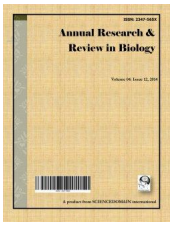

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Detection and Control of Foodborne Pathogenic Bacteria Using *Solanum nigrum* Extract as Antibacterial in Meat Products

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Authors' contributions

This work was carried out in collaboration between all authors. Author GMH designed the study,performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and work of experiment of the study. Author AMZ phytochemical analysis and managed the analyses of the study with Author GMH. Author MMAS Author work of Cytotoxicity assay. Author EEH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Foodborne pathogenic bacteria cause many diseases for the human after eating the spoiled food. For that reason, different meat products produced by different companies in Egypt were collected (during May-Sept 2017), and subjected to microbial analysis.

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Aims: The antibacterial activity of the *solanum nigrum* extract against the foodborne Pathogenic bacteria isolated from the collected meat samples such as; *Salmonella* sp, *E.coli*, *E.coli* H7, *Bacillus cereus,* Staphylococcus were evaluated and detection by Multiplex PCR.
Methodology: Multiplex PCR using different primers specific for either structure or function genes for the most common food born bacteria was approached for detection of the existing bacterial strains in the collected samples. However, the selective media results were insured by the multiplex PCR. Antibacterial activity of *S. nigrum* leaves extract against Foodborne Pathogenic Bacteria
Results: The antibacterial activity of *S. nigrum* leaves extract was tested against the isolated foodborn bacteria; *Salmonella* sp, *E.coli*, *E.coli* H7, *Bacillus cereus*, *Staphylococcus areus*, and streptococcus pyogenes) from the collected meat samples, the results showed that the diameter of the inhibition zones was ranged from 1.5 to 2.6 cm. The highest antibacterial activity was demonstrated against *Bacillus cerueus* and *E.coli* H7 but the lowest activity was observed with *Staphylococcus aureus*. The MIC for the plant extract was 12.5 mg/ml. The *S. nigrum* antibacterial activity may result in the high content of phenolic compounds and Flavonoids in its extract.
Conclusion: PCR is more acqurate than the selective media method to Detection Foodborn Pathogenic Bacteria and *S. nigrum extract* could be used as control agent against the foodborne Pathogenic bacteria in processed meats.

Keywords: Solanum nigrum; foodborne bacteria; multiplex PCR; antibacterial; meat products.

1. INTRODUCTION

Food is the main source of energy for both humans and animals. Meat as animal product could be easily contaminated by a large number of microbial pathogens which play an important role in human foodborne illness [1]. The WHO defines foodborne illnesses as diseases, usually either toxic or infectious in nature. There are more than 200 known causative agents can cause foodborne disease the symptoms and severity of foodborne illnesses vary, range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes [1].

The World Health Organization (WHO) reported that 1.8 million people died in 2005 from diarrheal diseases and a high proportion of these cases due to contamination of food and drinking water (WHO 2008). Although a large number of bacterial strains have been identified and be involved in foodborne diseases (WHO 2008). In developed counties, the annual incidence of microbiological foodborne illnesses is estimated to be around 30% of the population [2]. More than 250 different food borne diseases (FBD) have been described, and the bacteria are the causative agents of two thirds of food borne disease outbreaks [3]. The most common bacteria causing food borne illness are; *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeri smonocytogenes*, *Clostridium botulinus*, *Vibrio parahaemolyticus* and others [4,5].

Biswas and others (2008) [6] reported that both animals and poultry foods are the main reservoirs for different food borne pathogens. Furthermore, the sources of food borne bacteria could be the animal environment or contamination initiated during the food processing [7]. It was observed that animal feces considered the main source of meat contamination before and after the food processing [8]. More one scientist reported that Food borne illnesses resulted from eating the meat are mostly caused by different types of bacteria such as; *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* [9-12].

Different methods were development for controlling microbial diseases resulted from different types of pathogenic bacteria by food safety management and observation [13]. Cultivation, isolation and identification of food borne bacteria from the contaminated food is the first step for pathogen control [14]. Among the identification methods, Polymerase Chain Reaction (PCR) is heavily used in food microbiology for bacterial identification. But PCR reaction could be inhibited by different inhibitors existed in the contaminated food. Moreover, PCR cannot be able to differentiate between vegetative cells and dead cells [15]. On the other hand, other method based on instruments usage in the food industry which can help to detect pathogens such as the different developed sensors [16,15,17-24]. This kind of instrument has also been used to detect the *E. coli* in food samples [25] and also to detect the volatile

compounds produced in stored chicken according to storage time and temperature [26,27].

