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Research Article
Anti-*Escherichia coli* O157:H7 as Natural Preservative to Control and Prevent Food Contamination in Meat and Fish Products

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Abstract

Background and Objective: Shiga toxin-producing *Escherichia coli* O157:H7 is a troubled foodborne pathogen associated with contamination of meat, fish and poultry. The present work aimed to evaluate plant extracts as natural preservatives anti- *Escherichia coli* O157:H7 in meat and fish products. **Materials and Methods:** Antibacterial activity and minimum inhibitory concentrations (MICs) of seven herbal plants, clove, marjoram, sage, pomegranate peel, turmeric, *Cassia fistula* and black pepper and their different 6 mixes were examined against *Escherichia coli* O157:H7. Phytochemical qualitative analysis, phenolic compounds (HPLC), total phenolic, total flavonoid contents and antioxidant activities of individual extracts and their 6 mixes were evaluated. Combination Mix 5 extract was applied on meat and fish-fillet, then its antimicrobial effect against *E. coli* O157:H7 and sensory evaluation were assessed. **Results:** Five extracts exhibited good antibacterial activity against *Escherichia coli* O157:H7. The greatest inhibition zone was recorded by clove aqueous extract (25 mm). Mix 5 (clove, sage, pomegranate and *Cassia fistula*) showed the highest inhibition with MIC of 3.0 mg mL^{-1}. This mix exhibited strong anti-bactericidal effect against *E. coli* O157:H7 in meat and fish-fillet products throughout 8 days of cold storage (4°C). The sensory evaluation revealed that Mix 5 was acceptable by panelists with concentration of 0.50% in beef burgers and 0.25% in fish-fillet. **Conclusion:** This study suggests that the use of herbal extracts provide antibacterial potentials against food pathogens in meat and fish products.

Key words: Natural preservatives, antibacterial activity, antioxidants, *Cassia fistula*, marjoram, beef burgers, fish-filet

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Escherichia coli O157:H7 was in reality diagnosed as a pathogen. This bacterium is now appeared as the main reason of foodborne diseases, with reported outbreaks within the US, Canada and excellent Britain. Maximum outbreaks were associated with consuming half of-cooked minced meat or consuming crude milk less often. Retail meat and poultry surveys exposed the presence of *E. coli* O157:H7 in minced red meat, pork, poultry and lamb. *Escherichia coli* O157:H7 is like the different *E. coli* but it has one-of-a-kind features and could not possess β-glucuronidase interest or tolerate high temperature above 40°C. Consequently, heating minced meat which could be enough to kill it[1].

The maximum regularly occurring risk in food and water supply is a concept to be foodborne bacterial pathogens. Due to its combination of harm and pathogenicity, *Escherichia coli* O157:H7 has emerged as one of the deadliest foodborne pathogens[2]. The disease induced by this microorganism begins to life-threatening conditions such as hemorrhagic colitis and hemolytic uremic syndrome with bloody diarrhea. The center for disease control and prevention estimates an annual *E. coli* O157:H7 infection of 20,000 people[1].

The main cause of illness and death is foodborne pathogens in developing nations, costing billions of dollars in medical care and social expenses[2]. *E. coli* O157: H7 is one of the most prevalent sources of human infection due to low infectious dose, acid tolerance and may trigger multiple syndromes such as mild diarrhea, serious bloody diarrhea, hemorrhagic colitis or kidney failure owing to hemolytic uremic syndrome (HUS)[1].

The main reservoirs of *E. coli* O157:H7 are cattle and beef products which are known to be a primary source of foodborne pathogen transmission[1,3]. Carcass contamination occurs in processing animals during slaughter procedures while the pathogen is transferred from skin to carcass or fecal to carcass[4-6], leading risk factor for human infection. Several pre-harvest techniques such as direct-fed microbial, bacteriophage treatment and vaccination[7,8] and post-harvest involvement like skin, carcass cleaning and antimicrobial use that was used to decrease pathogen shedding[9].

The concern in natural antimicrobials has increased in recent years, particularly those extracted from plants. Furthermore, a big number of published researches have recorded the antimicrobial effect of plant extracts from fruits, spices, herbs and vegetables[10-12]. Though, it is not well documented the exact mechanism of action of these antimicrobial products.

In Gram-negative bacteria, the presence of lipo-polysaccharide cell wall stops the spread of hydrophobic compounds and makes it more resistant to plant extract. Otherwise, more antibiotic resistance has been acquired by pathogenic microorganisms. Consequently, researchers have recently concentrated on plant extracts as potential alternatives. Plant extracts appear to be the correct option for enhancing resistance to antibiotics and can also produce better results than synthetic preservatives. Furthermore, it has been shown that plant extracts reduce antibiotic resistance by encouraging synergistic effects between natural antimicrobials and antibiotics[13]. For all the above, the purpose of this study was to investigate selected plant extracts as a natural antibacterial agent against *Escherichia coli* O157:H7.

