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Antibacterial Activity of Antibiotics, Herbal Extracts and Commercially Available Essential Oils and Studies Leading to Shelf-life Extension of Chicken Meat

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ABSTRACT

This study was conducted to find out the antibacterial effects of different components including standard antibiotics, herbal extracts and commercially available essential oils by applying different bacteria and to evaluate their effect on chicken meat as a preservative. For this purpose, 8 different types of antibiotic discs of 0.5 mm were applied on the bacteria isolated from chicken obtained taken from the Laboratory of Microbiology, Institute of Microbiology and Biotechnology, The University of Lahore. Similarly herbal extracts and commercially available essential oils were also applied on the same bacteria by disc diffusion method. Then finally clove extract and clove oil were mixed in the concentrations of 5% and 10% v/w in the minced chicken meat to observe their preservative effects. The bacteria were not significantly seen to resist the action of antibiotics applied while ethanolic clove (*Syzygium aromaticum*) extract, clove oil and cinnamon (*Cinnamomum zeylanicum*) oil showed strong antibacterial potential. It was also found that clove extract and clove oil extend the shelf-life of the chicken meat samples upto 18 days instead of 12 days of the control samples kept at 4°C. It was concluded from the study that clove and cinnamon possessed strong antibacterial potential and clove had preservative effects.

So, clove can be further studied to be employed as a potential meat preservative

Keywords— antibacterial activity, herbal extracts, commercial essential oils, meat preservation, shelf-life extension

INTRODUCTION

Poultry industry is growing fast in Pakistan and production of broiler meat in 2006-07 has been increased upto 480 tons as compared to 463 tons in the year 2005-06 [1].

There is a high pressure for the selection of antibiotics in poultry because faecal flora of the poultry contains high proportion of resistant bacteria [2]. When the

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poultry are scarified, the resistant strains of their gut soil their carcasses and consequently multi-resistant bacteria contaminate the poultry meat. So, there is a probability that all known antibiotics are resistant to most of the pathogenic bacteria which threaten human health [3].

For primary health care, World Health Organization (WHO) noted that a considerable part of the world's population relies on traditional medicine [4]. The

non-antibiotic substances like essential oils [5] and their chemical constituents [6] have shown good potential against drug resistant pathogens [7,8].

There is a major concern of poultry industry to extend the shelf-life of the poultry products which depend on several factors like initial bacterial load, gaseous environment around the product and storage temperature [9]. In processed poultry, lipid oxidation is another factor which causes deterioration of meat and results in the development of rancid off flavors and undesired odors. In order to reduce the incidence of microbial contamination in the poultry meat products various methods are used including asepsis, irradiation, use of heat and low temperature, chilling, freezing, and preservation with adipic acid or succinic acid etc. at pH 2.5 [10].

WHO has desired to reduce the consumption of salt in order to reduce the incidence of cardiovascular diseases [11]. A Research has been conducted on the development of safe food which contains natural ingredients as antibacterial additives like essential oils instead of using salt in processed food, showing no hazardous effect on human health and ensured food preservation [12,13].

The shelf-life of the refrigerated meat can be extended by the addition of synthetic additives to the poultry meat. In recent years, the demand of the consumer is to use the natural ingredients as the alternative preservatives in food because the safety of the synthetic additives has been questioned [14]. Therefore, to ensure protection from spoilage organisms, use of natural food preservatives is one of the modern trends to achieve this goal [15].

Clove (*Syzygium aromaticum*) belonging to the family Mirtaceae is a plant indigenous to china and widely cultivated in Indonesia, Spice Island, Pemba and Zanzibar is used in seasoning of food [16,17] and has antimicrobial effects against many bacteria and some fungi as well [16] due to its constituents like eugenol and oleic acid [18]. Eugenol and eugenyl acetate in clove also have antioxidant properties

[19]. Clove and cinnamon are among the ten most inhibitory oils of the spices [16] and are most inhibitory to the growth of microorganisms [20].

After slaughtering, even under refrigeration temperature the deterioration of raw chicken meat tends to occur within 4-10 days. The deterioration time depends upon the environment of carcasses at the time of slaughter, type of packaging and condition of the storage [21]. There would be a need to reduce the initial bacterial load in the product to improve the shelf- life of the chicken meat [22]. The object of this study is to investigate the antibacterial potential of herbal extracts and essential oils in the preservation of chicken meat.

