

Plant Extracts as Inhibitors of Foodborne Pathogenic Bacteria

Soheir S. Abd-El Salam ^{(1)#}, Mohamed F. Ghaly ⁽²⁾, Mohamed H. Yassin ⁽¹⁾, Attia A.A. ⁽¹⁾ and Sally E. Sallam ⁽¹⁾

⁽¹⁾Microbiology Department, Faculty of Science, Benha University, Benha, Egypt;

⁽²⁾Microbiology Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

RECENT interest in natural materials, especially plant extracts, has increased as natural food preservatives following the rise of antimicrobial resistance, as well as the bad reputation of industrial preservatives of consumers. This study aims to evaluate the potential use of plant extracts as an effective and safe alternative for food preservation. Also, it provides factors influencing anti-bacterial effect using meat-based and milk-based models.

Ethanol plant extracts produced extremely important antimicrobial effects via inhibiting the tested multi-drug resistant bacteria. Sumac (*Rhus coriaria*) found to be the most effective extract producing inhibition zones average 27mm, followed by clove, rosemary and lemon (24mm, 21mm and 18mm, respectively), Whereas black pepper showed intermediate activity (10-16mm). The aqueous extracts showed lower activity. The combination of sumac with clove and clove with rosemary produced additive effect in most cases, whereas, sumac with rosemary produced synergistic effect in all cases. On the other hand, combination of sumac with clove and rosemary produced antagonistic effect.

The interaction or reaction of plant extracts with food components reduced the antibacterial effects of plant extracts; therefore, higher concentrations of used extracts were required to do the same effects in meat-based and milk-based models as in microbiological medium. The results indicated that plant extracts possessing antimicrobial activity can be used as ideal food preservatives after taken into account the reaction and interaction between food components and extract.

Keywords: Natural preservatives, Food models, Sumac, Clove, Rosemary.

Introduction

Contamination and poisoning of food products are major causes of morbidity and mortality in developing countries (Sapkota et al., 2012). Contamination of food products by Gram negative bacteria especially, *Salmonella* spp., *Escherichia coli* and *Pseudomonas aeruginosa* as well as Gram positives especially, *Bacillus cereus*, *clostridium* species, *Staphylococcus aureus* and *Listeria monocytogenes* is one of the main causes of food poisoning (Silva & Lidon, 2017 and Mostafa et al., 2018).

Contamination of food and foodborne pathogenic bacteria is regularly controlled using industrial preservatives. Even though their ability to prevent food contamination and epidemics, the

repeated use of chemical preservatives has led to the accumulation of their residues in the food chain and the emergence of microbial resistance to them as well as the adverse effects of these chemicals on the health of consumers (Nazir et al., 2017). For these reasons, concentrated efforts to provide natural alternatives that are strong and efficient and safe for consumer health and easy to prepare and use and therefore was the interest of plant extracts as natural preservatives (Negi, 2012).

There is a growing interest in antimicrobial plant extracts as alternatives to some synthetic food preservatives for sanitary, environmental, regulatory and marketing reasons (Pisoschi et al., 2018). Antibacterial effects of plant extracts against food spoilage and foodborne pathogenic bacteria have been researched and confirmed by

#Corresponding author email: dr.soheir_saad@yahoo.com

DOI: 10.21608/ejm.2018.3902.1058

©2018 National Information and Documentation Center (NIDOC)

many researchers around the world (Gutierrez et al., 2009; Pandey & Singh, 2011; Gyawali et al., 2015; Nazir et al., 2017; Mostafa et al., 2018 and Bouarab-Chibane et al., 2018).

Synergistic, antagonistic or additive effects can produced via combination of plant extracts as a result of the different interactions and reactions of the components of the extracts with each other and with the microbial targets. Synergy usually arises from component (s) assistance to other component (s) to produce better antibacterial effects. This may be a vital factor in using plant extracts as natural food preservatives (Baljeet et al., 2015). Even though numerous medicinal plants have been studied as antimicrobials, the effects (synergistic, additive or antagonistic) of mixing their extracts have not been well studied (Zhang et al., 2009; Arjun et al., 2014 and Baljeet et al., 2015).

Even though many studies have been conducted on the antibacterial effect of the extracts of medicinal plants, but few studies have been carried out on the application of food products may be because the effect of these extracts in food products less than the effect of pure compounds (Negi, 2012). The presence of proteins, carbohydrates, fats, salts and pH levels in foodstuffs may explain the activity differences produced *in vitro* experiments and *in vivo* on the food models or foodstuffs themselves (Gutierrez et al., 2009 and Weiss et al., 2015). Additionally, the accessibility of nutrients in great quantities in food products helps in rebuild and repair broken cells and the large amounts of fats and proteins may act as protection barriers for bacteria (Gyawali et al., 2015).

Mainly, foodstuffs are consists of carbohydrates, proteins, lipids, NaCl and water so analyzing the influence of these ingredients on the antibacterial effects of any suggested antibacterial compound is very important. Several authors have recently studied or reviewed the effects of these components on the antibacterial activity of natural products like plant extracts and essential oils (Cava et al., 2007; Gutierrez et al., 2009 and Bouarab-Chibane et al., 2018). Consequently, it is vital for the optimized application of natural products is the assessment of efficiency in foodstuffs or in food-based models that strongly similar to food composition, before applying to real foods. The present study was undertaken

to determine and compare the potential of some plant extracts as an antibacterial agents against foodborne pathogens isolated from some foods using food model media; an attempt to formulate natural food preservatives.

Materials and Methods

Collection, isolation and identification of foodborne pathogenic bacteria

A total of 200 food samples were aseptically and randomly collected in a sterile polyethylene bags from markets in Benha City, Egypt. Ten grams of each sample were homogenized in 90ml of sterile saline (0.9% NaCl) in a sterile polyethylene bag by stomacher for 2min. From each suspension 1ml were cultured in nutrient agar plate by pour-plate method. Then the plates incubated at 37°C24/h. A loopful of the positive bacterial growth was then transferred to selective media (TBX (*Escherichia coli*); XLD (*Salmonella* spp.); Cetrimide (*Pseudomonas aeruginosa*) and Baird-Parker (*Staphylococcus aureus*)) and incubated at 37°C48/h. Suspected colonies were picked up and re-streaked onto new plate of the same medium till obtaining pure separate colonies, then pure colonies transferred to nutrient agar slants and maintained at 4°C for further investigations (Sultana et al., 2014 and FDA, 2016). The purified cultures were identified and confirmed by investigating morphological characters and biochemical tests according to Bergey's Manual (Garrity et al., 2005 and Vos et al., 2009).

Antibiotic susceptibility test

Fourteen of different antibiotics (Oxoid, UK) listed in Table 1, were selected for disc diffusion bioassay. Briefly, 2-3 pure colonies of bacteria were picked then emulsified in sterile nutrient broth. The bacterial suspension was adjusted to the turbidity of 0.5 McFarland and the suspension was swabbed on to Muller-Hinton agar (MHA) plates on three directions to confirm a complete distribution. Antibiotic disks were applied to the surface of plates at constant distances. After incubation at 37°C24/h the entire diameter of the inhibition zone was measured and analyzed as per recommendations of Clinical and Laboratory Standards Institute (CLSI, 2008).