MATERIALS AND METHODS

The study was carried out at Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt from February-September, 2019.

Plant material samples and preparation: The plant material (Marjoram, clove, pomegranate peel, Turmeric, sage, *Cassia fistula* and black pepper) were bought from spices shop in Alexandria, Egypt. The plant samples were cleaned from strange materials, washed, air dried and grinded to a fine powder using a coffee grinder. The extraction was obtained by mixing Five gram of each single plant with 100 mL of boiling distilled water with stirring at 100 rpm for several times over night. The acquired extracts were centrifuged at 2147 rpm for 30 min and filtered using filter papers. The extracts were then lyophilized at -50°C (Telstar Model 50, Spain) and the powder collected was measured and placed in deionized water with several defined concentrations[14] (mg mL^{-1}).

Microorganisms and culture conditions: From the Microbiological Resources Center (MERCIN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt, *Escherichia coli* O157:H7 grown was acquired. It was then grown for 24 h at 37°C on tryptic soy broth (TSB).

Antibacterial activity: The ability of each individual extract to inhibit *Escherichia coli* O157:H7 development has been evaluated with some modifications[15,16]. In brief, about 100 µL of *Escherichia coli* O157:H7 overnight culture (10^6 CFU mL^{-1}) was spread over tryptic soy broth (TSB) media plates using sterile cotton swabs. Then, using sterile cork borer, the agar was perforated to create 9 mm well, 100 µL of each plant

extract was poured into the wells. The plates were then incubated at 37°C for 24 h. At last, measured and recorded in millimeter the diameter of the transparent areas created.

Preparation and antibacterial activity of mixes: To determine the activity of various combinations of extracts against *Escherichia coli* O157:H7, four separate mixtures of 2, 4, 5 and 7 plants were prepared and tested. Weighed in milligrams each individual lyophilized extract, dissolved in 50 mL deionized water and blended (1:1) with other extracts selected for each plant with a final mg mL^{-1} concentration. These mixtures were screened against *Escherichia coli* O157:H7 using a technique of agar diffusion[15,16]. The formed clear zones diameters were estimated in mm to select the strongest blend that inhibits *Escherichia coli* O157:H7 growth.

Minimum inhibitory concentration (MIC) determination: To determine minimum inhibitory concentration (MIC), various levels of Mix 5 (mg mL^{-1}) have been screened against the bacteria using the technique of agar well diffusion as outlined above. The bacterial strain (*Escherichia coli* O157:H7) was grown in tryptic soy broth (TSB) at 37°C for 24 h. Six concentrations of reconstituted plant water extracts (75, 50, 25, 12, 5, 6, 25 and 3,1 mg mL^{-1}) were examined to determine the minimum inhibitory concentration (MIC) against *Escherichia coli* O157:H7 strain. By calculating the diameter of the inhibition zone around the well (mm), including the diameter of the well, the inhibition zone was measured. The measurements were taken in three distinct fixed directions throughout all triplicates and the mean values were calculated.

Phytochemical qualitative analysis: Qualitative phytochemical analysis of alkaloids, flavonoids, tannins, volatile oils, amino acids, proteins, sugar reduction, glycosides, saponins (Foam test), steroids and terpenoids (Salkowski test) has been conducted[17,18].

Total phenolic content: Total phenolic content was determined by Folin-Ciocalteu spectrophotometric technique for each extract Cloves, sage, pomegranate and *Cassia fistula* and their mixture (Mix 5)[14]. A 0.1 mL Folin-Ciocalteu reagent fluid was added to 2 mL of purified extract. Then for 15 min the blend was permitted to stand. Three mL of saturated 2% (Na_2CO_3) was added to the mixture. The mixture was allowed to stand at ambient temperature for 30 min and the total phenolic content was measured at 760 nm using a spectrophotometer (Labo America, USA). Gallic acid has been used as standard total phenol values are displayed as mg of gallic acid equivalent g^{-1} of the sample using the linear regression equation from the standard gallic acid calibration curve y = 0.0067x+0.3481. All samples were evaluated in triplicate.

Total flavonoid estimation: The total content of flavonoids was estimated by colorimetric aluminum chloride assay[19]. Four milliliter of distilled water was added to 1 mL of methanol extract. Then 0.3 mL of 5% $NaNO_2$ was added to the above blend. The blends were allowed to stand for 5 min and then 0.3 ml of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added and the total volume was completed to 10 mL with distilled water. The solution was well blended and the absorption was measured at 510 nm against prepared reagent blank. The standard graph was prepared using various gallic acid concentrations.

HPLC conditions for phenolic compounds quantification: The quantification by high performance liquid chromatography (HPLC) of phenolic compounds of clove, sage, pomegranate and cassia fistula extracts and (Mix 5) was determined[20]. Compounds were characterized by comparing their retention times and UV-Vis spectra to standards, whereas their levels were calculated depending on the area under the peak of standards.

