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In Vitro ANTIBACTERIAL EFFECT OF SOME NATURAL PLANT EXTRACTS AGAINST *Escherichia coli O157:H7, Escherichia coli NRRL* AND *Salmonella sp.*

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ABSTRACT

Six extracts-based natural antimicrobials were evaluated for their ability to inhibit the growth of Escherichia coli O157:H7, Escherichia coli NRRL3008 and Salmonella sp. Degree of inhibition was determined by the disk-diffusion assay. Results revealed the inhibitory effect of extracts (Without dilution and the same volume). Garlic extract had the strongest effect against E.coli NRRL3008 (24.00mm), Escherichia coli O157:H7 (22.00mm) and Salmonella sp. (18.00mm). Clove extract had stronger action against E. coli O157:H7 (35.00mm), E. coli NRRL.3008 (27.00mm) than Salmonella sp. (23.00mm). The most powerful effect of ginger extract was against E. coli O157:H7 (30.00mm), E.coli NRRL.3008 (26.00mm) followed by Salmonella sp. (22.00mm). E. coli O157:H7 (28.00mm) and E. coli NRRL.3008 (23.00mm) were strongly influenced by cardamom extract. Cinnamon extracts effective action against E. coli O157:H7 (31.00mm) followed by E. coli NRRL3008 (28.00mm) than Salmonella sp. (23.00mm). Rosemary extract strongly affected against E. coli NRRL3008 (25.00mm), E. coli O157:H7 (23.00mm) and Salmonella sp. (22.00mm). Extract mixture (clove, cinnamon and ginger) at level of 0.6% had stronger effect against E. coli O157:H7 (36.00mm), followed by E. coli NRRL3008 (34.00mm). The effectiveness of the ethanolic extracts against the growth of E. coli O157:H7 during 7 days of storage period at 37ºC followed the order: ginger > cinnamon > rosemary > cardamom > garlic> clove. It can be concluded that natural extracts of clove, cardamom, cinnamon, rosemary, ginger and garlic differ in their inhibitory activity against Escherichia coli O157:H7, Escherichia coli NRRL and Salmonella sp. so, these natural extract could be considered *as natural food preservatives against* pathogenic toxic bacteria and generally recognized as safe.

Key words: *In Vitro*, natural plant extracts against, *Escherichia coli O157:H7*, *Escherichia coli NRRL*, *Salmonella sp*.

INTRODUCTION

Escherichia coli have been identified as a food borne pathogen since 1982 (Doyle, 1991). *Escherichia coli* O157:H7 generally, survives well in foods at refrigeration temperatures. Minimum water activity for growth is 0.95 (ICMSF, 1996). Meat probably becomes contaminated at the time of slaughter and grinding may compound the problem by introducing the pathogen into the interior of the meat, where it is more likely to survive cooking (Mead and Griffin, 1998).

The relatively recent emergence of *E. coli* O157:H7 as a food borne pathogen has a significant impact on the food industry (McClure, 2000).

Ground beef is the most susceptible form of meat to microbial contamination during processing and handling and it is the most susceptible to discoloration (Nam and Ahn, 2003).

Natural extracts are part of a group of substances considered as "tolerated" but not admitted as additives in a strictly legal way. Vegetal extracts would be included in the additive group classified as "aromatic and flavoring substances", in which are included all the natural products and relevant synthetic products and that can be used on all animal species, without any restriction regarding age of product dosage . Because these products are well accepted by consumers, they are a promising alternative to growth promoter antibiotics, and the search of new substances represents an important research area in the field of food additives. Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century. Naturally, occurring microbial inhibitors have been recovered from a wide variety of foods including onions, garlic, fruits, vegetables, cereals and spices, many of these antimicrobials contribute to the foodstuffs natural resistance to deteriorations. The flavour components consist of such compounds as alcohols, aldehydes, esters, terpens, phenols, organic acids and others, some of which have not yet been identified (Merih, and Buket, 2009).

