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ANTIMICROBIAL ACTIVITY OF SOME HERBAL PLANTS IN EL-JABAL AL AKHDAR AREA AND THEIR PRESERVATIVE EFFECTS AGAINST MICROBIAL LOADS IN MEAT SLICES DURING COLD STORAGE

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ABSTRACT

This study was carried out to investigate the antimicrobial activity of some herbs against some microorganisms (*Staph. aureus*, *B. subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albican*, *Aspergillus flavu*s and *penicillium chrysogenum*) which play an important role in spoilage of food and sometimes incriminated in food poisoning using the Disc Assay Procedure. In addition evaluation the effect of using of the powder of these plants as additives on the keeping quality of meat stored at refrigerator (microbiological and sensory properties of beef slices). It was found that the powders of these plants as well as their extracts possess an antimicrobial activity; therefore, they can be used in biotechnological fields as natural preservative ingredients in food which could prolong their shelf- life and improve their organoleptic characters.

Keywords: *Staph. Aureus,* food poisoning, Disc Assay, organoleptic .

INTRODUCTION

Meat is a highly favorable medium for growth of microorganisms, and it keeps well for only a short time at refrigeration temperature. The short shelf–life of meat is attributed to its perishable nature, sanitation, practices during handling and time and temperature of storage. Zheng et.al. (2005) reported that the growth of microorganisms on meat is one of the main factors that causes discoloration and spoilage, especially, in an environment of high relative humidity and

insufficient air exchange some microorganisms give rise to disagreeable odors and slime formation.

Furthermore, some microorganisms cause protein and fat degradation, changes in pigmentation and in turn reduce shelf- life of meat at refrigeration temperature. Among meat microbial flora are *E. coli*, *staphylococcus aureus*, Salmonella and yeasts and moulds (Buchanan and Gibbons, 1975). Antimicrobial agents including food preservatives have been used to inhibit food borne bacteria, fungi and extend the shelf life of processed food. Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Oussallah et al., 2006).

Spices and their essential oils are the most efficient natural antioxidants and antimicrobial agents which have long been used to preserve food (Mahmoud et al., 2006). The leafy part of plants such as Greek sage *Salvia fruticosa* (Family Lamiaceae), *Ocimum basilicum* (Family Lamiaceae) , *Pelargonium graveolans*(Family Geraniaceae) have been added to meat, poultry products , fish and food products for years. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic additives. It has been suggested that some synthetic chemicals convert some ingested materials into toxic substances or carcinogens by increasing the activity of microsomal enzymes (Farag, et al., 1989).

In restaurants, cafeterias and other food market shops, meat is handled in a couple of ways. The first way is to store meat in the freezer; then, thawed and refreeze for several times; which would destroy its nutritional and quality attributes. The other way of handling meat is to store it in the refrigerator for two or three days until consumed (This allows increasing microbial load which may lead to food poisoning). Hence, it is important to apply some treatments to refrigerated meat in order to inhibit growth; and in the same time , improve the flavor and other quality attributes of meat. El-Jabal AlAkhdar has a high

diversity of plant species that show both economic and medicinal importance. More than 100 species extensively used by Bedouins in folk medicine. Also many local plants are edible for human besides their traditional medicinal uses. El-Jabal Al Akhdar area possesses unique physiographic and climatic conditions that provides an excellent ecological niche and contributed to the restriction of many plant species.Among the most widely used Libyan plants are *Arbutus pavarii* [family Ericaceae], followed by *Pistacia lentiscus* [family Anacardiaceae] and *Myrtus communes* [family Myrtaceae]. There are apparently lack of published studies focusing on their chemistry and activities . The fruits of these plants are commonly considered as an edible fruits for human and animals.

So, the objective of this study was to investigate the antimicrobial activity of Greek sage, Basil, Pelargonium, Myrtle, Arbutus and Mastic against some microorganisms (*Staph. aureus*, *B.subtilis*, *E. Coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albican*, *Aspergillus flavu*s and *penicillium chrysogenum*) which play an important role in spoilage of food and food poisoning using the Disc Assay Procedure. Also evaluation the effect of using of these plants as additives on the keeping quality of meat stored at refrigerator (microbiological and sensory properties of beef slices) .