Antioxidant activity evaluation: Plant extract ability to scavenge DPPH free radicals has been evaluated using the standard and implemented with appropriate changes[14,18,21]. In short, at the initial concentration of 1 mg mL^{-1}, the stock solution of each sample was prepared in methanol and the final quantity consisted of 1 mL of ethanol. The DPPH solution was then prepared in 95% methanol (0.004% w/v). All tubes were filled with three mL of freshly prepared DPPH solution. For 30 min of incubation, the mixture was kept in the dark and analyzed with UV visible spectrophotometer at 517 nm. In distilled water at a concentration of 1000 µg mL^{-1}, a stock solution of ascorbic acid was prepared. From this stock, various concentration varying from 100-1000 µL were prepared and used as the reference standard. The blank solution was DPPH in methanol. IC_{50} values were measured using a non-linear regression algorithm from the inhibition percentage versus concentration plot after calculation of the inhibition ratio using the following equation[14]:

$$\text{Inhibition (\%)} = \frac{\text{A of control} - \text{A of sample}}{\text{A of control}} \times 100$$

Were:

A of control = Absorbance control
A of sample = Absorbance sample

Inhibitory effect of Mix 5 extract against *E. coli* O157:H7 in beef meat and fillet-fish: In this experiment, the method[22,23] was performed with minor changes as per this experiment[15,24,25]. In Borg El Arab, Alexandria, Egypt, post-rigor lean beef and fillet-fish were acquired from a slaughterhouse and fish supplier. Each piece was submerged for 5 min in boiling water. Under aseptic conditions, the cooked surface of beef meat and fillet-fish was removed with sterile knives and prepared pieces were minced in a sterile grinder. Then the samples were placed in high density polyethylene bags and 50 g each, were infected with 10^4 CFU of *E. coli* O157:H7/g. 1, 0.5 and 0.25% (w/w) of mixture Mix 5 extract were added to test the survival of pathogenic bacteria species prior to meat inoculation. The samples were homogenized, cooled (6±1°C) and examined for each sample after 0, 1, 2, 3, 4, 7, 10 and 15 days. Sterile water, instead of combination Mix 5 extract, was added in control samples Once beef and fillet-fish samples were prepared and inoculated at day 0 and upon incubation at 6±1°C, randomly selected meat-containing bags were examined for pathogenic bacteria. For 1 min, the samples were homogenized and 24 h incubated at 35°C. 1 mL was added to 9 mL of peptone broth from this pre-enrichment and incubated for 24 h at 35°C. The counts were undertaken for pathogenic bacteria on Tryptic Soy Blood Agar (TSBA) medium plates by placing the suitable sample dilutions aseptically in duplicate on the surface. In all the samples, three individual replicates were conducted from each experiment.

Sensory evaluation: Hundred grams from each beef meat and Fillet-fish for treatment by added various concentrations of combination Mix 5 extract. To test the Sensory properties, the concentrations of the potent combination Mix 5 extract were 1, 0.5 and 0.25% (w/w). The samples were homogenized for 5 min at normal speed. All samples of beef meat and Fillet-fish were refrigerated (7°C). then the Sensory evaluation for 5 treatments as control, without treatment (C), treatment with 1% Mix 5 (T 1%), treatment with 0.5% Mix 5 (T 0.5%), treatment with 0.25% Mix 5 (T 0.25%). Ten qualified panelists, members of the department of food science and technology, who had experience with meat and fish products, were chosen to evaluate the quality of fillets. Prior the panel was introduced, samples of meat and fish were cooked and served warmly to the panelists and coded using letters and submitted to the panelists randomly. Panelists were requested to examine the samples appearance, smell and taste. On a 10-point hedonic scale, appearance, smell and taste were scored. A zero score was used as a point of refusal attribute[26,27].

Statistical analysis: The statistical analysis was performed using analytical software SPSS® 13.0 (Statistical Package for Social Sciences)[28].

RESULTS

Antibacterial activity of *Escherichia coli* O157:H7: Results in Table 1 demonstrate that there may be no significant deference in inhibition zone of clove, pepper, *Cassia fistula*, sage or marjoram and had the highest antibacterial effect were their inhibition region values recorded 25, 24, 22, 22 and 20 mm, respectively. The percentage of inhibition recorded for extracts of clove (96.15%) and pepper (92.31%). In comparison, the lowest inhibition outcomes have been recorded for extracts of pomegranate (13%) and turmeric (10%).