The aim of this research was to find out possible antimicrobial effect of some plants extracts traditionally used as food spices on the growth of some pathogenic bacteria *E. coli 0157:H7*, *Escherichia coli NRRL* and *Salmonella sp. in vitro.*

MATERIALS AND METHODS

Materials:

Plants: Clove (*Syzygium aromaticum*), Cardamom (*Elettaria cardamom*), Cinnamon (*Cinnamomum zeylanicum J*), Rosemary (*Rosmarinus officinalis*), Garlic (*Allium sativum*) and Ginger*(Zingiben officinale*) were purchased from local market at Mansoura City, Dakahlia Governorate, Egypt, cleaned, air-dried at electric oven at 50 ºC for 12 hours, according to Mohdy (2001) and ground in the blinder. The powder of each sample was kept in polyethylene bags and preserved in deep freezer (- 18 ºC) until use.

Solvent: Ethanol 95% (L14100) was bought from Algomhoria company Mansoura city- Dakahlia Governorate, Egypt.

Culture media: Nutrient Broth (M002) and MacConkey Agar (w/0.15%Bilts, CV and NaCI M081) SS agar (Salmonella Shigella agar) B31were obtained from Algamhoria Company Cairo.

Experimental microorganisms' strains:-

Escherichia coli O157:H7 ATCC 51659, M, *Escherichia coli* ATCC8739-NRRL3008and *Salmonella sp.* strains were obtained from Cairo Microbiological Resources Center (MIRCENT), Faculty of Agriculture, Ain Shams University, Egypt.

Methods:

***Ethanolic extraction*:**

The extraction procedure for ethanolic extraction was carried out according to El-Badrawy (1996) who modified by (Ahmed *et al.*, 2009). Hundred grams of each milled plant samples were macerated in 250 ml of ethanol over night at room temperature, then filtered and the ethanolic crude extracts were collected. Another portion of 100ml of ethanol were added to plant residue and boiled for two hours under reflux condenser in a water bath and then filtered (with filter paper15.0cm), the filtrate was added to the previous crude extract. The solvents were evaporated under vacuum using rotary evaporator. The crude extract was kept in dark bottles and stored in a deep freezer until use.

Percentage of extraction=Weight of ethanol free extract/Weight of sample ×100

Microbial examination:

Preparation of mixture of natural ethanolic extract from cinnamon, clove and Ginger with (1:1:1):

Different concentrations were prepared (0.4-0.5-0.6%) of ethanol extracts of plants clove, ginger, cinnamon and mixed by 1 ml: 1 ml: 1 ml of each extract for studying their effects on some pathogenic toxic bacteria.

Determination of the antimicrobial activity:

The disc diffusion method was used to determine the antimicrobial activity of the natural extracts. The volume of 0.1ml (approximately 10^9 cells / ml) of the tested microorganisms grown in liquid growth media at 37ºC was inoculated on MacConkey growth media for *E. coli* and SS agar media for Salmonella sp. then spread on the entire surface of the Petri dish using a sterile swab. Discs (6mm diameter of whatmam.No.1 fitter paper) with 30 µl absorbed natural extracts were placed on the MacConkey agar by pressing gently. The plates were incubated at37ºC for 48 hours. After the incubation period, the inhibition zones around the paper discs were measured in millimeters. The sensitivity of the individual oil was classified by the diameter of inhibition zone as per the procedure of the experiment was repeated in duplicate for all of the test strains.

Non-sensitive (total diameter smaller than 8 mm)

Sensitive (total diameter between 9 – 14 mm)

Very sensitive (total diameter between 15 – 19 mm)

Extremely sensitive (total diameter larger than 20 mm) according to (Moreira *et al*., 2005).

Determination of inhibition percentage of ethanolic plants extracts against the growth of E. coli O157:H7 (toxic bacteria) during 7 days of storage period at 37ºC:

Natural extracts were added to Ethanol 95%, bottles after autoclaving to make concentration (0.4 - 0.5 - 0.6%) for each substance (except one bottle as a control) under aseptic condition. Each bottle was inoculated by *E. coli* O157:H7 to have a final number ranging from 10^6 to 10^7ctu ⁄ml. The osculated bottles were incubated at 37 ºC for 7days. Every day, 1 ml from each bottle was taken and *E. coli* O157:H7 was enumerated by using MacConkey agar media then the inoculated plates were incubated at 37 ºC for 24-48 hr.