Antibacterial activity of chemical food preservatives

Five of common chemical food preservatives

(sodium benzoate, potassium sorbate, calcium propionate, ascorbic acid and citric acid) were used. Stock solution (1.5mg/ml) from each preservative was prepared by sterile distilled water. Antibacterial activity of prepared preservatives was determined using disc diffusion method according to Selim et al. (2012) with some modifications. The density of bacterial suspension was adjusted equivalent to that of 0.5 McFarland. Sterile filter paper discs (Whatman No.3, 6mm diameter) were saturated by preservative stock solution and allowed to dry for 1h then placed on the surface of inoculated MHA plates (pH: 6). After incubation at 37°C/24h the entire diameter of the inhibition zones were measured in millimeters (mm) using a ruler including the diameter of the disk (6mm). The evaluation of inhibition zones was as following: resistant (≤ 14 mm), intermediate (15-19mm) and susceptible (≥ 20 mm).

Molecular identification of the most resistant isolates

The identification of the most resistant isolate in each bacterial group coded VF2, MP5, MP3 and D4 which resistant to most tested antibiotics and preservatives were confirmed by investigation of 16S rRNA gene sequences according to Kolbert & Persing, (1999). The obtained sequences were compared to published sequences in GenBank at NCBI web site (www.ncbi.nlm.nih.gov) and phylogenetic analysis using TREEVIEW program were used to assess the similarities of the obtained sequences.

Preparation of plant extracts

Five medicinal plants (Table 3) collected from local herbalists and markets in Cairo, Egypt were used to prepare ethanolic and aqueous extracts. The collected plant parts were air dried at the room temperature and complete dryness in oven at 45°C to constant moisture content, then grounded. One hundred grams of every plant was extracted with 500ml of 80% ethanol or distilled boiled water in a sterile conical flask for 72h with frequent shaking. Then it was centrifuged at 4000rpm for 10min and filtered by Whatman filter papers No.1. The supernatant was collected and concentrated under reduced pressure at 40°C in a rotary evaporator. The residual solvent was eliminated in an oven at 45°C to obtain powder extract. Each extract was solubilized in distilled water containing 2% dimethylsulfoxide (DMSO) to form stock solutions (50mg/ml). Then stock

solutions were kept at 4°C in refrigerator till use (Silva et al., 2014).

Antibacterial activity of plant extracts

Antibacterial activity of prepared extracts was determined using disc diffusion method against the most resistant (to preservatives and antibiotics) strain in each identified bacterial group (*P. aeruginosa* VF2, *E. coli* MP5, *S. enterica* subsp. *enterica* MP3 and *S. aureus* D4) according to Schwalbe et al. (2007) with some modifications. Sterile filter paper discs (Whatman No. 3, 6mm diameter & three layers) were saturated by extract stock solution (50 mg/ml) and allowed to dry for 1h then placed on the surface of inoculated MHA plates. The used 2% dimethylsulfoxide (DMSO) disks served as negative control. After incubation at 37°C/24h the entire diameter of the inhibition zones were measured in millimeters (mm) using a ruler including the diameter of the disk (6mm).

Combination between the most effective extracts

The most effective extracts (ethanolic extracts of sumac, clove and rosemary) were used. Mixtures from plant extract stock solutions (50mg/ml) were prepared by combination of sumac with clove (1:1 v/v from the stock solution), sumac with rosemary (1:1 v/v), clove with rosemary (1:1 v/v) and sumac with clove and rosemary (1:1:1 v/v/v). The antibacterial activity of each mixture was tested as previously described in section 2.5. Combinations of extracts can lead to additive or synergistic or antagonistic effects. Synergy: the interaction of compounds to create more profound antimicrobial action. The additive effect is equal to the individual effects, whereas the antagonistic effect is less potent than the individual effects (Baljeet et al., 2015).

Effect of food components on antibacterial activity of plant extracts by food models

The effect of food components on antibacterial activity of plant extracts were investigated by comparing the minimum inhibitory concentrations (MICs) of the most active ethanolic extracts (sumac and clove) on laboratory (control) and food model media. Agar dilution method was performed as described by Gutierrez et al. (2009) with some modifications. Control medium was Mueller Hinton agar. Meat model medium prepared from beef extract (1%) and agar (1.5%) while milk model medium was made by mixing semi skimmed milk powder

(1%) with agar (1.5%). Control, meat and milk model media were adjusted to pH 7.2 to separate pH effects. After autoclaving each medium was divided in sterile bottles in which each extract serially diluted to the appropriate concentrations (0.195, 0.390, 0.781, 1.56, 3.125, 6.25, 12.5 and 25mg/ml) which poured onto petri dishes and allowed to solidify. Target bacteria (the most resistant to preservatives and antibiotics) were previously grown (24h.) in Mueller Hinton broth or liquid model media to allow the cells to adapt to the food environment. Plates were then seeded with the target bacteria and incubated at 37°C/24h. The positive control consisted of control or model medium inoculated with the same amount of cells but without any extract, while uninoculated plates containing the extract served as negative control. Plates were evaluated for the presence or the absence of colonies after incubation period. MIC was defined as the lowest concentration of plant extract that completely suppressed colony growth.

Results and Discussion

Distribution of collected isolates

Out of total collected food samples 126/200 (63%) produced positive bacterial growth on nutrient agar (normal flora, lactic acid, spoilage, target and non target foodborne pathogenic bacteria) while 74 (37%) samples showed negative growth. Furthermore, 15 bacterial isolates (15/200 (7.5%) of total collected samples and 15/126 (11.9%) of total positive bacterial growth) showed suspected characters of target foodborne pathogenic bacteria on the selective media (Fig. 1). Meat and poultry products were the most contaminated food type where 8.75% were contaminated with foodborne pathogenic

bacteria, followed by vegetable and fruit products (8%), dairy products (6.66%), flour and bakery products (6.66%), respectively. While the least contaminated food type was canned fish products (5%).

Contamination of food products may occur throughout any stage of the farm to table process including utilizing of untreated water or sewage for irrigation, unsuitable or contaminated fertilizers or composted fertilizer; contamination of soil by animals grazing, as well as open vehicles transport contamination. Also, contamination can occur during processing, packing, distribution, or at retail markets (McEntire, 2013). Poultry and eggs are the major concern for salmonellosis. Additionally, other meat and dairy products were also implicated in previous outbreaks (Coburn et al., 2007). *E. coli* can contaminate water and soil through warm-blooded animal feces. Fruits and vegetables may also be contaminated if the untreated manures are used as fertilizers. Meats are also a common source of *E. coli*, since the meat may be contaminated by fecal contracts during slaughter (Armstrong et al., 1996). Moreover, foodborne outbreaks have been occurred by contaminated vegetables in many countries. Vegetables may be contaminated from the irrigation water, human sewage. Also, contaminated water used for rinsing and sprinkling vegetables to keep them fresh is extra probable source of contamination (Bukar et al., 2010). During the storage of raw milk, *Pseudomonas* species play a key role in milk spoilage they produce many thermo tolerant proteolytic and lipolytic enzymes which diminish together the shelf life and quality of processed milk (Dogan & Boor, 2003).