As shown in Table 2, the individual (IPE) and plant extracts mix (PEM) had the better own sturdy antibacterial activities in opposition to enterohemorrhagic coli O157:H7. Also, Mix 4, Mix 5 and Mix 6 were the most effective extracts towards the *E. coli* O157:H7 stress examined and recorded 23, 28 and 23 mm inhibition zone diameters, respectively, with inhibition as 88.46, 103.37 and 88.46%, respectively. Those consequences suggest that Mix 5 extract turned into the maximum efficient extract and confirmed effective antibacterial interest towards microorganism that poisoned food and made us to decide the MIC values of blend 5 most effective towards *E. coli* O157:H7, which found to be 3 mg mL^{-1} (Table 3). The determined version in antibacterial sports of the IPE may be as a minimum, due to the principal components of the plant extract[3] Because of its sizable components, i.e., gallic (GA) and chlorogenic acid (CGA), the IPE may additionally doubtlessly have an effective antibacterial interest. The statistics obtained in Table 5 can verify this assumption. Statistics represented in Table 1, 2 and 3, using t-test at 95% level, demonstrates that there may be variable significant deference ($p<0.05$) in inhibition zone of the examined extracts beneath have a look at.

Evaluation of the most effective extracts and their mix: The phytochemical characters of clove, sage, pomegranate, *Cassia fistula* water extracts and their mixture are summarized in Table 4. The aqueous extracts of clove and pomegranate have been found to incorporate tannins, alkaloids, glycosides,

Table 1: Inhibition zone diameter (mm) of individual plant extracts (IPE)

Plant extracts	*Inhibition zone of individual plant (IPE) extracts (mm)	Inhibition (%)
Control	0±0.00[a]	0.00
Clove	25±0.13[b]	96.15
Marjoram	20±0.11[b]	76.92
Sage	22±0.17[b]	84.61
Pomegranate	13±0.12[c]	50.00
Turmeric	10±0.01[c]	38.46
Cassia fistula	22±0.14[b]	84.61
Pepper	24±0.16[b]	92.31
**Ciprofloxacin	26±0.11[b]	100.00
LSD	6.17	-

*Each value represent mean of three replicates±SE, **Antibiotic reference standard used at 20 µg/disc

Table 2: Inhibition zone diameter (mm) of mix plant extracts (MPE)

Plant extracts mix	*Inhibition zone of individual plant extracts (mm)	Inhibition (%)
Control	0.0±0.0[a]	0.00
Clove+Marjoram (Mix 1)	20±3.14[b]	76.92
pomegranate+Pepper (Mix 2)	22±1.27[b]	84.61
Sage+*Cassia fistula* (Mix 3)	20±0.89[b]	76.92
Clove+Marjoram+Sage+Pomegranate+Turmeric+*Cassia fistula*+Pepper (Mix 4)	23±2.48[b]	88.46
Clove+Sage+Pomegranate+*Cassia fistula* (Mix 5)	28±1.15[c]	103.37
Marjoram+Pomegranate+*Cassia fistula*+Pepper (Mix 6)	23±3.01[b]	88.46
**Ciprofloxacin	26±0.17[c]	100.00
LSD	4.78	-

*Each value represent mean of three replicates ±SE, **Antibiotic reference standard used at 20 µg/disc

Table 3: Inhibition zone diameters and MICs of Combination Mix 5 (Clove, sage, pomegranate, *Cassia fistula*) aqueous extract against bacterial strains

	Inhibition zone diameter (mm)**						
Mix extract/Concentration	0.75*	0.50*	0.25*	0.125*	0.062*	0.031*	MIC
Mix 5	28±0.89	20±1.06	12±1.14	8±0.09	5±0.03	3±0.03	3±0.01

*Each value represent mean of three replicates ± SE, MIC: **Minimum Inhibition concentration (mg mL^{-1}) diameter included 5 mm well diameter

Table 4: Phytochemical screening of the tested aqueous extracts

Phytochemicals	Clove	Pomegranate	Sage	*Cassia fistula*	Mix
Tannins	+	+	+	+	+++
Reducing sugars	+	+	-	+	++
Glycosides	+	+	-	-	++
Alkaloids	+	+	+	-	++
Flavonoids	+	+	+	+	+++
Volatile oils	-	-	-	+	+
Amino acids/Proteins	-	+	-	+	++
Terpenoids	-	+	-	+	++
Saponins	-	+	+	+	++
Steroids	+	-	+	-	++

Data represented was confirmed in duplicates, +: Detected, -: Not detected

flavonoids, decreasing sugars. But an aqueous extract of Cassia fistula became determined to comprise tannins, flavonoids, amino acids/Proteins, unstable oils, terpenoids, lowering sugars and saponins. The Phytochemical screening and qualitative estimation screen that the medicinal flora used here has been rich in tannins, phenol, terpenoids, flavonoids and little volatile oils. The aqueous extracts of sage show case only tannins, alkaloids, flavonoids, saponins and steroids.

