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Research Article

Effect of Sumac and Thyme Extracts on *Salmonella typhimurium* and *Bacillus cereus* in Chicken Fillet

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Abstract

This research was carried out to evaluate the effectiveness of some spice extracts such as sumac and thyme for the control of the growth and survival of two pathogenic microorganisms of significant importance in food hygiene namely, *Salmonella typhimurium* and *Bacillus cereus*. Two different methods were conducted to examine the inhibitory effect of these spice extracts, agar cup method and in food model system (deboned poultry meat). Regarding agar cup method, by using two different concentrations of each spice extract (1.5 and 3%), the obtained results showed that *S. typhimurium* was more sensitive than *B. cereus* to the action of added spices and 1.5 and 3% sumac extracts demonstrating better activity against *S. typhimurium* with an inhibition zones of 20.1 and 32 mm, respectively, meanwhile, 1.5 and 3% of thyme extracts giving more inhibitory effect on *B. cereus* with an inhibition zones of 14 and 23.6 mm, respectively. The other method was performed on chicken fillet stored at 5 ºC for 7 days, the results showed that sumac extracts had the greatest rate of reduction of *S. typhimurium* populations after 7 days of storage, while the more inhibitory effect of *B. cereus* counts was by adding thyme extracts.

Keywords: Sumac; Thyme; Antimicrobial Activity; *S. typhimurium; B. cereus*

Introduction

Since ancient times spices and herbs have been added to foods, not only as flavouring agents but also as preservatives [1]. Certain spices prolong the storage life of food through bacteriostatic or bactericidal activity or by preventing rancidity through their antioxidant activity [2].

Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens [3]. Therefore, there has been increasing interest in discovering new natural antimicrobials such as spices, herbs and their extracts which cause no health problem to the handlers and consumers due to their availability, fewer side effects or toxicity as well as better biodegrability as compared to the available antibiotics and chemical preservatives which are corrosive and carcinogenic [1]. So plant products with antimicrobial properties have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth [4].

At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments. They are also extensively used in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also [5].

Sumac is commonly used as spice in the Mediterranean region especially in meat and fish dishes. The spice, produced by grinding the dried fruit with salt, is used as condiment and sprinkled over kebabs and grilled meat as well as over salads that often accompany these dishes. It is a very popular condiment in Turkey and Iran, where the ground fruits are liberally sprinkled over rice. The well-known Turkish fast food specialty *döner kebap* is sometimes flavoured with sumac powder. Another use of sumac is recorded from Lebanon, Syria and Egypt: The fruits are cooked with water to a thick, very sour essence, which is, then, added to meat and vegetable dishes [6,7]. In Palastine, sumac has been utilized extensively in many different meals, such as in Zahtar [dukka] which is a blend of sumac, thyme and citric acid with seasame seeds [8].

The genus Thymus is among the aromatic plants belonging to the Lamiaceae family. Thyme is commonly used [fresh and dried] as a culinary herb. The thyme essential oil is utilized as flavour ingredient in a wide variety of food, as well as in perfumery. Because of its antiseptic, antispasmodic and antimicrobial properties, it is also used for medicinal purposes [9,10].

Bacillus cereus is Gram-positive, facultative aerobic, beta hemolytic bacterium that can produce protective endospores. It is one of the most important pathogens responsible for food poisoning and foodborne illnesses throughout the world [2-5%] [11]. Generally, *Bacillus* foodborne illnesses occur due to survival of the bacterial spores when food is improperly cooked. This problem is compounded when food is then improperly refrigerated, allowing the spores to germinate [12]. Its pathogenicity is related to the production of two toxin, a thermostable emetic enterotoxin and a thermosensitive diarrhoegenic enterotoxin so *B. cereus* can cause two types of food poisoning: a diarrheal type and an emetic type [13].

Salmonella typhimurium is rod-shaped, Gram-negative, flagellated, facultatively anaerobic bacilli; cause gastrointestinal infections [diarrhea, abdominal cramps, and fever] of varying severity, classical food poisoning and infantile and travellers'diarrhaea. Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin [mainly meat, poultry, eggs and milk]. *Salmonella enteriditis* and *Salmonella typhimurium*, the two most important serotypes for salmonellosis are transmitted from animals to humans [14].

As *S. typhimurium* and *B. cereus* play a significant importance in food hygiene therefore, the purpose of this study was to determine the effectiveness of sumac and thyme extracts for the control of growth and survival of these two pathogenic microorganisms.

Materials and Methods

Bacterial strains

B. cereus strain was obtained from Animal Health Research Institute, Assiut Regional Laboratory, whereas, *S. typhimurium* strain was obtained from Fac. Vet. Med., Assiut Univ. (Food Hygiene Department). Tested bacterial strains were grown in tryptic soy broth an incubated at 37ºC.

Spices used

Sumac and thyme powder were purchased from a local market in Assiut city.

Extraction procedures

Extraction of active constituents of sumac and thyme powders using maceration technique [15]: 10 gm of each spice powder were soaked in 50 ml alcohol 70%, left for complete exhaustion then filtration in air until complete evaporation then diluted in Tween 80 to obtain 1.5 and 3% concentrations.