Inhibition percentage was calculated according to Sagdic, *et al.*(2002) as follows:

Inhibition percentage = Control count – Treatment count / Control count x 100

All experiments were carried out duplicates and the results are expressed as average values of inhibition.

RESULTS AND DISCUSSION

Data in Table (1) showed that the extraction rate of the plants was at the following decreasing order: ginger (7%), cinnamon (5%), clove (5%), cardamom (2.5%) and rosemary (2%) and garlic (1.5%).

Table 1. Ethanolic extraction percentage.

Name of sample	***Percentage of extraction***
Cinnamon	5.0
Cardamom	2.5
Ginger	7.0
Rosemary	2.0
Clove	5.0
Garlic	1.5

Antibacterial activity (inhibition zones (mm) of ethanolic extracts against various pathogenic bacteria (*Escherichia coli O157, Escherichia coli NRRL3008* and *Salmonella sp*):

Antimicrobial activity (inhibition zone (mm) of ethanolic extracts (Without dilution and the same volume) of garlic, clove, cardamom, ginger, cinnamon, and rosemary against *Escherichia coli O157*, *Escherichia coli NRRL3008* and *Salmonella sp*. is shown in Table (2). Results showed that *Escherichia coli* showed high susceptibility with natural extracts (Without dilution), for disc diffusion methods. Clove extract had the strongest effect against *E.* coliO157*:H7* (35.00mm), followed by cinnamon (31.00mm), ginger (30.00mm), rosemary (23.00mm) and garlic (22.00). However, cinnamon extract was the strongest antibacterial against *E.coli NRRL3008* (28.00mm), followed by clove (27.00mm) and ginger extract (26.00mm). On the other hand, clove and cinnamon had the same most powerful effect against

Table 2. Antibacterial activity of ethanolic extract of garlic, clove, cardamom, ginger, cinnamon and rosemary (without dilution) against various pathogenic bacteria:

Pathognic bacteria	Inhibition zone (mm)					
	Garlic	Clove	Cardamom	Ginger	Cinnamon	Rose-mary
E. coli O157:H7	22.00	35.00	28.00	30.00	31.00	23.00
***E. coli* NRRL3008**	24.00	27.00	23.00	26.00	28.00	25.00
Salmonella sp.	18.00	23.00	19.00	22.00	23.00	22.00

mm = Millimeter

Salmonella sp., followed by cardamom. Antibacterial activity of natural extracts of clove may be attributed to eugenol according to Wendakoon and Sakaguchi (1995) by inactivation of enzymes and genetic material. Also, Blaszyk and Holley (1998) concluded that eugenal inhibited the growth of *Escherichia coli O157:H7*.

Results obtained by Leuschner and Ielsch (2003) indicated that *Escherichia coli O157* was found to be highly sensitive to garlic action followed by *Escherichia coli NRRL*.

Antibacterial activity (inhibition zones (mm) of garlic ethanolic extracts against pathogenic bacteria:

Antibacterial activity (inhibition zones mm) of garlic ethanolic extracts against pathogenic bacteria (*E. coli O157:H7, E. coli NRRL3008 and Salmonella sp)* are show in Table (3). Data showed that the most effective concentration of garlic extract was at level of 0.6%. It had the strongest effect against *E.coli NRRL*3008 (22.00mm), followed by *Salmonella sp.* (16.00mm), then *E.coli O157*:*H7* (14.00). Also, the concentration at level of 0.5% take the same trend as it was more effective against *E.coli NRRL* (19.00mm) than *Salmonella* or *E.coli O157*:*H7* (13.00mm). However, the differences were at the lowest at level of 0.5%. The minimum inhibitory concentration of the garlic extract on the organism was observed to be 0. 6 %.Ethanol extract of garlic was highly effective against *Escherichia coli, and Salmonella* were sensitive to ethanol extract they also reported that 4% (w/v) fresh garlic in extract inhibited the growth of *E. coli*, and *Salmonella* (Al-Delaimy and Ali, 1971). Garlic is suggested as a natural herb could be used to extend the shelf life of meat