METHODOLOGY

Antibiotic Susceptibility Test (AST): The effects of eight different types of antibiotics were checked on the species of bacteria followed by the method of Soomro et al. [23]. The preserved strains were streaked on Mueller Hinton Agar (HIMEDIA) plates by sterile swabbing. Plates were kept for 5 minutes at room temperature and then diffusion disks with antimicrobial drugs were placed on the plates and incubated for 24 hours at 37°C. The antibiotic disks (OXOID) used were Amoxicillin (10µg), Imipenem (10µg), Ciprofloxacin (5µg), Erythromycin (15µg), Ampicillin (10µg), Penicillin (10µg), Vancomycin (30µg) and Gentamycin (10µg). Results were interpreted by measuring zones of inhibition in millimeters (ZOI in mm).

Collection of Plants and Condiments: Fresh required parts of different plants and condiments were collected from nursery. After collection, the leaves were washed with running tap water, dried at room temperature for 3-4 days, grounded into fine powder in an electrical grinder and finally stored in plastic bottles at room temperature. The condiments were grounded into fine powder with the help of mortar and pestle and stored in plastic bottles [24].

Method of Plant Extraction: Extraction of plants

was done by following the method of Gull et al. [24]. For extraction of leaves and condiments, a specific amount of each drug was weighed by digital weighing machine and placed in conical flasks. A 100 ml of quantity of ethanol was added in to each conical flask, shake well at 120 rpm and kept for 48-72 hours. The crude extract was centrifuged at 3000 rpm for 10 minutes at 25°C. The solution was then shifted to rotary evaporator for the evaporation of the ethanol at 50°C. Stock solutions of powdered extracts were made by dissolving in the solution of dimethyl sulfoxide (DMSO) in the falcon tubes labelled with the name of plant extract and preserved at 4°C in the refrigerator for later use.

Antibacterial Activity of Plant Extracts: Ethanolic extracts were prepared to check the in vitro antibacterial activity of the plants and condiments against the bacteria isolated from chicken meat samples. The antimicrobial assay was performed by disc diffusion method as described by Kirby-Bauer [25]. The bacteria were inoculated in Muller Hinton agar (HIMEDIA) by spread plate method. Small filter paper discs having a diameter of 6 mm were sterilized in a Hot air oven at 18°C for 30 minutes, soaked in 15µl of plant extracts and placed over MHA plates seeded with bacterial culture, with the help of sterilized syringe needle. The discs were pressed firmly to ensure their complete contact with the underneath media. The plates were then kept in an incubator aerobically at 37°C for 24 hours. Each plate was observed for the ZOI in mm. The diameter of the ZOI was measured including the diameter of the disc as described by Upadhyay et al. [26].

Antibacterial Activity of Essential Oils: Antibacterial activity as an in vitro study of 6 different types of commercially available essential oils was evaluated by the method of Chaudhari et al. [27] included these clove oil, cinnamon oil, coriander oil, almond oil, cardamom oil and olive oil and were checked against the 9 bacteria by disc diffusion method using the method of Kirby-Bauer [25]. Small filter paper discs of 6 mm sterilized by Hot air oven, and soaked in

15µl of oil were placed on MHA plates seeded with bacterial cultures. The plates were then kept in an incubator for 24 hours and after that they were observed for the ZOI in mm [26].

Application of Clove Extract and Clove Essential Oil for the Extension of Shelf Life: Minced chicken meat was purchased and transported to the laboratory within 30 minutes and held at 1°C for 1-2 h. A total of 6 samples, each of 100 g minced chicken meat were taken, packed in a sterile beaker and wrapped with parafilm were stored them. Two samples were considered as control, one stored at the freezing temperature and the other in the refrigerator at 4°C, and were labelled O and A, respectively. Clove extract was incorporated in the chicken meat samples [28] as 5% and 10% (v/w) which were labelled as B and C, respectively. Similarly 5% and 10% (v/w) of commercially available clove oil [27] was incorporated in the chicken meat samples and labelled them as D and E, respectively. All the samples were observed after 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days to check any change in their physical properties like colour, odour, pH and microbiological evaluation by making dilutions of each sample and swab on the nutrient agar as triplicate following the method of Akoachere et al. [29] with some modifications. Microbial alterations such as microbial load was observed in each sample and finally noted the increase in shelf- life of the chicken meat treated with clove extract and clove oil and compared them with the shelf- life of the control samples.

RESULTS

Antibiotic Susceptibility Test (AST): Eight different types of antibiotics were applied on the isolated strains of bacteria to check the sensitivity or resistance that is shown in the Table I. The data given in this table showed that Imipenem and Gentamicin had strongest activity against all the strains. Erythromycin also had strong action except against *S. epidermidis*.