Agar cup method

The method described by [16] was applied. Fifty milliliter Nutrient Agar (NA) cooled to 50 ºC after autoclaving at 121 ºC for 15 min, were inoculated well with 0.5 ml of both an overnight (12-18h) *B. cerus* and *S. typhimurium* cultures, mixed well and poured into standard Petri plates. After setting of medium after about 1 h, cups of 1cm diameter were prepared. The base of each cup was sealed with 50 ul of sterilized molten NA. The cups were filled by adding 300 ul of spice extracts (1.5 and 3%) concentrations while Tween 80 was added in one cup as a control. The plates having cups were incubated for 48h at 37 ºC. After incubation the growth inhibition zones around every cup (including cup) were measured with a caliper and recorded.

Effect of spice extracts on growth of the tested organisms in deboned poultry meat:

One Kilogram frozen boneless chicken breast was purchased from a local supermarket in Assiut city and transferred to the laboratory in an ice box with minimal of delay, then proved to be free from the tested organisms according to the method recommended by [17]. The samples were minced and divided into two equal groups; the first group was inoculated with *S. typhimurium* prepared inoculum (to obtain 7 log cfu/g inoculum), then subdivided into five equal portions each of 100 g in sterile plastic bags. The 1st and the 2nd bags received 1.5 and 3% sumac extracts, also, the 3rd and the 4th bags received 1.5 and 3% thyme extracts, respectively, while, the fifth portion was kept without adding any spice extracts (control). On the other hand, the second group was inoculated with *B. cereus* prepared inoculum (to obtain 7 log cfu/g inoculum), then subdivided into five equal portions each of 100 g in sterile plastic bags, then 1.5 and 3% sumac extracts were added into the 1st and the 2nd bags, while, 1.5 and 3% thyme extracts were added into the the 3rd and the 4th bags, respectively, whereas, the fifth portion was considered as control. Both the spice extracts and the bacterial inoculum were mixed uniformly in the minced poultry meat for 5 min. All samples and control were stored at 5º C for 7 days. Ten grams of each sample were taken aseptically at start of the experiment (0 day) and subsequently after 1, 3, 5 and 7 days of holding at 5 ºC and homogenized with 90 ml of 0.1 peptone water for 2 min. Serial dilutions of the homogenate were prepared by using 0.1-peptone water as diluents. The surface plating technique was used on S.S. agar for enumeration of *S. typhimurium* and KG medium for *B. cereus* [18] with an incubation at 37 ºC for 24h.

Results

The inhibitory effect of two concentrations of sumac and thyme extracts on the growth of *S. typhimurium* and *B. cereus* tested by agar cup method is represented in table 1. The obtained results showed that 1.5 and 3% sumac extracts exhibited better activity against *S. typhimurium* with inhibition zones of 20.1 and 32 mm, respectively. On the other hand, 1.5 and 3% thyme extracts demonstrated more action *on B. cereus* with inhibition zones of 14 and 23.6 mm, respectively.

Microorganism	Sumac		Thyme	
	1.5%	3%	1.5%	3%
Salmonella typhimurium	20.1	32	16.2	26
Bacillus cereus	12.2	21	14	23.6

Table 1: Growth inhibition zones (mm) by alcoholic extracts of sumac and thyme on *S. typhimurium* and *B. cereus.*

The results concerning the reduction in counts of *S. typhimurium* and *B. cereus* on deboned poultry meat with 1.5 and 3% concentrations of both sumac and thyme extracts at 0, 1, 3, 5 and 7 days of storage at 5º C (Tables 2 and 3 and Figure 1 and 2) provide additional confirmation. Here again *S. typhimurium* was found to be more sensitive, to some extent, than *B. cereus* to the action of the added spice extracts. Regarding spice extracts used, the results showed that sumac extracts had better activity against *S. typhimurium* than thyme extracts, meanwhile, thyme extracts was found to be more active against *B. cereus*. It could be evident that 3% sumac extract had the highest inhibitory effect in population of *S. typhimurium* in deboned poultry meat after 7 days of storage by 3 log cfu/g while, 1.5% sumac extract caused decline by 2.5 log cfu/g. However, 1.5 and 3% thyme extracts reduced *S. typhimurium* population after 7 days of storage by 2.2 and 2.6 log cfu/g (in comparison to control), respectively. Concerning *B. cereus,* the obtained results declared that addition of 1.5 and 3% thyme extracts to deboned poultry meat mixed with this pathogen at 5ºC for 7 days of storage resulted in reduction of its count by 1.9 and 2.3 log cfu/g, respectively, as well, 1.5 and 3% sumac extracts caused decrease in *B. cereus* population after 7 days of storage by 1.8 and 2 log cfu//g, respectively.

Days of storage	Control	Sumac		Thyme	
		1.5%	3%	1.5%	3%
0	7	7	7	7	7
1	7	7	6.8	6.8	6.7
3	7	6.5	5.9	6.3	5.7
5	7.1	5.9	5.3	5.7	5.1
7	7.2	5.2	5	5.1	4.7

Table 2: Inhibitory effect of sumac and thyme extracts on the growth of *S. typhimurium* mixed with deboned poultry meat for 7 days (log cfu/g).