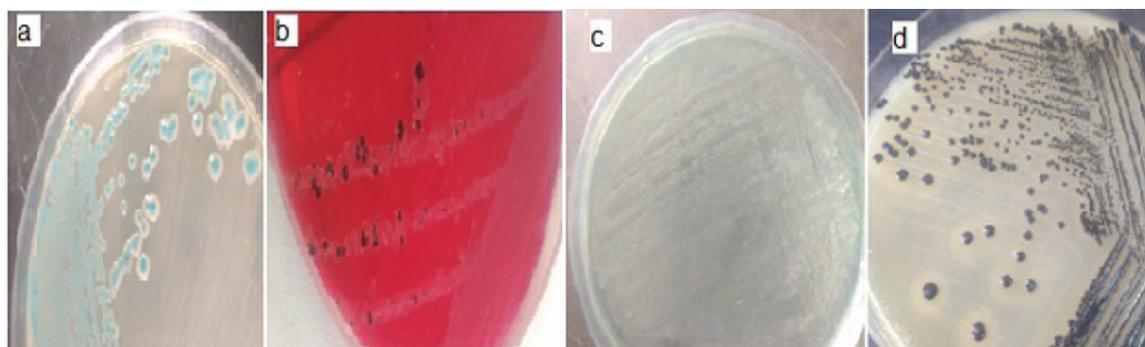


Fig. 1. Foodborne pathogenic bacteria on selective media: (a) TBX, *Escherichia coli*, (b) XLD, *Salmonella* spp., (c) Cetrimide agar, *Pseudomonas aeruginosa* and (d) Baird-Parker, *Staphylococcus aureus*.

In the current study, the most common and frequent pathogen was *Escherichia coli* representing 40% of collected bacterial isolates after that *Staphylococcus aureus* (26.7%) and *Pseudomonas aeruginosa* (20%), then *Salmonella enterica* subsp. *Enterica* (13.3%), respectively. Similar results were found in the previous studies which found that *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *S. aureus* are responsible for numerous worldwide cases of foodborne outbreaks (Ifediora et al., 2006; Ifeanyichukwu et al., 2014 and Mostafa et al., 2018). Likewise, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. were found to be the major frequent bacteria in the majority of food poisoning cases caused by contaminated raw or undercooked poultry and red meat (Dan et al., 2015).

The results were in line with the findings of Kumar et al. (2006) and Ghosh et al. (2007) who reported high prevalence of *S. aureus* and *E. coli* in street-vended foods. On contrast, in Nyenje et al. (2012) study, *Salmonella* spp. and *E. coli* were not isolated in any of the ready-to-eat food samples. Level of personal hygiene of the food handlers and geographical location differences may explain this disagreement. Recently meta-analysis paper (Paudyal et al., 2017) reviewed the predominance of foodborne pathogenic bacteria in some African countries. The prevalence of *Escherichia coli* was 37.6% and 31.6% in raw foods and ready-to-eat foods, respectively, *Staphylococcus aureus* (27.8% - 25.1%), *Salmonella* (19.9% - 21.7%) and *Listeria monocytogenes* (19.5% - 6.7%). The average prevalence of foodborne pathogenic bacteria in this study was 34.2%.

Antibiotic susceptibility

In recent years, pathogenic bacteria have caused several foodborne diseases caused by contaminated foods. These bacteria have shown severe resistance to various common antibiotics (Ifediora et al., 2006; Ifeanyichukwu et al., 2014 and Dan et al., 2015). The results revealed that tested isolates showed high percentages of multidrug-resistant (Table 1). Imipenem antibiotic was the best one against the tested bacteria (93.3% susceptibility) followed by ofloxacin, amikacin, ciprofloxacin and vancomycin nitrofurantoin with 80%, 73.3%, 66.7% and 53.3%, respectively. On the other hand, the tested bacterial isolates showed highly resistance to oxacillin, sulphamethoxazole/trimethoprim and amoxicillin. Our results are in line with that of Reddy et al. (2016). They found

that imipenem was the most effective antibiotic (82%), after that amikacin (71%), ciprofloxacin (63%) and ofloxacin (63%), respectively. They found also highly resistance to doxycycline (85%), ceftriaxone (66%) and co-trimoxazole (54%). Furthermore, Dan et al. (2015) reported high percentage (23%) of multidrug-resistant (MDR) pathogenic bacteria in the tested food samples. The tested bacteria showed high resistance to sulfonamides, fluoroquinolones and tetracycline. The resistant *E. coli* and *Salmonella* strains were investigated for the occurrence of typical resistance genes (*tetA*, *tetB*, *tetG*, *Kn*, *aadA1a*, *DfrIa*, *Sul*, and *bla_{TEM}*). They concluded that, these isolates represent an important reservoir in the spread of antibiotic resistance phenomenon.

Antibacterial activity of chemical food preservatives

The increasingly appearance of bacterial strains resistant to common antimicrobial agents originated from uncontrolled use of chemical preservatives and misuse of antibiotics (Gyawali et al., 2015). The results of this study revealed that the most effective chemical preservative was potassium sorbate with 66.7% susceptibility followed by sodium benzoate (53.3%) and calcium propionate (46.7%), respectively. In contrast, 40% of tested isolates were resistant to citric acid and ascorbic acid (Table 2 and Fig. 2).

The most regularly used low cost preservatives are the conventional preservatives including potassium sorbate, calcium propionate and sodium benzoate (Silva & Lidon, 2017). The efficiency of sorbates on *Salmonellae*, *S. aureus*, psychotropic spoilage bacteria, *V. parahaemolyticus* and coliforms were investigated. The usage of sorbates on vacuum packaged poultry products, fresh poultry meat, perishable fruits and fresh fish improved the shelf life extensions (Jay et al., 2005). A lot of organic acids such as: Citric acid, tartaric acid, ascorbic acid, lactic acid and acetic acid were used widely as preservatives in beverages like fruit juices and carbonated drinks. Moreover, organic acids were used widely in other foods for their bactericidal properties for instance whipping cream, canned artichokes, frankfurters, salad dressings and figs (Silva & Lidon, 2017). As an antimicrobial agent, citric acid is poorly effective (Winniczuk & Parish, 1997) and is required at high concentrations for activity, 0.3% citric acid affected *salmonellae* (Thomson et al., 1967), while 2% extended the shelf-life of ground beef slightly (Shelef et al., 1997).