MATERIALS AND METHODS

1. Preparation of microbial cultures

Eight microorganisms composed of bacteria , moulds and yeast used as test organisms. These microorganisms includes *Staph. aureus*, *B.subtilis*, *E. Coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albican*, *Aspergillus flavu*s and *penicillium chrysogenum* . They obtained from food control department of Omar El-Mukhtar University. Stock culture of bacterial species were grown on blood sheep agar at 37^0C for 24h Nebahat et al. (2008). Yeast strain grown on PDA (Potato Dextrose Agar) for 3days at 25^0C while mould at 25 ^{0}C for 5 -7 days. Final cell concentration ranged from 10^6-10^7 cfu/ml (APHA,1992).

2. Preparation of plant materials

The leaves and stems of the six tested plants were collected from Al – Jabal Al Akhdar (El – Bieda city – Libya during 2010) .The plant materials were kindly identified by Prof. Dr. Mahmoud Ali Hassanan , Professor of medicinal and aromatic plants, Faculty of Agriculture, Omar EL-Mukhtar University, El Bieda. Leaves and stems of the plants under investigation were separately air-dried, powdered and kept in tightly closed amber colored containers .

3. Preparation of total alcohol extracts:

Thirtygrams of the air dried aerial parts (leaves and stems) of each studied plant were separately extracted with alcohol 90% using soxhlet apparatus till exhaustion. Each of the resulted extract was concentrated under vacuum by rotary evaporator. The residues left after distillation of solvent were weighed and kept in a desiccators (AOAC,1980).The percentages of alcohol extract was calculated and recorded in table (1).

Table (1): Percentage of alcohol extracts obtained from the studied leaves

Extract	Total alcohol
Myrtus communus	20.76 %
Pistacia lenticulus	45.00 %
Arbutus pavarii	83.73 %
Ocimum basillicum	16.30 %
Pelargonium graveolans	15.30 %
Salvia fruticosa	47.60 %

4. Preparation of beef slices

Fresh lean beef was purchased from the butcher shop at El – Bieda city. It was transferred to the laboratory in an ice box. The lean beef was boneless and trimmed of fat and connective tissues. Then ,the

beef was cutted into slices (weighing about 50 g with a size of about 5× 5 × 2 cm for each slice). The beef slices were covered by herbal additives powder as designed in table (2). Five slices of each treatment were packaged in polyethylene bags and were stored in the refrigerator (4 ± 1 ^{0}C). The polyethylene bags were held in single layers to ensure that each bag had similar exposure to surrounding air and light. Examination of beef slices were carried out at zero, 3rd, 6th, and 9th day of refrigerated storage.

Table (2): Formulations of herbal additives.

herbal additives	Constituents of herbal additives
1st treatment	Free from any herbal additives (control).
2nd treatment	5g onion+ 0.1 g black pepper+ 5 g Myrtus communus.
3rd treatment	5g onion+ 0.1g black pepper + 5 g Arbutus pavarii.
4th treatment	5g onion+ 0.1 g black pepper +5 g Pistacia lenticulus.
5th treatment	5g onion+ 0.1g black pepper +5 g Ocimum basillicum.
6th treatment	5g onion+ 0.1g black pepper +5 g Pelargonium graveolans.
7th treatment	5g onion+ 0.1 g black pepper +5 g Salvia fruticosa.

5- Paper Disc plate method

1ml of the bacterial and fungal culture was inoculated into 15 ml of sterile nutrient agar and Potato dextrose agar medium respectively. The inoculated agar was poured aseptically into sterile Petri plates. The agar was allowed to solidify. A sterile filter paper of 0.5 cm diameter was saturated with 0.6 µl of each alcohol extract solution and then it placed in the center of each Petri plates containing the inoculated specific agar. The plates were incubated at 37^{0}C/ 24hr while in fungi at 25^{0}C for 5 days according to (Lorian , 1980 ; Collins et al., 1989). The diameter of each inhibition zone was determined in mm. Diameter less than 5 mm. indicates no effect. Disc impregnated with alcohol is used as a negative control as well as discs of Cefotrioxan, and Nystatin were used as positive control for each microorganism.

6 -Microbial load

Aerobic plate count (APC), *Staph. aureus*, Enterobacteriaceae, Psychrophilic and mould & yeast counts of beef slices were determined

by using Nutrient agar , Baird Parker, Violet Red Bile Glucose, Crystal Violet Tetrazolium, Potato Dextrose agar media, respectively according to the procedures described by (APHA, 1992).Incubation were carried out at 32^0C/48hr for TPC; at 37^0C/24hr for *Staph. aureus*, Enterobacteriaceae; at 7^0C/10 day for Psychrophilic and 25^0C/ 5 day for yeasts and moulds counts.