Phenolic compounds are very essential factors of plants because of their scavenging capability and their hydroxyl groups. The existence of 17 Phenolic compounds in Table 5 is present in HPLC evaluation. In clove, sage, pomegranate, cassia fistula and mix extracts, gallic acid, catechin, rutin and quercetin have been verified. Table 5 shows the presence of 17 Phenolic compounds in extracts.

Overall phenolic content (TPC) of clove, sage, pomegranate, Cassia fistula water extract and their combination have been expressed in phrases of phenol equal and the mean values of their phytochemical contents are shown in Fig. 1. The TPC for aqueous extracts below looks at ranged from 26.79±1.06 mg GAE/g dry weight in pomegranate to 244.50±9.12 mg GAE/g dry weight in clove.

Table 5: Phenolic compounds analysis of clove, sage, pomegranate, *Cassia fistula* aqueous extract and their mix via HPLC

	Concentration (μg mL^{-1})				
Phenolic compounds	Clove	Sage	Pomegranate	*Cassia fistula*	Mix
Gallic acid	837.97±3.02	79.31±2.45	20.59±0.11	48.05±0.56	246.48
Chlorogenic acid	69.21±1.14	5.37±0.08	0.00±0.00	10.59±0.11	21.29
Catechin	0.00±0.00	4.51±0.03	46.45±2.10	0.00±0.00	12.74
Caffeine	0.00±0.00	0.00±0.00	0.00±0.00	1.68±0.002	1.68
Caffeic acid	4.74±0.003	14.19±0.09	0.87±0.001	2.68±0.002	5.62
Syringic acid	0.00±0.00	3.75±0.005	0.00±0.00	2.67±0.001	1.60
Rutin	0.00±0.00	4.04±0.001	0.00±0.00	31.67±1.001	8.92
pyrocatechol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00
Ellagic acid	0.00±0.00	8.23±0.05	0.00±0.00	0.00±0.00	8.23
Coumaric acid	1.06±0.001	2.08±0.002	0.00±0.00	6.45±0.08	2.39
Vanillin	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00
Ferulic acid	0.00±0.00	0.64±0.004	0.00±0.00	0.00±0.00	0.64
Naringenin	15.03±0.012	0.00±0.00	0.00±0.00	0.00±0.00	15.03
Propyl gallate	0.00±0.00	1.96±0.003	0.00±0.00	8.66±0.012	2.65
4.7-Dihydroxyisoflavone	0.00±0.00	5.21±0.01	0.00±0.00	1.34±0.001	1.63
Quercetin	1.04±0.008	49.94±3.02	2.32±0.002	1.20±0.001	1.14
Cinnamic acid	0.09±0.001	1.57±0.002	0.41±0.002	0.40±0.001	0.61

Data are means of 3 replicates (n = 3)±standard error

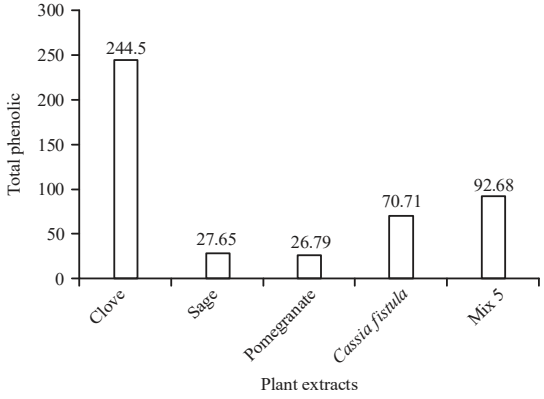

Fig. 1: Total phenolic content of clove, sage, pomegranate, *Cassia fistula* aqueous extract and their mix (mg GAE/100 g of DW)

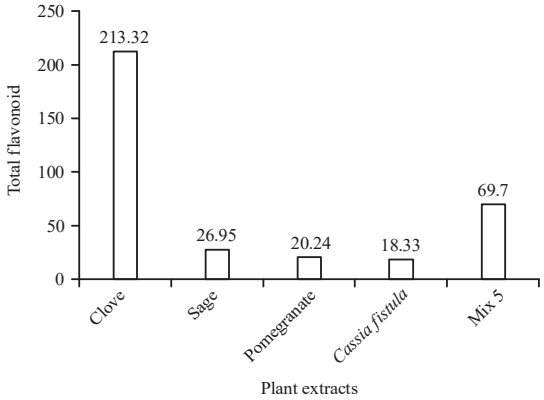

Fig. 2: Total flavonoids content of clove, sage, pomegranate, *Cassia fistula* aqueous extract and their mix (mg GAE/100 g of DW)

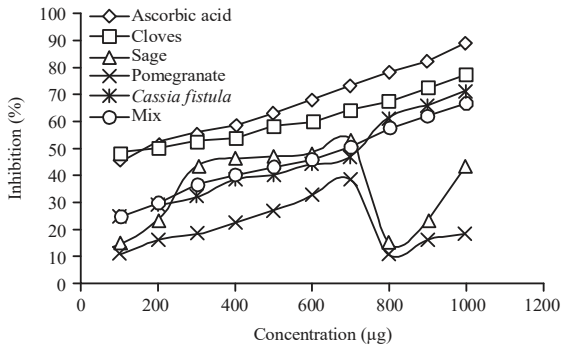

Fig. 3: DPPH radical scavenging activity by the different aqueous extracts tested

Clove became extensively higher than the ones of different aqueous extracts ($p<0.05$). Additionally, TPC in sage and pomegranate and *Cassia fistula* showed no massive differences and have been notably decreased than clove (Fig. 2).