TABLE 1. The susceptibility of isolated bacteria to selected antibiotics.

Antibiotics	Code	µg/disc	Susceptible(%S)	Intermediate (% I)	Resistant (%R)
Imipenem	IPM	10	93.3	6.7	0
Ofloxacin	OFX	5	80	6.7	13.3
Amikacin	AK	30	73.3	6.7	20
Ciprofloxacin	CIP	5	66.7	0	33.3
Vancomycin	VA	30	53.3	6.7	40
Nitrofurantoin	F	300	46.7	20	33.3
Ceftriaxone	CRO	30	33.3	20	46.7
Azithromycin	AZM	15	33.3	6.7	60
Amoxicillin/clavulanic acid	AMC	20/10	20	13.3	66.7
Cefaclor	CEC	30	13.3	20	66.7
Cephalothin	CL	30	6.7	13.3	80
Amoxycillin	AX	25	0	13.3	86.7
Oxacillin	OX	1	0	0	100
Sulphamethoxazole/trimethoprim	SXT	25	0	13.3	86.7

$$\%S = \frac{\text{No. of sensitive isolate}}{\text{Total count of isolate}} \times 100; \quad \%I = \frac{\text{No. of intermediate isolates}}{\text{Total count of isolate}} \times 100; \quad \%R = \frac{\text{No. of resistant isolate}}{\text{Total count of isolates}} \times 100$$

TABLE 2. Comparative susceptibility of bacterial isolates against chemical food preservatives.

Preservatives	Resistant (%)	Intermediate (%)	Susceptible (%)
Sodium benzoate	26.7	20	53.3
Potassium sorbate	20	13.3	66.7
Calcium propionate	33.3	20	46.7
Citric acid	40	26.7	33.3
Ascorbic acid	40	20	40

Molecular identification of the most resistant isolates

The identification of the most resistant isolate in each group (VF2, MP5, MP3 and D4) was confirmed by using 16S rRNA gene sequencing as: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica subsp. enterica* and *Staphylococcus aureus*, respectively. The obtained sequences were submitted to GenBank in accession numbers: KY630713, KY630714, KY630715 and KY630716, respectively. Phylogenetic analysis using TREEVIEW program were used to assess the DNA similarities of the obtained 16S rRNA gene sequences (Fig. 3).

Antibacterial activity of plant extracts against MDR bacteria

The current study showed that all tested ethanolic plant extracts produced extremely

important antimicrobial effects via inhibiting the tested multi-drug resistant bacteria (Table 3). Sumac (*Rhus coriaria*) found to be the most effective extract producing inhibition zones average 27mm, followed by clove, rosemary and lemon (24mm, 21mm and 18mm, respectively). Whereas, the ethanolic extract of black pepper produced intermediate activity (13mm). Moreover, the aqueous extracts showed lower activity (Table 3).

The obtained results are in agreement with the findings of earlier studies. The antibacterial activity of ethanolic extract of sumac fruits was investigated by Nasar-Abbas & Halkman (2004) on Gram negative bacteria (*E. coli*, *C. freundii*, *Hafnia alvei*, *S. enteritidis* and *Proteus vulgaris*) and Gram positives (*S. aureus*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus*

subtilis, *Bacillus thuringiensis* and *Listeria monocytogenes*). They found the extract highly effective on all tested bacteria and the Gram positives were more sensitive. Ali-Shtayeh et al. (2013) investigated the antibacterial activity of 56 Palestinian plants. They found sumac to have the maximum antibacterial effect against *E. coli*, *P. aeruginosa* and *S. aureus*. Similarly, Aliakbarlu et al. (2014) found sumac (*Rhus coriaria*) extract to have the maximum antibacterial effect on *S. typhimurium*, *B. cereus*, *E. coli* and *L. monocytogenes*.

Antimicrobial activity of clove against foodborne pathogens was investigated by several researchers (Pandey & Singh, 2011; Saeed et al., 2013 and Mostafa et al., 2018). Pandey & Singh (2011) reported that, clove extract was highly effective on *E. coli*, *P. aeruginosa* and *S. aureus* and its minimum inhibitory concentrations were 0.1 to 2.31mg/ml. Mostafa et al. (2018) reported that clove extract was found to be effective on *S. aureus*, *B. cereus*, *P. aeruginosa* and *E. coli* producing inhibition zones of 15.8, 14.6, 13.4 and 11.9mm, respectively, with MICs from 2.5 to 5.0mg/ml. Zhang et al. (2009) investigated the antibacterial activity of ethanolic extracts of 14 plants against *P. fluorescens*, *E. coli*, *L. sake* and *L. monocytogenes*. The extract of rosemary produced strong antibacterial activity. The antibacterial activities of lemon peel and fruit extract were previously studied using different microorganisms. The results supported the possibility of using these extracts in various applications including food preservation (Dhanavade et al., 2011 and John et al., 2017).

The antimicrobial activity of plant extracts are associated with their chemical composition which are divided into main groups such as flavonoids, alkaloids, coumarins, iridoids, steroidal, saponins, xanthenes, tannins, flavones, phenols, essential oils, lactones and steroids. The release of these compounds in plant extracts depends on the solubility of these chemicals in the solvents used (Cowan, 1999). The accurate pathways by which plant compounds apply their antibacterial effects are not clearly defined, even though, a number of mechanisms have been reported. These mechanisms contain interruption of bacterial cell membrane leading to leakage of intracellular contents, loss of membrane potential and impaired ATP production. Additionally, interruption of DNA/RNA synthesis and functions may occur. Also, coagulation of cytoplasmic constituents and disruption of normal cell communication may occur leading to cell death (Radulovic et al., 2013 and Gyawali et al., 2015).

The obtained data showed that Gram-negative bacteria are more resistant to plant extracts than Gram-positive bacteria. This observation was reported by many studies (Saeed et al., 2013 and Kozłowska et al., 2015). The resistance of Gram negative bacteria to herbal extracts can be due to the complexity of the bilayer cell wall of these bacteria, compared to the glycoprotein-teichoic acid cell wall of Gram positive bacteria (Nazir et al., 2017). Moreover, the Gram negative bacteria have an outer membrane which is known to make a barrier to penetration of many antimicrobial agents. Also cell surface hydrophobicity can also be proposed as an effective factor (Gyawali et al., 2015).