Table 3. Minimum inhibitory zone of various concentrations of garlic ethanolic extract on the growth of pathogenic bacteria

Pathogenic bacteria	Concentrations of garlic ethanolic extract (%)		
	0.4	0.5	0.6
	Minimum inhibition zone (mm)		
E. coli O157:H7	8.00	13.00	14.00
E. coli NRRL.3008	9.00	19.00	22.00
Salmonella sp.	8.00	13.00	16.00

products, providing the consumer with food containing natural (Tsai *et al.*, 1982). Chowdhury *et al.*(2000) recorded that garlic extract inhibited the growth of *Salmonella* on l agar plate with diameter of zone of inhibition averaging 18mm which agree with our results.

Garlic is rich in anionic components such as nitrates , chlorides and sulfates as well as other water soluble components common in most plants which may be responsible for its anti bacterial activity (Chah *et al.*, 2003).

Antibacterial activity (inhibition zones (mm) of cinnamon ethanolic extracts against pathogenic bacteria:

Antibacterial activity (inhibition zones (mm) of cinnamon ethanolic extract against of pathogenic bacteria is presented in Table (4). The ethanol extracts of cinnamon showed better results as compared to Garlic. The most effective concentration of cinnamon extract was at level of 0.6%. It had the strongest effect against *E. coli O157:H7* (26.00mm) followed by *E. coli NRRL*3008 (19.00mm), then Salmonella *sp*, (17.00mm). In addition, the concentration at level of 0.5% takes the same trend as it was more effective against *E.coli O157:H7or E. coli NRRL3008* (19.00mm) than *Salmonella sp.* (14.00mm). However, the weakest effect was at the level of 0.5%. The activity of the ethanolic extracts is mainly due to the Vanillin, Zingiberene, which are responsible for antimicrobial activity. The most representative compound called o-methoxy cinnamaldehyde and other compounds to achieve the inhibition of microorganisms Morozumi, (1978) characterize cinnamon. Srinivasans, *et al.*, (2001) found that ethanol extract of cinnamon showed better zones at all concentrations against *E.coli* as compared to the aqueous extracts, as well as its effect against *Escherichia coli* (Friedman

Table 4. **Minimum inhibitory zone of various concentrations of cinnamon ethanolic extract on the growth of pathogenic bacteria.**

Pathogenic bacteria	Concentrations of cinnamon ethanolic extract(%)		
	0.4	**0.5**	**0.6**
	Minimum inhibition zone (mm)		
***E. coli* O157:H7**	12.00	19.00	27.00
E. coli NRRL.3008	9.00	18.00	20.00
***Salmonella sp*.**	7.00	14.00	17.00

et al., 2002). Kalemba and Kunicka (2003) reported that cinnamon oil was among the strongest antimicrobials from plants and species. Valero and Salmeron (2003) also indicated a very strong effect of cinnamon extract against *Salmonella*. Cinnamon extract possess effective antibacterial properties against *Salmonella* and *E.coli* (Ooi *et al*., 2006).

Antibacterial activity (inhibition zones (mm) of rosemary ethanolic extract against pathogenic bacteria:

Antibacterial activity (inhibition zones (mm) of ethanolic extracts of rosemary against pathogenic bacteria is show in Table (5). Data showed that the most effective concentration of rosemary extract was at level of 0.6%. Rosemary extract had the strongest effect against *E. coli O157:H7* (19.00mm) Followed by *E.coli NRRL3008* (18.00mm), then *Salmonella sp* (14.00 mm). In addition, the concentrations at level of 0.5% take the same trend, as it was more effective against *E.coli O157:H7 or E. coli NRRL3008* (14.00mm) than *Salmonella sp* (9.00 mm). However, the differences were at the lowest at level of 0.5%. As the extract of rosemary leaves contains α-pinene and camphor, which are responsible for antimicrobial activity (Daferera, *et al.*, 2003). These results came in agreement with what was mentioned by (zinka, 2004). Moderate antimicrobial activity of rosemary have been reported by several authors (Quispe, 2005), (Celiktas, 2007) and (Gachkar, 2007).