Medicinal plants have the antimicrobial properties could be used as a good substitution for the biogenic antibiotics to control or prevent bacterial and fungal growth in different food products [28,29]. In the last decade scientists gave good attention and good interest in using natural extracts, such as extracts of spices and herbs, for food preservation [30-33]. *Solanum nigrum* plant contains steroid, alkaloids such as Solamargine, solasonine, solanine and saponin which used for antitumor therapeutics [34]. solanum nigrum derived compounds of saponins and other alkaloids have antimicrobial and antioxidant activity reported [35,36]. Solanum nigrum derived glycoproteins have a dose dependent radical scavenging activity against many free radical of DPPH, OH, Superoxide anion and also induce apoptosis in HT-29 cells [37-39].

The aim of this work is to compare between two detection methods for food born bacterial pathogens. The two methods are; the multiplex PCR and the selective media. Moreover, testing the *S.nigrum* leaves extract as antibacterial agent.

2. MATERIALS AND METHODS

2.1 Sample Collections

Meat samples; thirty six meat product samples were randomly collected (12 from each product Beef, Sausage and Pastirma) from different Super Markets at Alexandria and New Borg El Arab Cities show Fig. 1A. The collected samples were immediately transported in insulated ice containers to the laboratory for microbial analysis using both conventional method and PCR technique. On the other hand, leaves samples of *Solanum nigram* wild plant were collected from the field of Borg El Arab City, Alexandria, Egypt. The plant was identified and authenticated by the taxonomists of Botany Dep., Faculty of Science, Mansoura University, Egypt (Fig.1B).

2.1.1 Collection and Preparation for Meat Samples

Ten gram of the solid sample was weighed and aseptically taken into a sterile bottle containing 90 ml sterile normal saline. It was homogenized with sterile blender (Retsch, GM 200, and Australia) at 3000 rpm for 5-10 min. A 1mL aliquot of homogenate was transferred to a test tube containing 9 mL of sterile buffered peptone water 0.1% to make 10-2 dilution and shaken well with vortex mixer (Digosystem, VM-1000, Taiwan). Serial dilutions up to 10-5 were prepared to be used for DNA extraction. The same sample subcultured on selective media (Brilliant Green Agar, Mannitol Salt Agar (MSA), Blood Agar, MacConkey, Brilliance Bacillus Cereus Agar) for further confirmation and microbiological analysis.

2.2 Isolation the Pathogenic Bacteria from the Collected Meat Samples on Selective Media

Different selective media were used for isolation of the expected pathogenic bacteria which not detected by the Multiplex PCR. In briefly, Brilliant Green Agar medium was used for Salmonellae isolation, Mannitol Salt agar (MSA) used for *Staphylococcus aureus* and *Staphylococcus epidermidis*, whereas Blood Agar medium was used for isolation of *Streptococcus pyogenes*. MacConkey medium was used to isolate *E.coli* while, Brilliance *Bacillus Cereus* Agar was used for isolation of Bacillus cereus and the medium Chromogenic *E.coli* O157: H7 Agar was used for isolation *E. coli* O157:H7 from Sausage samples according to [40].

2.3 Bacterial Detection Using Multiplex PCR

A 100 mg of each meat sample was subjected to DNA extraction using DNA extraction kit (Qiagene, USA) according to the manufacture procedures. Eight different specific primers for different specific genes of bacterial species were used in this study (Table 1).The PCR reaction mixture for 50 µl contains: 10µl of 10X reaction buffer, 3 µl of 25 mM MgCl2, 0.5 µl of 10 mM dNTPs, 2 µl of each primer in (Table 1) (10 pmol/µl), 0.25 µl Taq DNA polymerase Promega, 2 µl DNA (100ng), and the volume was completed up 50 with dH2O. A GeneAmp PCR System 9700 (Perkin Elmer, Norwalk, CT) thermo-cycler device was used with the following program: 94ºC for 5 min, as initial denaturation cycle and 35 cycles consisting of; denaturation at 94ºC for 30 sec, annealing of primer at 65ºC for 30 sec and extension for 1 min at 72ºC and finally addition of 3` terminal at 72ºC for 10 min. The DNA Quantity and quality was determined by spectrophotometry

(OD260/OD280) and agarose gel electrophoresis according to [40].

2.4 Plant Taxonomy and Extract Preparation

Solanum nigraum (Enab El-Deeb) was collected from New Borg El Arab City, (El Banger Region), Alexandria, Egypt. The plant was classified by the department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt. The *S. nigrum* is wild plant belongs to family Solanacae. A 20g of dry plant leaves was extracted using water by cold maceration method according to [41]. The water plant extract was lyophilized at -50ºC (Telstar Model 50, Spain) and kept at 4ºC until be used.