7- Organoleptic evaluation

Organoleptic evaluation of cooked beef slices was carried out according to Watts et al. (1989)

Table (3): Antimicrobial activity of different extracts prepared from the six plant species under *investigation:*

Microorganism / Total Alcohol extract	*Staph. aureus*	*B. subtilis*	*E. Coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*A. flavus*	*p. chrysogenum*
Myrtus communus	**18**	**16**	**21**	**25**	**6**	**13**	**15**	**25**
Pistacia lenticulus	**18**	**17**	**15**	**5**	**19**	**16**	**--**	**12**
Arbutus pavarii	**15**	**20**	**6**	**25**	**--**	**6**	**--**	**13**
Ocimum basillicum	**--**	**6**	**13**	**16**	**--**	**6**	**--**	**19**
Pelargonium graveolans	**20**	**17**	**9**	**13**	**16**	**11**	**15**	**11**
Salvia fruticosa	**10**	**12**	**13**	**15**	**--**	**12**	**--**	**7**
Ceftrioxan	**7**	**13**	**30**	**23**	**26**	**--**	**--**	**--**
Nystatin	**--**	**--**	**--**	**--**	**--**	**21**	**13**	**22**

Inhibition zones = <5 [-(negative)] , 6-15 [+(weak)], 16-25 [++(moderate)], >25 [+++(high)]

Data in table (3) concerning the antibacterial and antifungal effect of Ethanol extract prepared from the six studied plants showed that these extracts possessed a broad spectrum effect against both the tested Gram positive and Gram negative bacteria , in addition to their

moderate and high inhibitory effect against both tested fungal strains *C. albican , A. flavus* and *P. chrysogenum*.Ethanolic extract obtained from *Myrtus communus* showed the least antimicrobial activity against *Klebsiella pneumoniae* with the high activity against the other tested Gram negative bacteria. At the same time this extract showed a moderate activity against the tested Gram positive organisms. In addition to its moderately high antifungal effect against *C. albicans* and *A. flavus* and highly antifungal effect against *P. chrysogenum*.

Ethanolic extract obtained from *Pistacia lentiscus* showed moderate antibacterial activity against most of the tested Gram positive bacteria , with the low activity against *P. areugenosa*. Moreover, this extract showed a moderately high activity against the tested Gram negative organisms. In addition to , the negative effect on *A. flavus.* and moderately high activity against the tested *C. albicans.* Ethanolic extract obtained from *Arbutus pavarii* showed the least antibacterial activity against most of the tested Gram negative bacteria [*Klebsiella pneumonia* and *E. coli*] with the high activity against *P. areugenosa*. Otherwise this extract showed a moderately high activity against the tested Gram positive organisms with a low antifungal effects.

Ethanol extract obtained from *Ocimum basillicum* showed the least antibacterial activity against Gram positive bacteria strains, while that of *pelargonium graveolans* and *Salvia fruticosa* possess higher inhibitory effects against *Bacillus subtilis* with a moderate effect on *Staphylococcus aureus* . Ethanolic extract obtained from *Pelargonium graveolans* showed the highest antibacterial activity against the Gram negative bacteria .Moreover, both extracts obtained from *Ocimum basillicum* and *Salvia fruticosa* showed a moderately high activity against *E. Coli* and *Pseudomonas aeruginosa* .In addition to ,their negative effect on *Klebsiella pneumonia*. Ethanolic extract obtained from *Pelargonium graveolans* showed the highest antifungal activity against the three tested strains, while the *Ocimum basillicum* extract demonstrated a highly inhibitory activity against the *P. chrysogenum* while a moderate inhibitoryactivity against *Candida albicans* only with no effect on *Asparagillus flavus*.

The total ethanol extracts obtained from *six plants species under investigation* exhibited a significant anti-bacterial effect against the examined Gram positive and Gram negative bacteria under investigation in comparison with the standard antibiotic Ceftrioxan and moderate antifungal effects in comparison with the standard anti fungal Nystatin. These results agreed with what is mentioned in the previous studies dealing with both the antibacterial and antifungal activities (Shin, 2003; Adam et al., 1998; Burt, 2004; Pepeljnjak et al., 2005; Oussallah et al., 2006; El Akrem et al., 2008; Nebahat et al., 2008 ; Oral et al., 2008).