Days of storage	Control	Sumac		Thyme	
		1.5%	3%	1.5%	3%
0	7	7	7	7	7
1	7	6.8	6.6	6.9	6.7
3	7.2	6.1	5.2	6.3	5.5
5	7.3	5	4.7	5.6	4.9
7	7.3	4.5	4	4.8	4.4

Table 3: Inhibitory effect of sumac and thyme extracts on the growth of *B. cereus* mixed with deboned poultry meat for 7 days (log cfu/g).

Figure 1: Effect of spice extracts on Sat. typhimurium mixed with deboned poultry meat stored at 5 C for 7days.

Figure 2: Effect of spice extracts on B. cereus mixed with deboned poultry meat stored at 5 C for 7days.

Discussion and Conclusion

In recent years, several studies from different countries were published showing the antimicrobial activities of sumac and thyme extracts on various food poisoning microorganisms [5,6,8,19,20].

From the achieved results, it could be observed that *S. typhimurium* (Gram-negative) was found to be more sensitive, to some extent, than *B. cereus* (Gram-positive) to the effect of sumac and thyme extracts. This result disagree with other reports which found that Gram- positive bacteria are more sensitive to the spice than Gram- negative ones [6,8].

Sumac extracts exhibited better activity against Gram- negative bacteria (*S. typhimurium*) than against Gram-positive ones (*B. cereus*). This result agree with the report published by [21] who found that sumac showing better overall activity against the Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) than against the Gram-positive bacterium *S. aureus.*

In a similar study, alcoholic extract of thyme (10%) caused decrease in *S. typhimurium* counts in deboned poultry meat after 7 days of storage by 0.78 log cfu/g [22].

[6] investigated the inhibitory effect of alcohol extract of sumac on the growth of 12 bacterial strains (6 Gram-positives and 6 Gram-negatives). They found that among the Gram-positives, Bacillus species (*B. cereus*, *B. megaterium*, *B. subtilis* and *B. thuringiensis*) were the most sensitive, surviving up to only 500 mg/L of the spice, followed by *S. aureus* (1000 mg/L), and then by *L. monocytogenes* (1500 mg/L), while, among the Gram-negative bacteria, *S. enteritidis* and *E. coli* type 1 were found to be more resistant, surviving up to 3000 mg/L of the spice.

The antimicrobial activity of smooth sumac (Rhus glabra), which is considered to be the most potent of the five non-toxic species of Rhus, was evaluated against 11 microorganisms in vitro. From ground, dried smooth sumac branches extracted in methanol, three antibacterial compounds were isolated: two methylated gallic acid derivatives, and gallic acid. The first two compounds are reported in this study for the first time; they appear to be 2.5-80 times more active than the gallic acid [21].

Moreover, [23] pointed out that antibacterial, antifungal and antioxidant properties of sumac make it a great and versatile tool to be used in the food industry, where it can be used as an efficient food preservative and natural, harmless food additive.

Inhibitory action of ground leaves and extracts of thyme (Thymus vulgaris), mint (Mentha piperita) and laurel (Laurus nobilis) were investigated on *S. aureus*, *S. typhimurium* and *V. parahaemolyticus.* Thyme was most prominent antibacterial product being active up to concentration 0.5% (w/v) for ground and 5000 ppm (v/v) for extracts [24]. Also, [25] determined the antimicrobial properties of thyme oil and their main components against various strains of food spoilage and pathogenic bacteria (including *B. cereus* and *S. typhimurium*) using a broth microdilution method. These researchers emphasized that thyme essential oil have powerful antimicrobial activity.

Furthermore, thyme and balm essential oils effectively reduced deteriorative processes in chicken meat and extended the shelf life of this fresh product; thus the essential oils of thyme and balm can protect the chicken meat from decomposition during the storage time [26].

Recently in a related study [27] found that thyme oil has good potential to increase the antimicrobial efficacy of lemon juice marinade against *Salmonella* on raw chicken breast and enhance the microbial safety of this popular poultry product.

Numerous scientific citations support antimicrobial properties of thyme extracts. The efficacy of thyme extracts toward the tested microorganisms may be attributed to thymol and carvacrol and the hydrocarbons γ-terpinene and ρ-cymene [10,25]. Many papers on the antimicrobial activity of plant extracts have been published; data show a great discordance between the same essences. The reasons for this variability could be attributed to the variation in the type, chemical composition and concentration of plant oils and extracts according to climatic, seasonal and geographic conditions, harvest period, the method used to assess antimicrobial activity and the choice of test organism (s) [28].

Finally, different studies have concluded that the use of thyme increases stability and reduces lipid oxidation during the shelf-life period of foods, which makes thyme a promising source of natural additives [29].

In conclusion, the ultimate goal of studies concerning medicinal plants was to develop safe, effective, and inexpensive food formulations and processes to reduce pathogens in foods. In this respect, extracts such as sumac and thyme extracts, could be considered as preservative materials for some kinds of foods; they could find an application as additives to poultry products in storage to protect them from *S. typhimurium and B. cereus* contamination.

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