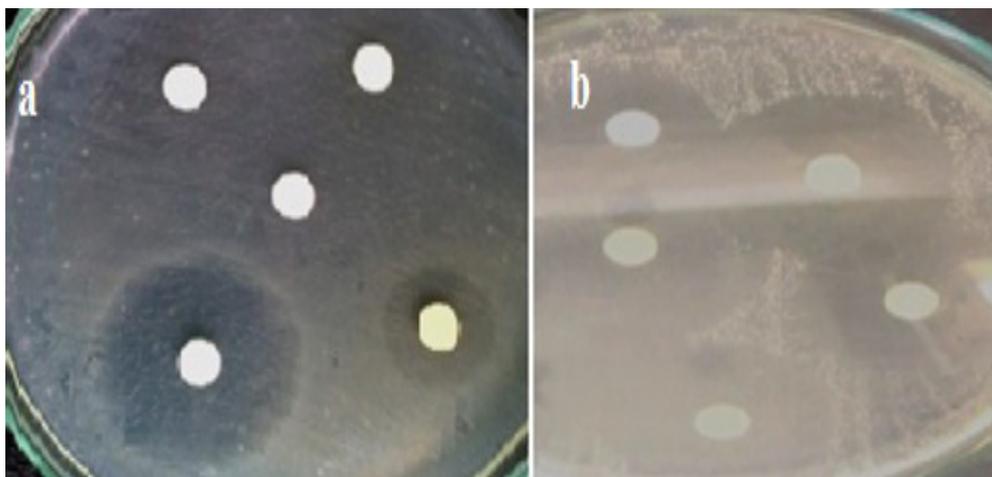


Fig. 2. Antibacterial activity of chemical food preservatives by disc diffusion method, (a) *Pseudomonas aeruginosa*, (b) *Staphylococcus aureus*.

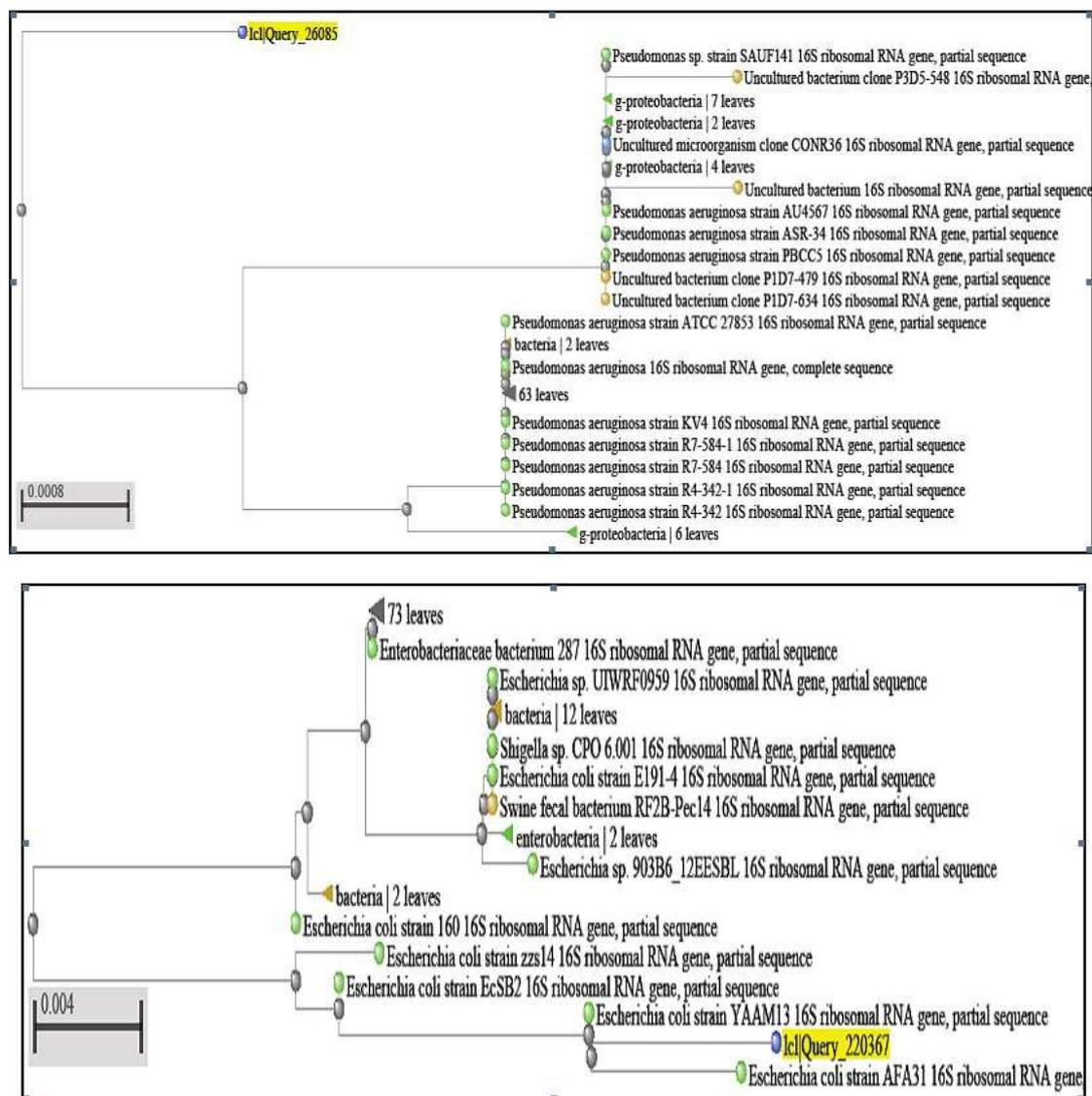


Fig. 3. Phylogenetic trees of the most resistant isolates, (a) *P. aeruginosa* VF2, (b) *E. coli* MP5, (c) *S. enterica* subsp. *enterica* MP3 and (d) *S. aureus* D4.

TABLE 3. Antimicrobial activity of plant extracts on MDR bacteria.

Plant name	Family	Part used	Solvent	Inhibition zone (mm)*			
				<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>	<i>E. coli</i>
<i>Rhus coriaria</i> (sumac)	<i>Anacardiaceae</i>	Fruits	Ethanol	31	23	28	25
			Water	23	15	20	18
<i>Rosmarinus officinalis</i> (rosemary)	<i>Lamiaceae</i>	Aerial parts	Ethanol	24	17	21	19
			Water	13	0	13	11
<i>Piper nigrum</i> (black pepper)	<i>Piperaceae</i>	Seeds	Ethanol	16	10	14	12
			Water	12	0	10	0
<i>Syzygium aromaticum</i> (clove)	<i>Myrtaceae</i>	Floral buds	Ethanol	27	21	25	25
			Water	20	13	15	17
<i>Citrus limon</i> (lemon)	<i>Rutaceae</i>	Fruits	Ethanol	21	15	20	17
			Water	14	0	10	9

* Negative control (2% DMSO) disks were inert.

Effect of combination between the most effective extracts

Mixing of plant extracts can help to minimize required concentrations and accordingly reduce sensory impact. In addition, these mixtures may control some resistant bacteria (Arjun et al., 2014 and Rada et al., 2016). The combination of sumac with clove and clove with rosemary showed additive effect in most cases (equal to the individual effects), whereas, combination of sumac with rosemary produced synergistic effect in all cases (more antibacterial action than that of the individual effects). On the other hand, combination of sumac with clove and rosemary produced less potent effect than the individual effects (antagonistic effect) (Table 4).