Antioxidant activity evaluation of the most effective extracts and their mix are shown in Fig. 3 and 4. As shown, the aqueous extract of clove, sage and *Cassia fistula* at 1000 μg mL^{-1} revealed potential radical scavenging interest with a percentage decrease of 78.05, 43.6 and 71.37%, respectively. While pomegranate supplied particularly low unfastened radical scavenging interest of 18.52%. In evaluation with industrial antioxidant L-ascorbic acid (89.56%), pomegranate confirmed better IC_{50} cost (103.82) as it had weaker DPPH scavenging pastime. As shown in Fig. 4, the aqueous extracts of clove, sage, *Cassia fistula* and mix extracts showed much less IC_{50}

Table 6: Antimicrobial effect of combination Mix 5 extract on *E. coli* O157:H7 in beef meat and fillet-fish (log10 CFU g^{-1})

	Populations of *E. coli* O157:H7 in beef meat and fillet-fish throughout the refrigerated storage time at 6±1 °C (CFU g^{-1})								
Extract/days	0	1	2	3	4	6	8	10	15
Beef meat									
Control	3.6×10^{3Aa}	3.7×10^{4Ab}	2.6×10^{5Ac}	3.5×10^{5Ab}	2.3×10^{6Ad}	1.3×10^{8Ae}	1.8×10^{9Af}	3.6×10^{9Ag}	5.6×10^{9Ah}
0.25 mg g^{-1}	3.6×10^{3Aa}	2.4×10^{3Ba}	2.0×10^{3Ba}	2.8×10^{2Bb}	2.4×10^{2Bb}	0.9×10^{2Bc}	0.0^{Bd}	0.0^{Bd}	0.0^{Bd}
0.50 mg g^{-1}	3.6×10^{3Aa}	1.3×10^{3Cb}	1.4×10^{3Cb}	2.3×10^{2Cc}	0.7×10^{2Cd}	0.00^{Ce}	0.0^{Be}	0.0^{Be}	0.0^{Be}
1 mg g^{-1}	3.6×10^{3Aa}	0.8×10^{3Db}	1.1×10^{3Db}	2.4×10^{2Dc}	0.9×10^{2Dd}	0.00^{Ce}	0.0^{Be}	0.0^{Be}	0.0^{Be}
Fillet-fish									
Control	1.0×10^{4Aa}	3.2×10^{4Ab}	2.5×10^{5Ac}	3.7×10^{5Ad}	2.6×10^{6Ae}	1.6×10^{8Af}	1.7×10^{9Ag}	3.4×10^{9Ah}	5.1×10^{9Ai}
0.25 mg g^{-1}	1.0×10^{4Aa}	3.3×10^{3Bb}	3.0×10^{3Bb}	3.5×10^{2Bc}	2.7×10^{2Bc}	1.2×10^{2Bd}	0.0^{Be}	0.0^{Be}	0.0^{Be}
0.50 mg g^{-1}	1.0×10^{4Aa}	2.5×10^{3Cb}	2.4×10^{3Cb}	3.2×10^{2Cc}	1.9×10^{2Cd}	1.0×10^{2Cd}	0.0^{Be}	0.0^{Be}	0.0^{Be}
1 mg g^{-1}	1.0×10^{4Aa}	1.7×10^{3Db}	1.1×10^{3Db}	2.7×10^{2Dc}	0.7×10^{2Dd}	0.0^{De}	0.0^{Be}	0.0^{Be}	0.0^{Be}

Data represented are average of triplicates, means with different capital letters within a column are significantly different at $p\leq0.05$, means with different small letters within a row are significantly different at $p\leq0.05$

Table 7: Sensory evaluation of beef meat and fish after treatment of Mix 5 extract with different concentration (0, 25, 0.50 and 1%)