Concentration of Herbal Extracts: Nine herbal extracts were prepared in ethanol to check their

antimicrobial susceptibility in concentrations mentioned in the Table II.

All the extracts had different concentrations depending upon the amount of drug and ethanol. **Antibacterial Activity of Herbal Extracts**

All the 9 herbal extracts including clove, cinnamon, oregano, rosemary, black pepper, papaya, cumin, turmeric and mint were applied with Imipenem used as a positive control and DMSO used as a negative control against the isolated bacteria to check their antibacterial activity by disc diffusion method and measured their ZOI in mm. Activity of all extracts are shown in the table III below.

It was concluded that extracts of clove were more effective as antibacterial agents against the bacteria while oregano, rosemary and black pepper had lesser activity as compared to clove. However, cinnamon, papaya, cumin, turmeric and mint had shown no activity against the isolates. Imipenem showed strong antibacterial property while DMSO itself had no activity against the bacteria (used as a positive and negative control respectively).

Antibacterial Activity of Essential Oils: Antibacterial activities of six different essential oils including clove oil, cinnamon oil, coriander oil, almond oil, cardamom oil and olive oil with Imipenem used as a positive control were tested against the bacteria and measure their ZOI in mm which are shown in the table IV below.

It was found that Clove and Cinnamon oils had antibacterial susceptibilities against 9 isolated bacteria, similar to Imipenem (positive control) while other oils showed no such activity.

The observations in the Table V above showed that the sample mixed with 5% clove extract changed its color from dark brown to fawn colour after 14 days but its pH and odour remained unchanged upto 18 days. Application of 10% clove extract had more positive effect as the change in color occurred after

Table 1: ANTIBACTERIAL ACTIVITY OF THE DIFFERENT ANTIBIOTICS

IPM = Imipenem, AMC = Amoxicillin, CIP = Ciprofloxacin, E = Erythromycin, AM = Ampicillin, P = Penicillin, VA = Vancomycin, GM = Gentamicin

Sr. No.	Bacterial Isolates	Antibiotics										
		IPM (10ug)	AMC (10ug)	CIP (5ug)	E (15ug)	AM (10ug)	P (10ug)	VA (30ug)	GM (10ug)	Susceptible %	Intermediate %	Resistant %
1	<i>S.aureus</i>	S	S	R	I	S	S	R	S	62.5	12.5	25
2	<i>S.epidermidis</i>	S	I	S	R	R	R	S	S	50	12.5	37.5
3	<i>S.enterica</i>	S	I	I	I	I	R	R	S	25	50	25
4	<i>S.sonnei</i>	S	R	R	R	R	R	R	S	25	0	75
5	<i>Paeruginosa</i>	S	R	R	R	R	R	R	S	25	0	75
6	<i>E.coli</i>	S	R	R	R	S	S	S	S	62.5	0	37.5
7	<i>B.cereus</i>	S	R	I	R	R	R	I	S	12.5	25	62.5
8	<i>E.facalis</i>	S	S	S	S	S	S	S	S	100	0	0
9	<i>M.luteus</i>	S	I	S	I	S	S	S	S	75	25	0
Susceptible %		100	22.2	33.3	11.1	44.4	44.4	44.4	100			
Intermediate %		0	33.3	22.2	33.3	11.1	0	11.1	0			
Resistant %		0	44.4	44.4	55.5	44.4	55.5	44.4	0			

Table 2: CONCENTRATIONS OF THE EXTRACTS FORMED

Sr. No.	Name of Plant Extracts	Quantity of Powdered Plant (g)	Quantity of Ethanol (ml)	concentration of final Extract
1	Clove	20	100	2
2	Cinnamon	10	60	1.6
3	Oregano	12	80	1.5
4	Rosemary	10	80	1.5
5	black Paper	10	90	1.1
6	Papaya	10	60	1.6
7	Cumin	10	70	1.4
8	Tumeric	16	100	1.6
9	Mint	15	90	1.6

Table 3: ANTIBACTERIAL ACTIVITY OF HERBAL EXTRACTS AS ZOIN MM AGAINST BACTERIA

CL = clove, CM = cinnamon, OG = rosemary, BP = black paper, PP = papaya, CU = cumin, TM = turmeric, MT = mint, -- = 0mm(resistant), + = 7-12mm(resistant), ++ = 12-18mm(intermediate), +++ = >18(susceptible)