Different plant compounds may affect bacteria in different ways, so, combination of plant extracts treatments may produce better activity by targeting a range of targets in bacterial cell or improving the action of one compound. Researchers have examined the activity of plant extracts in combination with other plant extracts, essential oils or plant compounds (Zhang et al., 2009; Arjun et al., 2014 and Baljeet et al. 2015). The results gained are in agreement to the findings of previous studies. Zhang et al. (2009) investigated the antibacterial activity of ethanolic extracts of 14 plants against *P. fluorescens*, *E. coli*, *L. sake* and *L. monocytogenes*. They found synergistic effect in the combination of rosemary and liquorice extracts which produced the best inhibitory effects. Azizkhani & Tooryan (2015) evaluated the synergistic effects of combination of rosemary and mint against microorganisms from the sausages. Also, they investigated the antibacterial effects of rosemary, mint and a mixture of tocopherols. Arjun et al. (2014) reported that combination of three extracts (*Opuntia ficus indica*, *Larrea tridentata*, and *Flourensia cernua*) by 1:1:1 v/v/v proportion showed synergical effects as it was the best treatment for inhibiting the tested bacteria. On the other hand, no synergistic effects of combinations from extracts of different spices were observed and additive effect only was observed (Baljeet et al., 2015).

Effect of food components on antibacterial activity of plant extracts:

Data presented in Table 5 showed that, when the ethanolic extracts were mixed into food models the antibacterial activity was reduced and the required MICs were duplicated in most cases. MICs of sumac extract was from 0.390 to 1.562, 0.781 to 3.125 and 1.562 to 6.250mg/ml when tested on microbiological (control) medium, meat model and

milk model respectively, while the MICs of clove was from 0.781 to 3.125, 1.562 to 3.125 and 3.125 to 6.250mg/ml when tested on control medium, meat model and milk model, respectively. Similar results were obtained by various studies which have been reported that, the interaction or reaction of plant extracts with food components reduced the antibacterial effects of plant extracts; therefore, higher concentrations of used extracts were required to do the same effects in meat-based and milk-based models as in microbiological medium. (Uhart et al., 2006; Gutierrez et al., 2009; Azazy et al., 2017 and Bouarab-Chibane et al., 2018).

The presence of proteins, carbohydrates, fats, salts and pH levels in foodstuffs may explain the activity differences produced in *in vitro* experiments and in *in vivo* on the food models or foodstuffs themselves (Ozdal et al., 2013 and Weiss et al., 2015). Additionally, the accessibility of nutrients in great quantities in food products helps in rebuild and repair broken cells and the large amounts of fats and proteins may act as protection barriers for bacteria (Gyawali et al., 2015). In the presence of milk proteins a reaction between carvacrol (a component of a variety of plant extracts) and these proteins have been proposed as a limiting factor on the antibacterial effects on *L. monocytogenes* and *B. cereus* (Pol & Smid, 1999). Likewise, in diluted low fat cheese the protein interaction has been proposed as a factor limiting the action of clove oil against *Salmonella* species (Smith-Palmer et al., 2001). In the same line, Uhart et al. (2006) reported that spices extracts inhibited *Salmonella typhimurium* in direct contact, but the activity reduced when applied to ground beef. Furthermore, interactions between proteins and polyphenols (component of various plant extracts) have been extensively reviewed (Ozdal et al., 2013). The obtained results showed that, the antibacterial activity were reduced on semi-skimmed milk model more than that on meat model. This may be due to the protection action of fats, the hydrophobic antimicrobials may migrate to the fatty compounds of the foods, leaving the aqueous fraction, where the microbe develop, free of antimicrobials or may be due to the difference in complexity of the foods. In general, more complex foods are affected less by natural antimicrobial compounds (Gutierrez et al., 2009 and Weiss et al., 2015). This effect was also reported by Cava et al. (2007). Similarly, the increase of natural antimicrobials MICs observed in whole milk containing 3.6% (w/w) milk fat in comparison to TSB 1% (w/w) reported by Bouarab-Chibane et al. (2018).

TABLE 4. Combination between the most effective ethanolic extracts.

Bacteria	Inhibition zone (mm)			
	S & C	S & R	C & R	S, C & R
<i>S. enterica</i> MP3	28	32	25	18
<i>E. coli</i> MP5	24	27	24	16
<i>S. aureus</i> D4	32	35	29	20
<i>P. aeruginosa</i> VF2	23	27	20	14

S= Sumac, C= Clove, R= Rosemary

TABLE 5. The MICs (mg/ml) of sumac and clove ethanolic extracts on laboratory and food model media.

Bacteria	Control medium		Meat model		Milk model	
	Sumac	Clove	Sumac	Clove	Sumac	Clove
<i>S. enterica</i> MP3	0.781	0.781	1.562	1.562	1.562	3.125
<i>E. coli</i> MP5	0.781	1.562	1.562	3.125	3.125	6.25
<i>S. aureus</i> D4	0.39	0.781	0.781	1.562	1.562	3.125
<i>P. aeruginosa</i> VF2	1.562	3.125	3.125	3.125	6.25	6.25

Conclusion

Consumer's interest in natural materials, especially plant extracts, has increased as natural food preservatives following the rise of antimicrobial resistance, as well as the bad reputation of industrial preservatives. This study evaluated the potential use of plant extracts as an effective and safe alternative for natural food preservation. Also, it provides factors influencing antibacterial effect including strains of microorganisms, extraction method and type and compositions of food. These factors must be taken into consideration when plant extracts are used as natural food preservatives.

References

- Aliakbarlu, J., Mohammadi, S. and Khalili, S. (2014) A study on antioxidant potency and antibacterial activity of water extracts of some spices widely consumed in Iranian diet. *J. Food Biochem.* **38**, 159–166.
- Ali-Shtayeh, M.S., Al-Assali, A.A. and Jamous, R.M. (2013) Antimicrobial activity of Palestinian medicinal plants against acne-inducing bacteria. *Afr. J. Micro. Res.* **7**, 2560–2573.
- Arjun, D., Kumar, R. and Singh, C. (2014) The effects of ethanol plant extracts on food-borne pathogen bacteria. *Adv. Food. Sci. Technol.* **2**(10), 271–275.
- Armstrong, G.L., Hollingsworth, J. and Morris, J.G. (1996) Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epid. Rev.* **18**(1), 29–51.
- Azazy, W.M. (2017) Antimicrobial activity of some plant extracts using food model media. The Twelfth Inter. *Environmental Conf.*, Faculty of Science, Zagazig Univ., Egypt. pp. 302–320.
- Azizkhani, M. and Tooryan, F. (2015) Antioxidant and antimicrobial activities of rosemary extract, mint extract and a mixture of tocopherols in beef sausage during storage at 4°C. *J. Food Saf.* **35**, 128–136.
- Baljeet, S.Y., Simmy, G., Ritika, Y. and Roshanlal, Y. (2015) Antimicrobial activity of individual and combined extracts of selected spices against some pathogenic and food spoilage microorganisms. *IFRJ*, **22**(6), 2594–2600.
- Bouarab-Chibane, L., Oulahal, N., Dumas, E., Thanh, N.T., Bouajila, J., Souchard, J. and Degraeve, P. (2018) Effect of interaction with food constituents on plant extracts antibacterial activity. *Food Sci. Appl. Biotech.* **1**(1), 77–85.
- Bukar, A., Uba, A. and Oyeyi, T.I. (2010) Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready-to-eat foods in Kano metropolis, Nigeria. *Afr. J. Food Sci.* **4**, 32–36.
- Cava, R., Nowak, E., Taboada, A. and Marin-Iniesta, F. (2007) Antimicrobial activity of clove and cinnamon