Samples	Color (10)	Odor (10)	Taste (10)	Texture (10)	Appearance (10)	Overall acceptance (10)
Beef burger						
Control	8.6±0.115	8.7±0.145	9.5±0.125	8.4±0.135	9.6±0.057	9.4±0.047
T 0.25%	8.4±0.145	8.0±0.078	9.0±0.088	8.7±0.115	8.1±0.037	8.2±0.047
T 0.50%	8.5±0.230	9.6±0.120	9.2±0.152	8.6±0.176	9.5±0.088	9.3±0.135
T 1%	7.5±0.185	7.6±0.057	8.1±0.047	7.3±0.057	8.2±0.057	8.0±0.115
Fish burgers						
Control	9.2±0.115	8.6±0.145	9.7±0.145	8.3±0.115	9.8±0.057	9.9±0.057
T 0.25%	8.5±0.135	9.8±0.088	9.6±0.058	8.4±0.115	9.4±0.027	9.7±0.027
T 0.50%	9.5±0.230	9.6±0.120	9.5±0.152	8.6±0.176	8.5±0.088	9.0±0.155
T 1%	8.5±0.185	8.0±0.070	8.7±0.057	7.3±0.057	8.2±0.057	8.5±0.115

Data represented are average of triplicates±standard error

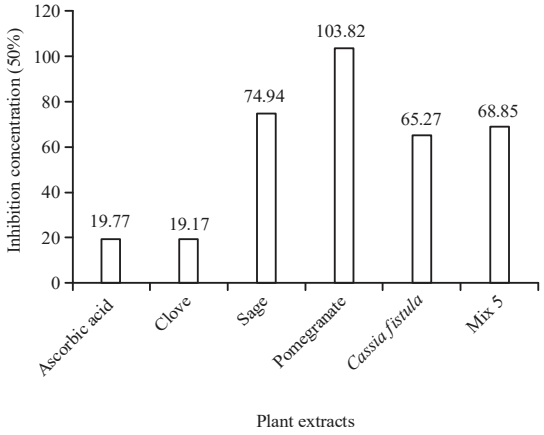

Fig. 4: IC_{50} by the different aqueous extracts tested

values akin to the standard which had very less IC_{50} price as they'd more potent DPPH scavenging activity.

Inhibitory impact of Mix 5 extract against in minced beef meat and fillet-fish: It's far nicely recognized the *in vitro* impacts of plant extracts on pathogenic bacteria but few researches have discussed the influences of these compounds on muscle food -related pathogens. In this look at *E. coli* O157:H7 colonies changed into inoculated onto red meat and fillet fish uncovered to surface spray programs with three different concentrations of Mix 5 had been investigated throughout the refrigerated storage time at 6±1 °C.

Data represented in Table 6 sincerely suggests great differences ($p\leq0.05$) between means of bacterial populations as affected either by different concentrations of Mix 5 or throughout the refrigerated storage time. Compared to control 0.25, 0.50 and 1.00 mg g^{-1} Mix 5 significantly reduced numbers of *E. coli* O157:H7 in beef meat by 3.6 log CFU g^{-1} after 8, 6, 6 days of refrigerated storage time, respectively. However, the above 3 concentrations decreased numbers of *E. coli* O157:H7 in fillet fish via 1.00 log CFU g^{-1} after 8, 8, 6 days of refrigerated storage time, respectively. Prolonging the shelf life of fish and fishery products by reducing *E. coli* and other microbial loads. It is of interest to mention that *E. coli* counts exceeded the recommended limits of more than 2 log CFU g^{-1}, indicating poor hygiene practices.

Sensory evaluation: Contemplating the maximum appropriate parameters which include smell, taste and ordinary acceptability (Table 7), sensory evaluation of pork meat and fish fillet samples turned into done. Our information of sensory assessment suggests that Mix 5 at 0.50 mg g^{-1} concentration significantly influenced ($p<0.05$) all attributes evaluated.

The overall acceptability and taste of fish burgers handled with Mix 5 at 0.25 and 1.00 mg g^{-1} became unacceptable by the panelists. The sensory evaluation of beef burgers supplied with Mix 5 was acceptable by the panelists at the level of 0.25 mg g^{-1} and unacceptable at the level of 0.50 and 1.00 mg g^{-1}. Preliminary sensory trials, however, stated that it would be inappropriate to apply PIEO at levels greater than 1 mg g^{-1}. For this reason, 0.25-1.00 mg g^{-1} of Mix 5 levels were applied to fish burgers and beef burgers in this study.

DISCUSSION

Antibacterial efficiency of seven individual plants extracts (IPE) under study against *E. coli* O157:H7, have been evaluated qualitatively and quantitatively, by following the existence or lack of inhibition zone and measuring place diameters.

As proven from Table 1-3, clove, pepper and Mix 5 had been extra effective against the tested bacterial microorganism[29,30]. The antimicrobial influences of spices and herbs on *E. coli* have been suggested. The discrepancy in antibacterial efficiency may be attributed to variations in chemical composition because of the complex chemical composition of coli and other gram-negative bacteria. As mentioned previously, plant sources with phenolic compounds were well known to have broad-spectrum antimicrobial functions[31].