Sr. No.	Bacterial Isolates	IPM (control)	CL	CM	OG	RM	BP	PP	CU	TM	MT
1	<i>S.aureus</i>	+++	+++	-	+	-	+	-	-	-	-
2	<i>S.epidermidis</i>	+++	+++	-	-	+++	-	-	-	-	-
3	<i>S.enterica</i>	+++	+++	-	+	+	-	-	-	-	-
4	<i>S.sonnei</i>	+++	+++	-	+++	+	+	-	-	-	-
5	<i>Paeruginosa</i>	+++	+++	-	-	-	-	-	-	-	-
6	<i>E.coli</i>	+++	+++	-	+	-	+	-	-	-	-
7	<i>B.cereus</i>	+++	+++	-	-	+++	-	-	-	-	-
8	<i>E.facalis</i>	+++	+++	-	+++	+++	+++	-	-	-	-
9	<i>M.luteus</i>	+++	+++	-	+++	-	-	-	-	-	-
Susceptible%		100	100	0	22.2	44.4	11.1	0	0	0	0
+++>= 18mm											
Resistant% (0mm) & =(12mm)		0	0	100	77.8	55.6	88.9	100	100	100	100

Table 4: ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AS ZOI IN MM AGAINST BACTERIA

CL = clove, CM = cinnamon, CR = coriander, AL = almond, CD = cardamom, OL = olive, -- = 0mm(resistant), + = 7.12mm(resistant), ++ = 12.18mm(intermediate), +++ = >18(susceptible)

Sr. No.	Bacterial Isolates	Essential Oils						
		IPM (control)	CL	CM	CR	AL	CD	OL
1	<i>S.aureus</i>	+++	+++	-	-	-	-	-
2	<i>S.epidermidis</i>	+++	+++	+++	-	-	-	-
3	<i>S.enterica</i>	+++	+++	+++	-	-	-	-
4	<i>S.sonnei</i>	+++	+++	+++	-	-	-	-
5	<i>P.aeruginosa</i>	+++	+++	+++	-	-	-	-
6	<i>E.coli</i>	+++	+++	+++	-	-	-	-
7	<i>B.cereus</i>	+++	+++	+++	-	-	-	-
8	<i>E.facalis</i>	+++	+++	+++	-	-	-	-
9	<i>M.luteus</i>	+++	+++	+++	-	-	-	-
Susceptible%		100	100	88.9	0	0	0	0
+++=> 18mm								
Resistant%		0	0	11.1	100	100	100	100
-(0mm) & =(12mm)								

Figure 1: COLOR COMPARISON OF CHICKEN IN PRESERVED MEAT



Table 4: EFFECT OF CLOVE EXTRACT AND CLOVE OIL FOR SHELF LIFE EXTENSION

Sample + Preservative	Days	pH	Color	Odor	Log10 CFU/g
Control (A)	0	6	Pinkish	Normal	8.43
	1	6	Pinkish	Normal	8.44
	2	6	Pinkish	Normal	8.41
	4	6	Pinkish	Normal	8.4
	6	6	Pinkish	Normal	8.43
	8	6	Pinkish	Normal	7.45
	10	6	Pinkish	Normal	7.46
	12	6	Pinkish	Normal	7.47
	14	7	Dull Pink/fainted	Slightly putrid	7.46
	16	7	Dull Pink/greenish	Putrid	7.47
5% Clove extract (B)	0	5	Dark Brown	Clover	3.69
	1	5	Dark Brown	Clover	3.54
	2	5	Dark Brown	Clover	3.5
	4	5	Dark Brown	Clover	3.6
	6	5	Dark Brown	Clover	3.66
	8	5	Dark Brown	Clover	3.71
	10	5	Dark Brown	Clover	3.79
	12	5	Dark Brown	Clover	3.63
	14	5	Dark Brown	Clover	3.61
	16	5	Fawn	Clover	3.67
5% Clove oil (C)	0	5	Light Brown	Clover	2.54
	1	5	Light Brown	Clover	2.65
	2	5	Light Brown	Clover	2.49