- essential oils against *Listeria monocytogenes* in pasteurized milk. *J. Food Prot.* **70**(12), 2757–2763.
- CLSI (Clinical and Laboratory Standards Institute) (2008) Performance standards for Antimicrobial susceptibility testing; 18th informational supplement. M100-S18, Wayne, PA.
- Coburn, B., Grass, G.A. and Finlay, B.B. (2007) *Salmonella*, the host and disease: A brief review. *Immunol. Cell Biol.* **85**, 112–118.
- Cowan, M.M. (1999) Plant products as antimicrobial agents. *Clin. Micro. Rev.* **12**(4), 564–582.
- Dan, S.D., Tăbăran, A., Mihaiu, L. and Mihaiu, M. (2015) Antibiotic susceptibility and prevalence of foodborne pathogens in poultry meat in Romania. *J. Infect. Dev. Ctries.* **9**(1), 35–41.
- Dhanavade, M.J., Jalkute, C.B., Ghosh, J.S. and Sonawane, K.D. (2011) Study antimicrobial activity of lemon (*Citrus lemon* L.) Peel Extract. *Br. J. Pharma. Toxicol.* **2**(3), 119–122.
- Dogan, B. and Boor, K.J. (2003) Genetic diversity and spoilage potentials among *Pseudomonas* from fluid milk products and dairy processing plants. *Appl. Env. Micro.* **69**, 130–138.
- FDA (2016) Food and Drug Administration: Bacteriological Analytical Manual (BAM). Rockville, MD.
- Garrity, G.M., Brenner, D.J., Krieg, N.R. and Staley, J.T. (2005) "*Bergey's Manual of Systematic Bacteriology*". 2nd ed. Vol. 2: The Proteobacteria. Part B: The Gammaproteobacteria. Springer, New York.
- Ghosh, M., Wahi, S., Kumar, M. and Ganguli, A. (2007) Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. *Int. J. Environ. Health Res.* **17**, 151–156.
- Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2009) Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiology*, **26**(2), 142–150.
- Gyawali, R., Hayek, S.A. and Ibrahim, S.A. (2015) Plant extracts as antimicrobials in food products: Mechanisms of action extraction methods, and applications. In: "*Handbook of Natural Antimicrobials for Food Safety and Quality*", Taylor, T.M. (Ed.), pp.31–47. Woodhead Publishing Series in Food Science, Technology and Nutrition.
- Ifeanyichukwu, I., Ngozi, A., Emmanuel, N., Chika, E., Anthonia, O. and Donathus, I. (2014) Antibigram of food-borne pathogens isolated from ready-to-eat foods and Zobo Drinks Sold Within and Around PRESCO Campus, Nigeria. *J. Toxicol. Env. Health Sci.* **6**, 1–4.
- Ifediora, A.C., Nkere, C.K. and Iroegbu, C.U. (2006) Weaning food preparations consumed in Umuahia, Nigeria: Evaluation of the bacteriological quality. *J. Food Technol.* **4**, 101–105.
- Jay, J.M., Loessner, M.J. and Golden, D.A. (2005) "*Modern Food Microbiology*", 7th ed. Springer Since+Business Media, LLC. New York, NY.
- John, S., Monica, S.J., Priyadarshini, S., Sivaraj, C. and Arumugam, P. (2017) Antioxidant and antimicrobial efficacy of lemon (*Citrus limonum* L.) Peel. *Int. J. Pharm. Sci. Rev. Res.* **46**, 115–118.
- Kolbert, C.P. and Persing, D.H. (1999) Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Cur. Opin. Microbiol.* **2**(3), 299–305.
- Kozłowska, M., Laudy, A.E., Przybył, J., Ziarno, M. and Majewska, E. (2015) Chemical composition and antibacterial activity of some medicinal plants from *Lamiaceae* family. *ACTA Pol. Pharm.* **72**, 757–767.
- Kumar, M., Agarwal, D., Ghosh, M. and Ganguli, A. (2006) Microbiological safety of street vended fruit chats in Patiala city. *Indian J. Med. Micro.* **24**, 75–76.
- McEntire, J. (2013) Foodborne disease: the global movement of food and people. *Inf. Dis. Clin. North Am.* **27**(3), 687–693.
- Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N. and Bakri, M.M. (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J. Biol. Sci.* **25**, 361–366.
- Nasar-Abbas, S.M. and Halkman, A.K. (2004) Inhibition of some foodborne bacteria by alcohol extract of sumac (*Rhus coriaria* L.). *J. Food Safety*, **24**, 257–267.