From table (4) it could be concluded that, the lowest aerobic plate count (APC) was recorded at zero time of first treatment and at 3rd day of storage of 6th and 7th treatments (*Pelargonium graveolans* and *Salvia fruticosa*) .On the other hand APC increase during refrigerated storage by using 5th treatment (*Ocimum basillicum*).The psychrophilic bacterial count tend to be low for 3rd , 4th ,6th and 7th treatments by prolonged refrigeration , while the count increased in case of 5th treatment .

Staphylococcus *aureus* count was lowest for 4th , 6th and 7th treatments at the end of cold storage (9th day) . Enterobacteriaceae counts was lowest for 4th , 6th and 7th treatments while increased in 5th treatment .From the observed results it was found that the highest growth of mould and yeast occurred at 3rd treatment. Many studies have been conducted on the antimicrobial properties of herbs , spices and their derivatives such as essential oils and extracts. For instance, El Akrem et al. (2008) recorded that the antibacterial activities of essential oils in minced beef meat stored at 4-7 ^{0}c for 15 days were clearly evident.

Table (4): Bacterial growth in meat slices during refrigerated storage.

Treatments / Storage period	APC (cfu/g)	Psychrophilic Bacteria counts (cfu/g)	*Staph. aureus* (cfu/g)	Enterobacteriaceae counts (cfu/g)	mould & yeast counts (cfu/g)
zero time	2.1×10^2	8.0×10^2	2.0×10^2	3.5×10^2	1.1×10^2
1st treatment					
3 day	7.5×10^3	3.0×10^3	3.5×10^2	6.2×10^3	4.3×10^2
6 day	2.1×10^4	2.0×10^4	5.1×10^3	7.3×10^3	1.5×10^3
9 day	2.2×10^5	3.1×10^5	6.2×10^3	2.1×10^4	3.4×10^3
2nd treatment					
3 day	3.1×10^2	1.7×10^2	1.5×10^2	3.6×10^2	2.3×10^2
6 day	3.1×10^2	2.0×10^2	1.6×10^2	3.8×10^2	1.8×10^2
9 day	3.1×10^2	2.0×10^2	1.4×10^2	4.0×10^2	1.8×10^2
3rd treatment					
3 day	4.0×10^2	$<10^2$	2.0×10^2	1.9×10^2	2.5×10^2
6 day	3.2×10^2	$<10^2$	1.8×10^2	1.5×10^2	3.1×10^3
9 day	3.2×10^2	$<10^2$	2.0×10^2	1.5×10^2	2.4×10^3
4th treatment					
3 day	3.4×10^2	$<10^2$	$<10^2$	$<10^2$	5.1×10^2
6 day	3.5×10^2	$<10^2$	$<10^2$	$<10^2$	5.0×10^2
9 day	5.0×10^2	$<10^2$	$<10^2$	$<10^2$	5.0×10^2
5th treatment					
3 day	2.0×10^3	2.5×10^3	9.0×10^2	8.2×10^2	3.0×10^2
6 day	2.3×10^3	2.4×10^3	1.5×10^2	1.0×10^3	3.1×10^2
9 day	2.3×10^3	2.8×10^3	1.6×10^2	1.0×10^3	3.1×10^2
6th treatment					
3 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	1.3×10^2
6 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	1.2×10^2
9 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	1.2×10^2
7th treatment					
3 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	5.1×10^2
6 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	2.5×10^2
9 day	$<10^2$	$<10^2$	$<10^2$	$<10^2$	3.0×10^2

Recently, there has been much research into the health benefits conferred by the essential oils found in basil. Scientific studies have established that compounds in basil oil have potent antioxidant, anti-

cancer, antiviral, and anti-microbial properties (Bozin et al., 2006 ; Benedec et al., 2007). In addition, basil has been shown to decrease the occurrence of platelet aggregation and experimental thrombus in mice. It is traditionally used for supplementary treatment of stress, asthma and diabetes in India. The Chinese also use fresh or dried basils in soups and other foods. In Taiwan, people add fresh basil leaves to thick soups They also eat fried chicken with deep-fried basil leaves. Basil is commonly steeped in cream (Suppakul et al., 2003) .

Salvia *fruticosa* (Greek sage) family Lamiaceae is a perennial herb or sub-shrub native to the eastern Mediterranean, southern Italy, Israel, the Canary Islands, and North Africa. It has a long tradition in Greece-valued for its beauty, medicinal value, and culinary use, along with its sweet nectar and pollen. In Western cooking, it is used for flavoring fatty meats, cheeses and some drinks. In the United States, Britain sage is used with onion for poultry or pork stuffing and also in sauces. In French cuisine sage is used for cooking white meat and in vegetable soups. Germans often use it in sausage dishes, and sage forms the dominant flavoring in the English Lincolnshire sausage. Sage is also common in Italian cooking. Caution is indicated when used in conjunction with central nervous system stimulants or depressants(En.Wikipedia) .