Sample + Preservative	Days	pH	Color	Odor	Log10 CFU/g
10% Clove extract (C)	4	5	Light Brown	Clover	2.56
	6	5	Light Brown	Clover	2.63
	8	5	Light Brown	Clover	2.65
	10	5	Light Brown	Clover	2.74
	12	5	Light Brown	Clover	2.78
	14	5	Light Brown	Clover	2.53
	16	5	Light Brown	Clover	2.56
	18	5	Pale Yellowish	Clover	6.46
5% Clove oil (D)	0	5	Fawn	Clover	3.47
	1	5	Fawn	Clover	3.5
	2	5	Fawn	Clover	3.53
	4	5	Fawn	Clover	3.62
	6	5	Fawn	Clover	3.5
	8	5	Fawn	Clover	3.54
	10	5	Fawn	Clover	3.64
	12	5	Fawn	Clover	3.7
	14	5	Fawn	Clover	3.63
	16	6	Pale Yellowish	Clover	3.66
18	6	Pale Yellowish	Clover	6.47	
10% Clove oil (E)	0	5	Fawn	Clover	2.65
	1	5	Fawn	Clover	2.51
	2	5	Fawn	Clover	2.57
	4	5	Light Yellow	Clover	2.61
	6	5	Light Yellow	Clover	2.55
	8	5	Light Yellow	Clover	2.71
	10	5	Light Yellow	Clover	2.54
	12	5	Light Yellow	Clover	2.66
	14	5	Light Yellow	Clover	2.49
	16	5	Light Yellow	Clover	2.72
18	5	Pale Yellowish	Clover	6.46	

16 days from light brown to pale yellowish. Similarly sample with 5% clove oil changed its colour after 14 days from fawn to pale yellowish and change in pH from 5 to 6 after 14 days but odour remained same. In the last sample, mixed with 10% clove oil changed its colour after 2 days from fawn to light yellow and then after 16 days from light yellow to pale yellowish but its pH and odour remained unchanged. The microbial load of all the four test samples reached maximum on 18th day. The controlled group (A) had maximum load from the day first while its pH on 0 day to 12th day was 6

and after that changed to 7, its colour was pinkish for first 12 days then changed

to dull pink and after 14 days its colour changed to somewhat greenish due to fungal growth, its odour changed to foul smell after 14 days. Similarly the controlled group (O) which was placed in the freezer also had fungal growth after 14 days which caused change in their colour to greenish and odour to foul smelling. There was no fungal growth in four test samples upto the 18th days.

DISCUSSION

In the present study 8 different types of antibiotics were tested against 9 bacterial species isolated from the meat samples and observed that all 9 bacterial species showed 100% sensitivity to both Imipenem (IPM) and Gentamicin (GM), followed by 44.4% sensitive to Vancomycin (VA), 33.3% sensitive to Ciprofloxacin (CIP), 44.4% sensitive to Ampicillin (AM), 44.4% sensitive to Penicillin (P), 22.2% sensitive to Amoxicillin (AMC) and 11.1% sensitive to Erythromycin (E).

Antibiotic susceptibility testing showed that *Enterococcus faecalis* was 100 sensitive to all antibiotics, followed by *Staphylococcus aureus* 62.5% sensitive, *Escherichia coli* 62.5% sensitive, *Staphylococcus epidermidis* 50% sensitive, *Salmonella enterica* 25%, *Shigella sonnei* 25% and *Pseudomonas aeruginosa* 25% sensitive. The Danish Integrated Antimicrobial Research Monitoring and Research Programme reported that resistance level of *Enterococcus faecalis* was 45% for broilers which is contrary to the findings of the present study. Other contrary findings reported in Nigeria by Majolagbe et al. [30] showed that resistance was 20% for Erythromycin, 70% for Gentamicin and 10% for Ciprofloxacin. *Pseudomonas aeruginosa* isolated from poultry showed that it was completely resistant to Amoxicillin and Ciprofloxacin while 3.3% sensitive to both Gentamicin and Imipenem as reported in Egypt by Gehan et al. [31].

Floristean et al. [32] reported that *Bacillus cereus* was resistant to Penicillin and Amoxicillin while sensitive to Gentamicin. Similarly Zahraei and Farashi (2006) in Iran reported that *Escherichia coli* isolated from broiler chicken showed resistance of 97% with Erythromycin, 67% with Ciprofloxacin that is in agreement with the present study. Essential oils or phenolic volatile compounds are the main active constituents in most herbs e.g., carvacrol in Rosemary (*Rosmarinus officinalis*) and Oregano (*Origanum vulgare*); menthol in Peppermint (*Mentha piperita*) and eugenol in Clove (*Syzygium*

aromaticum) [33].