- Nazir, F., Salim, R., Yousf, N., Bashir, M., Naik, H. R. and Hussain, S. Z. (2017) Natural antimicrobials for food preservation. *J. Pharma. Phytoch.* **6**(6), 2078–2082.
- Negi, P.S. (2012) Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *Int. J. Food Micro.* **156**, 7–17.
- Nyenje, M.E., Odjadjare, C.E., Tanih, N.F., Green, E. and Ndip, R.N. (2012) Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province; South Africa: Public health implications. *Int. J. Environ. Res. Public Health*, **9**, 2608–2619.
- Ozidal, T., Capanoglu, E. and Altay, F. (2013) A review on protein-phenolic interactions and associated changes. *Food Res. Int.* **51**(2), 954–970.
- Pandey, A. and Singh, P. (2011) Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. *Asian J. Plant Sci. Res.* **1**(2), 69–80.
- Paudyal, N., Anihouvi, V., Hounhouigan, J. and Matsheka, M.I. et al. (2017) Prevalence of foodborne pathogens in food from selected African countries – A meta-analysis. *Int. J. Food Micro.* **249**, 35–43.
- Pisoschi, A.M., Pop, A., Georgescu, C., Turcus, V., Olah, N.K. and Mathe, E. (2018) An overview of natural antimicrobials role in food. *Eur. J. Med. Chem.* **143**, 922–935.
- Pol, I.E. and Smid, E.J. (1999) Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes*. *Lett. Appl. Microbiol.* **29**, 166–170.
- Rada, X., Todd-Searle, J., Friedman, M., Patel, J., Jaroni, D. and Ravishankar, S. (2016) Combining essential oils and olive extract for control of multi-drug resistant *Salmonella enterica* on organic leafy greens. *SDRP. J. Food Sci. Techn.* **1**(2), 1–9.
- Radulovic, N.S., Blagojevic, P.D., Stojanovic-Radic, Z.Z. and Stojanovic, N.M. (2013) Antimicrobial plant metabolites: structural diversity and mechanism of action. *Curr. Med. Chem.* **20**, 932–952.
- Reddy, P.S., John, M.S., Devi, P.V. and Kumar, S.S. (2016) Nosocomial infections among patients admitted in general ICU: Study from a tertiary-care hospital in South India. *Int. J. Med. Sci. Pub. Health.* **5**, 21–27.
- Saeed, M., Nadeem, M. and Khan, M.R. et al. (2013) Antimicrobial activity of *Syzygium aromaticum* extracts against food spoilage bacteria. *Afr. J. Microbiol. Res.* **7**(41), 4848–4856.
- Sapkota, R., Dasgupta, R., Nancy, S. and Rawat, D.S. (2012) Antibacterial effects of plants extracts on human microbial pathogens and microbial limit tests. *Int. J. Res. Pharm. Chem.* **2**(4), 926–936.
- Schwalbe, R., Steele-Moore, L. and Goodwin, A.C. (2007) "Antimicrobial Susceptibility Testing Protocols". CRC Press, Taylor & Francis Group, LLC.
- Selim, S.A., El Alfy, S.M., Abdel Aziz, M.H., Mashait, M.S. and Warrad, M.F. (2012) Evolution of bactericidal activity of selected food additives against food borne microbial pathogens. *Biosci. Biotech. Res. Asia*, **9**(1), 07–17.
- Shelef, L.A., Mohammed, S., Tan, W. and Webber, M.L. (1997) Rapid optical measurements of microbial contamination in raw ground beef and effects of citrate and lactate. *J. Food Protection*, **60**, 673–676.
- Silva, I.F., Oliveira, R.G., Soares, I.M., Alvim, T.C., Ascêncio, S.D. and Martins, D.T (2014) Evaluation of acute toxicity, antibacterial activity, and mode of action of the hydroethanolic extract of *Piper umbellatum* L., *J. Ethnophar.* **151**, 137–143.
- Silva, M.M. and Lidon, F.C. (2017) Food preservatives: An overview on applications and side effects. *Emir. J. Food Agric.* **28**(6), 366–373.
- Smith-Palmer, A., Stewart, J. and Fyfe, L. (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. *Food Micro.* **18**, 463–470.
- Sultana, T., Rana, J., Chakraborty, S.R., Das, K.K., Rahman, T. and Noor, R. (2014) Microbiological analysis of common preservatives used in food items and demonstration of their *in vitro* antibacterial activity. *Asian Pac. J. Trop. Dis.* **4**(6) 452–456.
- Thomson, J.E., Banwart, G.J., Sanders, D.H. and Mercuri, A.J. (1967) Effect of chlorine, antibiotics, β -propiolactone, acids, and washing on *Salmonella*

- typhimurium* on eviscerated fryer chickens. *Poultry Sci.* **46**, 146–151.
- Uhart, M., Maks, N. and Ravishankar, S. (2006) Effect of spices on growth and survival of *Salmonella typhimurium* DT 104 in ground beef stored at 4 and 8°C. *J. Food Safety*, **26**(2), 115–125.
- Vos, P.D., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. and Whitman, W.B. (2009) "*Bergey's Manual of Systematic Bacteriology*". 2nd ed. Vol. 3: The Firmicutes. Springer, New York.
- Weiss, J., Loeffler, M. and Terjung, N. (2015) The antimicrobial paradox: Why preservatives lose activity in foods. *Curr. Op. Food Sci.* **4**(8), 69–75.
- Winniczuk, P.P. and Parish, M.E. (1997) Minimum inhibitory concentrations of antimicrobial against microorganisms related to citrus juice. *Food Microbiol.* **14**, 373–381.
- Zhang, H.Y., Kong, B.H., Xiong, Y., Sun, X. (2009) Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4°C. *Meat Sci.* **81**, 686–692.

(Received 22/ 5 /2018;
accepted 30/ 8 /2018)

المستخلصات النباتية كمثبطات للبكتيريا المسببة للأمراض التي تنتقل عن طريق الأغذية

سهير سعد عبد السلام⁽¹⁾، محمد فاروق غالي⁽²⁾، محمد هشام ياسين⁽¹⁾، عطية أحمد عطيه إبراهيم⁽¹⁾، و سالي عزت سلام سلام⁽¹⁾
⁽¹⁾ قسم الميكروبيولوجي – كلية العلوم – جامعة بنها – مصر و ⁽²⁾ قسم الميكروبيولوجي – كلية العلوم – جامعة الزقازيق – الزقازيق – مصر.

ازداد الإهتمام مؤخراً بالمواد الطبيعية، وخاصة المستخلصات النباتية، كمواد حافظة غذائية طبيعية بعد ارتفاع المقاومة لمضادات الميكروبات، فضلاً عن السمعة السيئة للحافظات الصناعية للمستهلكين. تهدف هذه الدراسة إلى تقييم الإستخدام المحتمل للمستخلصات النباتية كبديل فعال وأمن لحفظ الطعام. أيضاً، فإنه يوفر العوامل التي تعمل كمضاد للبكتيريا باستخدام النماذج القائمة على اللحوم والحليب.

أظهرت المستخلصات النباتية الإيثانولية تأثيرات مضادة للميكروبات بالغة الأهمية عن طريق تثبيط البكتيريا المقاومة للعقاقير المتعددة المختبرة. وجد أن السماق هو المستخرج الأكثر فعالية لإنتاج مناطق تثبيط 27 مم، يليه القرنفل وإكليل الجبل والليمون (24 مم، 21 مم و 18 مم على التوالي)، بينما أظهر الفلفل الأسود نشاطاً متوسطاً (10 - 16 ملم). أظهرت المستخلصات المائية نشاطاً أقل. إن تركيبة السماق مع القرنفل والقرنفل مع إكليل الجبل تنتج تأثيراً مضافاً في معظم الحالات، في حين أن السماق مع إكليل الجبل ينتجان تأثيراً تآزرياً في جميع الحالات. ومن ناحية أخرى فإن تركيبة من السماق مع القرنفل وإكليل الجبل أنتجت تأثيراً معادياً.

أدى تفاعل المستخلصات النباتية مع مكونات الطعام إلى تقليل التأثيرات المضادة للجراثيم للمستخلصات النباتية. لذلك كانت هناك حاجة إلى تركيزات أعلى من المستخلصات المستخدمة للقيام بنفس التأثيرات في النماذج المعتمدة على اللحوم والحليب.

أشارت النتائج إلى أن المستخلصات النباتية التي تمتلك نشاطاً مضاداً للميكروبات يمكن استخدامها كمادة حافظة غذائية مثالية بعد أخذ التفاعل بين مكونات الطعام ومستخلصها في الحسبان.