Antibacterial activity (inhibition zones (mm) of ginger ethanolic extracts against pathogenic bacteria

Antibacterial activity (inhibition zones (mm)) of ginger ethanolic extracts against pathogenic bacteria is presented in Table 6. Data showed that the most effective concentration of ginger extract was at level of 0.6%.it had the strongest effect against *E. coli O157:H7* (27.00mm), followed by *E.coli NRRL.3008* (19.00mm) then *Salmonella sp.* (14.00mm).

Table 5. Minimum inhibitory zone of various concentrations of rosemary ethanolic extract on the growth of pathogenic bacteria:

Pathogenic bacteria	Concentrations of Rosemary ethanolic extract,%		
	0.4	0.5	0.6
	Minimum inhibition zone (mm)		
***E. coli* O157:H7**	9.00	11.00	17.00
***E. coli* NRRL.3008**	10.00	13.00	19.00
Salmonella sp.	4.00	9.00	14.00

Table 6. Minimum inhibitory zone of various concentrations of ginger ethanolic extract on the growth of pathogenic bacteria

Pathogenic bacteria	concentrations of ginger ethanolic extract, %		
	0.4	0.5	0.6
	Minimum inhibition zone (mm)		
***E. coli* O157:H7**	11.00	17.00	27.00
***E. coli* NRRL.3008**	6.00	9.00	19.00
Salmonella sp.	6.00	10.00	14.00

Also, the concentration at level of 0.5% was more effective against *E.coli O157:H7* and *salmonella* sp.(17.00mm). However, the lowest inhibition zone was recorded for *E. coli NRRL3008* (9.00mm). On the other hand, the differences were at the lowest level at the concentration of 0.5%. The results obtained in our study agree with those reported by Roy *et al.* (2006) who explained that bioactive compounds of ginger rendering microbial activity are volatile in nature and antimicrobial activity of ginger extract decreases upon storage. It was reported that sesquiterpenoids are the main component of ginger that attributes to its antibacterial activity *(Malu et al., 2008).*

Antibacterial activity (inhibition zones (mm) of Cardamom ethanolic extracts against pathogenic bacteria:

Antibacterial activity (inhibition zones (mm) of cardamom ethanolic extracts against pathogenic bacteria is show in Table (7). Results showed that the antimicrobial activity assayed indicated that cardamom extract at different concentration had inhibitory activity on *E. coli, salmonella*. Data showed that the most effective concentration of cardamom extract was at level of 0.6%., it

Table 7. Minimum inhibitory zone of various concentrations of cardamom ethanolic extracts on the growth pathogenic bacteria

Pathogenic bacteria	Concentrations of cardamom ethanolic extracts		
	0.4%	**0.5%**	**0.6%**
	Minimum inhibition zone (mm)		
E. coli O157:H7	14.00	17.00	23.00
E. coli NRRL.3008	12.00	17.00	21.00
Salmonella sp.	9.00	11.00	16.00

had the strongest effect against *E. coli O157:H7* (23.00mm) followed by *E.coli NRRL.3008* (21.00mm) than *salmonella sp*. (16.00mm). Moreover, the concentrations at level of 0.5% take the same trend as it was more effective against *E.coliO157:H7* or *E. coli NRRL3008* (17.00mm) than *salmonella* sp. (11.00mm). However, the differences were at the lowest level of 0.5%.Antimicrobial characteristics of the plant are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue (Baytop, 1984) and (Con *et al*., 1998). The obtained results agree with Fardiaz (1995) and Sema *et al.* (2005) who studied the antimicrobial effect of seed extract of cardamom.

