the effect of extract on total viable count of treatments. In this matter, all concentrations of extract influenced on total viable count decreased. On the 45th day, the difference was significant.

**FIG. 1. CHANGE OF COUNT *STAPHYLOCOCCUS AUREUS* DURING 45 DAYS STORAGE**
(P < 0.05) between T4 and other treatments. With respect to the effect of extract on total viable count, the highest and lowest effect was related to T4 and T1, respectively. T1 treatment showed similar trend with control and no significant difference was seen between T1 and control treatments during 45 days storage.

CONCLUSION

According to previous research on antimicrobial properties of extract of *Salvia leriifolia* leaves, similar results were obtained in microbial suspensions by Modarres. In that study, the highest effect was related to 20,000 mg/kg extract concentration and there was no significant effect for 5,000 mg/kg. No significant difference was shown between 5,000 and 10,000 mg/kg and between 15,000 and 20,000 mg/kg. Prediction of growth behavior of *S. aureus* is very difficult in various food products including hamburger due to meat nature and other additives, available oxygen (Eh), water activity (Aw), temperature, pH and synergism and antagonism among microorganisms. With respect to the emphasis of World Health Organization for the use of natural preservations in foods and the results of this research, we can use leaf extract powder of *Salvia leriifolia* leaf in foods as a natural antimicrobial because of increasing oxidative stability and other nutritional properties as well as decreasing microbial count.
REFERENCES