It is of interest to mention that terpenoid, alkaloid and phenolic compounds are well known to have broad antimicrobial properties against the growth of food-borne bacteria and spoilage. Many scientists connected the inhibitory impact of those substances to their affiliation with microbial membrane enzymes and proteins, causing their disruption to spread a flux of protons out of doors the cell which can result in cellular dying or inhibit enzymes required for amino acid biosynthesis[32]. Others counseled that these substances have hydrophobic traits them to react with microbial cellular membranes and mitochondrial proteins which disturb their structures and regulate their permeability[12]. Our consequences of clove obtained right here have been according those said through[33].

Studies of the phytochemical screening and qualitative estimation well-known shows that the components of the plant used had been wealthy in phenol, flavonoids, terpenoids and little cardiac glycosides[34]. GA brought about irreversible adjustments in cellular permeability and membrane capabilities through reducing poor ground load, changes in hydrophobicity and incidence of neighborhood rupture or pores in the cell membranes with consequent leakage of a critical intracellular component[35]. The reason for the low antibacterial interest of the pomegranate and turmeric water extracts in our study is due to the evaporation of the important active ingredients during boiling[36].

Total flavonoid contents of tested extracts had been expressed in terms of gallic acid equal and aqueous extract of the TPC for the identical extracts. The antioxidative effect of phenols has attracted the interest of several researchers and this may be revered to their redox properties which increase their capability to adsorb and scavenge free radicals[37,38]. Our consequences found out a more phenolic content than studies on numerous sweet and warm peppers indicated. This difference may be attributed to extraordinary cultivars used as well as the growing conditions[36].

Flavonoids free radical scavenging potential is attributed to their organic activity which includes antioxidants, anticancer and anti-inflammatory[37]. Ordinary, the considerable amount of aqueous extracts of flavonoids and phenolic substances relative to the norms on these studies showed important versions in antioxidant interest.

The evaluation of plant extract's antioxidant activity became explored with the aid of its capacity to decrease DPPH, a stable free radical and any molecule that can give DPPH an electron or hydrogen[30]. In evaluation, an aqueous extract of pomegranate, showed maximum IC_{50} cost as it had low DPPH scavenging activity and those results are within the equal fashion with the ones observed[38]. The excessive phenolic contents of clove and Cassia fistula show the linear correlation between phenolic content and antioxidant interest and this turned into in accordance with the statistics posted[12]. In evaluation, an aqueous extract of pomegranate, showed maximum IC_{50} cost as it had low DPPH scavenging activity and those results are within the equal fashion with the ones observed[39]. The excessive phenolic contents of clove and Cassia fistula show the linear correlation between phenolic content and antioxidant interest and this turned into in accordance with the statistics posted[12]. The antioxidative effect of phenols has attracted the interest of several researchers and this may be revered to their redox properties which increase their capability to adsorb and scavenge free radicals[40,41].

Sensory evaluation of plant extracts has been studied by Solomakos *et al.*[42], who observed that the addition of 0.6 mg/100 g of thyme oil was organoleptically acceptable. Other researches also demonstrated the sensory viability of adding essential oils to meat products. It is indicated[43] that marjoram EO added 0.11 mL/100 g of fresh sausages had the same acceptability as a non-essential oil item[44]. The addition of oregano EO (0.01 mL/100 g) to chicken promoted desirable

odor, according to a panel of skilled evaluators. It is also illustrated that 0.02 mL/100 g of mortadella oregano, rosemary and thyme essential oils acquired comparable or greater ratings than vital oil-free samples[45-48]. Finally, this study explains the importance of using natural plant extracts (clove, marjoram, sage, pomegranate peel, Turmeric, *Cassia fistula* and black pepper) to preserve meat and meat products from pathogenic bacteria such as *E. coli*, an alternative of industrial preservatives that have harmful effects on humans. These results will be considered as a pioneer data which will help other researchers to continue using natural plant extracts in food preservation.

CONCLUSION

Among the herbal plants tested, only five had strong antimicrobial activity for *E. coli* O157:H7. The Mix 5 aqueous extract demonstrated the greatest inhibition of *E. coli* O157:H7 and reduced its populations in beef and filet fish. This research indicates that the use of herbal extracts can lead to reduced pathogens populations on beef meat surfaces. According to the result, the selected herb extracts can afford antibacterial potentials against *E. coli* O157:H7 in beef and fish products and also work as natural antioxidant so it could be used as a natural preservative for the control and prevention of meat and fish contamination.

SIGNIFICANCE STATEMENT

This study explains the importance of using natural plant extracts (clove, marjoram, sage, pomegranate peel, turmeric, *Cassia fistula* and black pepper) to preserve meat and meat products from pathogenic bacteria such as *E. coli*, an alternative of industrial preservatives that have harmful effects on humans. These results will be considered as a pioneer data which will help other researchers to continue using natural plant extracts in food preservation.

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