Antibacterial activity (inhibition zones (mm) of clove ethanolic extracts against pathogenic bacteria:

Antibacterial activity (inhibition zones (mm) of clove ethanolic extracts against pathogenic bacteria *(E.coli O157:H7, E. coli NRRL3008 and Salmonella sp.)* is shown in Table (8). Data showed that the most effective concentration of clove extract was at level of 0.6%.It had the strongest effect against *E. coli O157:H7* or *E. coli NRRL.3008* (27.00mm) than *salmonella sp.* (18.00mm). In addition, the concentration at level of 0.5% was more effective against *E.coli O157:H7* or *E. coli NRRL*3008 (19.00mm), followed by *salmonella sp.* (13.00mm). However, the differences were at the lowest at level at the concentration of 0.5%. Clove has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and is used traditionally as flavoring agent and antimicrobial material in food (Lee and Shibamoto, 2001), (Huang *et al*., 2002) and (Velluti *et al*., 2003). These results agree with Saeed, and Tariq (2008) who studied the chemical analysis and antimicrobial activity of the essential oil of *Syzigiumaromaticum* (clove).

Antibacterial activity (inhibition zones (mm) of ethanolic extracts of cinnamon, clove and ginger with (1:1:1) against pathogenic bacteria:

Antibacterial activity (inhibition zones (mm)) of ethanolic extracts of mixture against pathogenic bacteria is show in Table (9). *E. coli* showed high

Table 8. Minimum inhibitory zone of various concentrations of clove ethanolic extracts on the growth pathogenic bacteria.

Pathogenic bacteria	Concentrations of clove ethanolic extracts, %		
	0.4	**0.5**	**0.6**
	Minimum inhibition zone (mm)		
E. coli O157:H7	14.00	19.00	27.00
E. coli NRRL.3008	12.00	19.00	27.00
Salmonella sp.	9.00	13.00	18.00

Table 9. Antibacterial activity zone of mixture of ethanol extract of cinnamon clove and ginger (1:1:1) on the growth pathogenic bacteria.

Pathogenic bacteria	*Concentrations of cinnamon clove and ginger (1:1:1), %*		
	0.4	**0.5**	**0.6**
	Minimum inhibition zone (mm)		
E.coliO157:H7	27.00	32.00	36.00
E.coliNRRL3008	25.00	30.31	34.00
Salmonella SP.	23.00	26.00	28.00

susceptibility with high concentration of extracts, for disc diffusion methods. Data showed that the most effective concentration of mixture extract was at level of 0.6%. It had stronger effect against *E. coli O157:H7* (36.00mm), *E.coli NRRL3008* (34.00mm) than *Salmonella sp,* (28.00mm). Also the concentration at level of 0.5% take the same trend as it was more effective against *E.coli O157:H7* (32.00mm) *or E.coli NRRL3008* (30.00mm) than *salmonella* sp. (26.00mm). However, level of 0.5% the produce lower effect. The antibacterial activities of the extracts are expected perhaps due to the compounds like flavonoids and volatile oil, which were dissolved in organic solvents. The results obtained in our study agreed with those reported by Roy *et al.* (2006) who explains that bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and antimicrobial activity of ginger extract decreases up on storage. The main compound present in Cinnamon extracts were Vanillin .The antimicrobial activity of combinations of clove, ginger and Cinnamon extracts have not been reported before Individual extracts contains complex components, when combined with each other, may lead to additive, synergistic or antagonistic effects. It is reported that trans-6-shogaol are the main component of ginger, which attributes to its antibacterial activity (Malu *et al*., 2008).