There are ± 220 species within the genus *Pelargonium*, *Pelargonium graveolens* is a species in the *Pelargonium* genus, which is indigenous to various parts of southern Africa, and in particular South Africa. It is often called geranium This specific species has great importance in the perfume industry. It is cultivated on a large scale and its foliage is distilled for its scent. *P. graveolens* cultivars have a wide variety of smells, including rose, citrus, mint, coconut and nutmeg, as well as various fruits. Anti Bacterial & Anti Microbial: This property does not let bacteria or microbes develop on wounds and otherwise and keeps you safe from infections. The leaves are aromatic, balsamic, haemostatic and tonic. Recent research has revealed a substance in the plant that has an antibiotic action.

Myrtus (myrtle) leaves are aromatic, balsamic, haemostatic and tonic Recent research has revealed a substance in the plant that has an antibiotic action (Aidi Wannes et al., 2010). *Pistacia lentiscus* (Mastic) A 1985 study by the University of Thessaloniki and by the Meikai University discovered that mastic can reduce bacterial plaque in the mouth by 41.5 percent. A 1998 study by the University of Athens found that mastic oil has antibacterial and anti-fungal properties Alem et al. (2008). Another 1998 University of Nottingham study, published in the *New England Journal of Medicine*, claims that mastic can heal peptic ulcers by killing *Helicobacter pylori*, which causes peptic ulcers, gastritis, and duodenitis. Some *in vivo* studies have shown that mastic gum has no effect on *Helicobacter pylori* when taken for short periods of time **Al-Said et al. (1986) and Al-Saimary et al. (2002)** . However a recent and more extensive study showed that mastic gum reduced *Helicobacter pylori* populations after an insoluble and sticky polymer (poly-β-myrcene) constituent of mastic gum was removed and taken for a longer period of time. Further analysis showed the acid fraction was the most active antibacterial extract, and the most active pure compound was isomastic adienolic acid (Afef et al., 2006).

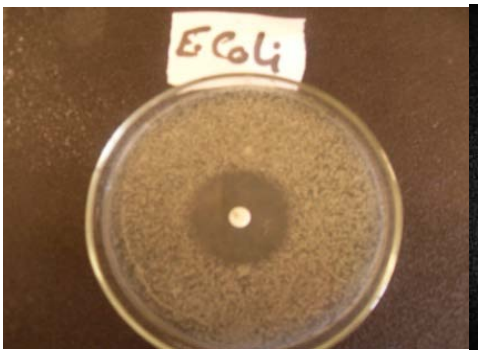
Table (5): Sensory evaluation of meat slices of different treatments at 9th day of refrigeration storage of meat.

Treatments	Evaluation of the sensory properties				
	Taste	texture	odour	colour	Overall acceptability
1st treatment	Appearance signs of decomposition (slimness, abnormal odour, proteolysis)				
2nd treatment	6.50	6.67	6.67	8.25	7.02
3rd treatment	8.92	8.75	6.75	8.42	8.21
4th treatment	4.42	4.17	6.08	8.25	5.73
5th treatment	6.58	6.58	6.25	8.25	6.92
6th treatment	8.50	8.92	9.92	8.17	8.88
7th treatment	8.83	8.75	6.50	8.67	8.19

From data recorded in table (5) it could be observed that sensory properties of beef slices (taste, odour, texture and overall acceptability) were significantly affected by constituents of each herbal. Hence, *Arbutus pavarii* ,*Pelargonium graveolans* and *Salvia fruticosa* had the best taste ,odour ,texture and colour. Treatment by *Pistacia lenticulus* came in the third order after *Myrtus communes & Ocimum basillicum*. Furthermore, control sample had signs of decomposition (slimness, abnormal odour, proteolysis).

Pelargonium graveolans herb

Salvia fruticosa herb



The inhibitory effects of standard antibiotic

The inhibitory effects of the alcohol extracts obtained from studied plant

CONCLUSION

It can be concluded that, the powdered plants or their extracts possess antimicrobial activity, and therefore, they can be used in biotechnological fields as natural preservative ingredients in food which prolong their shelf life and improve their Organoleptic characters.

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