Antibacterial activity of 9 herbal extracts against 9 isolated bacterial species showed that extract of clove had 100% sensitive effects followed by Rosemary (*Rosmarinus officinalis*) with 44.4% sensitivity, Oregano (*Origanum vulgare*) with 22.2% sensitivity and Black pepper (*Piper nigrum*) with 11.1% sensitivity while Cinnamon (*Cinnamomum zeylanicum*), Papaya (*Carica papaya*), Cumin (*Cuminum cyminum*), Turmeric (*Curcuma longa*) and Mint (*Mentha piperita*) showed no antibacterial activity against the isolated bacterial species. Oregano found Sensitive against *Shigella sonnei* and *Enterococcus faecalis*; Rosemary sensitive against *Staphylococcus epidermidis*, *Bacillus cereus*, *Enterococcus faecalis* and *Micrococcus luteus* and Black pepper found sensitive against *Enterococcus faecalis*.

Badhe et al. [34] reported that a 100% aqueous extract of clove was sensitive against *Staphylococcus aureus* and *Bacillus cereus* while resistant against *Salmonella* and *Escherichia coli*. They also found that essential oil of clove was better than aqueous extract in bringing down the microbial count. Similar findings on bacteriostatic property of aqueous clove extract and oil against *Staphylococcus aureus* were reported by Nzeako et al. [35]. Kaushik et al. [36] reported that Ethanolic extract of *Elettaria cardamomum* showed antibacterial effects against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus* which is contrary to the findings of the present study.

Antibacterial activity of 6 essential oils against 9 isolated bacterial species showed that clove oil (*Syzygium aromaticum*) was found as 100% sensitive and cinnamon oil (*Cinnamomum zeylanicum*) was found as 88.9% sensitive while coriander oil (*Coriandrum sativum*), almond oil (*Prunus amygdalus*), cardamom oil (*Elettaria cardamomum*) and olive oil (*Olea europaea*) showed no antibacterial activity against isolated bacterial species.

Ethanol is usually added to the essential oils as solvent to enhance the aromatic and volatility [37]. In order to avoid the possible antimicrobial effect of the solvent, commercially available, non-diluted by any solvent, not chemically altered essential oils were used in this study.

Prabuseenivasan et al. [4] reported that clove oil and cinnamon oil have strong activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Sanla-Ead [38] reported that clove oil and cinnamon oil showed antimicrobial activity against *Bacillus cereus*, *Enterococcus faecalis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* except *Pseudomonas aeruginosa* which is in agreement with the present study.

Contrary to the findings of the present study, Nirosha and Mangalanayaki in India [39] reported that Ethanolic extracts of *Carica papaya* showed antibacterial activities against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus* and *Bacillus cereus*; Baskaran et al. in India [40] also reported the same results and El-Kady et al. [41] reported the antibacterial effects of menthol against Gram-positive and Gram-negative bacteria.

Contrary to the findings of the present study olive oil and almond oil showed susceptibility against *Escherichia coli*, *Micrococcus luteus*, *staphylococcus aureus* and *Bacillus cereus* as reported by Upadhyay et al. [26] and Prabuseenivasan et al. [4] in different bacterial strains like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* species. Some plant essential oils have shown growth inhibitory effects against *Escherichia coli* [42], *Bacillus* species [43], *Staphylococcus aureus* [44,45] and *Salmonella enteritidis* [46].

Application of clove extract and clove oil in the minced meat for the extension of its shelf- life showed that microbiologically clove extract (5% and 10%) and clove oil (5% and 10%) increased the shelf- life of the meat upto the 18 days when stored in

refrigeration at 4 °C. While the sensory characteristics of the meat like pH and odour remained the same throughout the 18 days and the color of the meat changed on 16th day with 5% extract and oil and on 18th day with 10% extract and oil which lead to the conclusion that clove extract and clove oil increased the shelf- life upto 6 days more when compared to the control group which became putrid after 12th day.

Similar positive effects with Oregano (*Origanum Vulgare*) essential oil were found in the study of Skandamis and Nychas [47] and with Sage (*Salvia Officinalis*) reported by Ahmed Ismail in Egypt [28].

CONCLUSION

It is concluded from the study that bacteria isolated from the chicken meat have higher rate of sensitivity than resistance against 8 types of antibiotics. Moreover, the antibacterial effect of clove extract is highest (100%) as compared to 8 others used in experiment and clove oil and cinnamon oil both have 100% antibacterial potential compared to all the 6 essential oils used in this study. The application of clove extract and clove essential oil in the minced chicken meat extend its shelf- life 6 days more at 4 °C due to their antibacterial and anti-oxidant properties.

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