Effect of ethanolic extracts of plants on the growth of E. coli O157:H7 during 7 days of storage period at 37ºC:

Antimicrobial activity (inhibition percentage (%) of ethanolic extracts mixture of garlic, clove, cardamom, ginger, cinnamon and rosemary against *Escherichia coli O157:H7*. Results in Table (10) showed that *E.coli O157:H7*showed high susceptibility with high concentration of natural extracts, for disc diffusion methods. Therefore, the effect of ethanolic extracts against growth of *E. coli O157:H7* on broth media was investigated. Data showed that the most effective concentration of ethanolic extracts of garlic, clove, cardamom, ginger, cinnamon and rosemary was at level of 0.6% throughout the 7 days of the storage period. On the first day, the highest effect was by cinnamon extract (53.76%**),** followed by ginger (46.08%), rosemary (43.05%) and garlic (37.85%), cardamom (33.59%), and then clove (27.31%). On the second day, ginger extract had the highest effect (100.00%), followed by cinnamon (75.84%), rosemary (57.62%), cardamom (52.03%), clove (51.47%) and garlic (48.10%). On the third day, the highest effect was recorded by ginger and cinnamon (100.00%) followed by garlic (74.63%), rosemary (72.21%),while clove had the lowest effect (57.20%).On day4, ginger, cinnamon and rosemary (100.00%) were the most effective followed by garlic (67.41%),cardamom (66.42%) and clove extract (61.47%) .On day 5, data showed that the most effective concentration of extracts was ginger, cinnamon and rosemary at level of 0.6% ,it had the strongest effect against *E. coli O157:H7* (100.00%), however clove had the weakest effect (56.49%). In addition, the concentrations at level of 0.5% take the same trend and it was more effective against *E.coli O157:H7* by ginger extract.

Data showed that, on day 6, the most effective concentration of ethanolic extracts of ginger, cardamom, cinnamon and rosemary was at level of 0.6%. it had the strongest effect against *E. coli O157:H7* (100.00%), followed by clove (71.89%) and garlic(61.44%).Also the concentrations at level of 0.5% take the same trend as it was more effective against *E.coli O157:H7* for ginger and cinnamon. On day7, the most effective concentration of ethanolic extracts of ginger, cinnamon, rosemary and garlic was at level of 0.6%.it had the strongest effect against *E. coli O157:H7* (100.00%), followed by clove (74.09%),garlic (61.44%). In addition, the concentrations at level of 0.5% tended to the same behavior, as it was more effective against *E.coli O157:H7* for ginger, cinnamon, cardamom, and rosemary. Some workers such as Cosentino *et al*. (1999), Dorman and Deans (2000) and Rauha *et al*. (2000) reported that ginger extract showed an inhibitory effect on *E. coli* O157:H7. Cinnamon at 0.5 and 0.6 % showed the bactericidal effect on the third and sixth days, respectively, Which are coinciding with Burt and Reinders (2003).

Table 10. Effect Inhibition percentage (%) of ethanolic plants extracts against the growth of *E. coli* O157:H7 during 7 days of storage period at 37°C.

Extracts	Additives, %	First day	2nd day	3rd day	4th day	5th day	6th day	7th day
Cardamom	**0.4**	12.35	39.54	42.90	37.01	53.39	45.10	73.68
	0.5	32.65	55.21	63.45	64.54	62.11	61.69	100.00
	0.6	33.59	52.03	58.47	66.43	67.13	100.00	100.00
Cinnamon	**0.4**	34.68	52.31	53.35	49.98	35.71	29.03	45.79
	0.5	43.77	50.76	62.01	64.81	63.03	100.00	100.00
	0.6	53.76	75.84	100.00	100.00	100.00	100.00	100.00
Ginger	**0.4**	24.62	45.64	35.84	49.93	55.03	57.12	61.68
	0.5	35.84	46.04	62.02	56.68	100.00	100.00	100.00
	0.6	46.08	100.00	100.00	100.00	100.00	100.00	100.00
Rosemary	**0.4**	33.03	47.54	50.71	56.59	66.35	56.38	62.58
	0.5	39.83	46.93	60.25	66.82	71.23	78.34	100.00
	0.6	43.05	57.62	72.21	100.00	100.00	100.00	100.00
Garlic	**0.4**	25.17	44.13	42.41	34.03	41.15	30.23	46.49
	0.5	38.50	49.23	54.84	38.39	62.27	61.44	64.85
	0.6	37.85	48.10	74.36	67.41	75.04	65.35	100.00
Clove	**0.4**	16.34	36.52	54.44	49.93	50.92	54.14	57.38
	0.5	25.56	44.13	56.16	62.81	56.06	60.3	62.06
	0.6	27.31	51.47	57.20	61.74	65.49	71.89	74.09

Rosemary extract at 0.5 and 0.6% showed bactericidal effect for seventh and third days, respectively. Which are in agreement with Hammer *et al*. (1999) and Pintore *et al.* (2002).Cardamom extract has bactericidal effect at0.5 and 0.6% for sixth and seventh days, respectively. These results are coinciding with those obtained by Palmer *et al.*(1998), Hammer *et al.*(1999) and Dzcan and Erkmen (2001).Three percent of garlic extract was bactericidal at seventh day. While, clove extract has a bacteria static effect with all concentrations. These results are in harmony with (Palmer *et al.,* 1998) and (Hammer *et al.*, 1999).

Conclusively, it can be concluded that natural extracts of clove, cardamom, cinnamon, rosemary, ginger and garlic differ in their inhibitory activity against Escherichia coli O157:H7, Escherichia coli NRRL and Salmonella sp. so, these natural extract could be considered as natural food preservatives against *pathogenic toxic bacteria and generally* recognized as safe.

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التأثير المثبط للمستخلصات النباتية الطبيعية على بكتيريا القولون الممرضة والسامة والسالمونيلا فى البيئات

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تم دراسة تاثير ستة مستخلصات نباتيه من مضادات الميكروبات الطبيعية وقدراتها على تثبيط نمو بكتيريا القولون السامة(*E.coli O157:H7* وبكتيريا القولون الممرضة *E.coli NNRL 3008,* والسالمونيلا وتم تحديد درجة التثبيط بقياس قطر المنطقة الخالية من البكتيريا حول القرص وكشفت النتائج ان التأثير المثبط للمستخلصات بدون تخفيف كان أقوى تاثيرللثوم على *E.coli NNRL 3008,* (٢٤مم) و*E.coli O157:H7* (٢٢مم)) تليها السالمونيلا (١٨مم)وكان لمستخلص القرنفل اقوى تاثير ضد *E.coli O157:H7*(٣٥مم) يلية *E.coli NNRL 3008* (٢٧مم) والسالمونيلا (٢٣مم).بينما التاثير الاقوى لمستخلص الزنجبيل ضد *E.coli O157:H7* (٣٠مم) و*E.coli NNRL 3008* (٢٦مم) تليها السالمونيلا (٢٢مم)وكان للحبهان نفس التأثير *E.coli O157:H7* (٢٨مم) يلية *E.coli NNRL 3008* (٢٣مم) والسالمونيلا (١٩مم).أما مستخلص القرفه وجد أن أعلى تأثير له على*E.coli O157:H* (٢٧مم) يلية *E.coli NNRL 3008* (٢٨مم) والسالمونيلا (٢٣مم) وكان التاثير الاقوى لمستخلص الروز مارى ضد *E.coli NNR*L 3008 (٢٥مم) يلية *E.coli O157:H7* (٢٣مم) والسالمونيلا (٢٢مم).وكان لخليط المستخلصات المكون من (القرفه-القرنفل والزنجبيل بنسب متساوية) عند تركيز ٠.٦% تاثير اقوى ضد *E.coli O157:H7* (٣٦مم)، تليها *E.coli NNRL 3008*(٣٤مم). ولوحظ ان درجة تثبيط مستخلصات الايثانول لنمو *E.coli O157:H7* خلال سبعة ايام من التخزين على درجة ٣٧ درجة مئوية كانت كالاتى. الزنجبيل >القرفه> الروزمارى > الحبهان > الثوم > القرنفل ويمكن ان نخلص الى ان المستخلصات الطبيعية من القرفة والقرنفل والحبهان والروزمارى والزنجبيل والثوم تختلف فى نشاطها المثبط ضد بكتيريا القولون *E.coli O157:H7*.

التوصية: يمكن اعتبار هذه المستخلصات الطبيعية كمادة حافظة للطعام ضد البكتيريا المسببة للامراض والسامة والمعترف بها عموما على انها أمنة.