2.5 Antibacterial Activity Test for the *S. nigrum* Extract

The antibacterial activity for the crude water extract of *S. nigrum* leaves against five different pathogenic bacteria isolates isolated in this study from the collected meat samples; *Salmonella* sp, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus* and *streptococcus pyogenes*. The antibacterial activity of the plant extract was tested using the well diffusion method according to [42,41]. Wells were made on the agar surface with 5mm cork borer. The extract was added into the wells and the plate was incubated at 37+2ºC for 24 hrs. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter and recorded.

2.5.1 Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using descending concentrations of the *S. nigrum* extract against the five pathogenic bacteria; *Salmonella* sp, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus* and *streptococcus pyogenes* all of them isolated from meat products were tested by the well diffusion method and MIC was determined using descending concentrations of the *S. nigrum* of extract. The crude extract of was diluted using sterile H_2O and different concentrations (3.12, 6.25, 12.5, 25, 50, 75, 100 mg/mL) were tested for their antibacterial activity against; *Sausagelmonella* sp, *E. coli*, *Bacillus cereus*, *Staphylococcus aureus* and *streptococcus pyogenes* according to [43,41]. The formed clear zones were measured and recorded and the MIC for each strain was determined.

2.5.2 Inhibitory effect of *Solanum nigrum* extract against pathogenic bacteria inoculated in minced beef meat

The procedure described by [44,45] was followed in this experiment with slight modifications according to [40]. Post-rigor lean beef were obtained from a slaughter house in Borg El Arab, Alexandria, Egypt. Each piece was immersed in boiling water for 5 min, in order to reduce the number of the microorganisms attached to the surface of the beef meat. The cooked surface of the beef meat was eliminated with sterile knives under aseptic conditions. The pieces of meat prepared as above were minced in a sterile grinder, and portions of 250±0.1 g were put in high-density polyethylene bags. 50 g of the meat samples were inoculated with 10^4 CFU of *S. pyogenes* /g of meat, and 50 g of the meat samples were inoculated with 10^4 CFU of *E. coli*, 50 g of the meat samples were inoculated with 10^4 CFU of *E. coli*-O157:H7, 50 g of the meat samples were inoculated with 10^4 CFU of *Bacillus Cereus*, 50 g of the meat samples were inoculated with 10^4 CFU of *S. aureus*, 50 g of the meat samples were inoculated with 10^4 CFU of *Salmonella* sp. Prior to the inoculation of the meat, the samples were added different concentrations of *Solanum nigrum* extract. For testing the survival of bacteria pathogenic species the concentrations of the potent *Solanum nigrum* was 1%, 0.5% and 0.25% (w/w). The samples were homogenized using a Stomacher at normal speed for 5 min. All the bags containing the samples of meat were refrigerated (7ºC) and examined after 0, 1, 2, 3, 4, 7, 10 and 15 days of storage for each microorganism. The untreated controls were added sterile water (instead of *Solanum nigrum* extract), inoculated with the test bacteria, and stored under the same conditions as the other samples. After preparing and inoculating beef samples at day 0 and following incubation at 6±1ºC, randomly selected bags containing meat were examined for bacteria pathogenic. The bacteria pathogenic isolation was done by adding 25 g of meat to a plastic bag containing 225 ml of 1% peptone water. The samples were homogenized for 1 min using the Stomacher, and incubated at 35ºC for 24 h. From this pre-enrichment (for the resuscitation of possible injured living cells), 1 ml was added to 9 ml of peptone broth and incubated at 35ºC for 24 h. The counts were taken on Tryptic Soy Blood Agar (TSBA) medium plates for pathogenic bacteria by surface plating the appropriate dilutions of the samples aseptically in duplicate.

Three individual replicates of each experiment were performed, in all cases.

2.5.3 Cytotoxicity assay

In 96-well plate, 1 x 10^5 WBCs / well were mixed with 100 µL of serial dilutions of extracts and standard anti-inflammatory drug (hydrocortison). The plate was incubated in humidified atmosphere at 37ºC, 5% CO_2 and 90% relative humidity for 72h. At the end of the incubation period, 20 µL of MTT (5 mg/mL in PBS) was added per well and incubated in CO_2 incubator for 3h. After that plates were centrifuged at 2000 rpm for 10 min to discard MTT solution and then 100 µL of DMSO was added and the absorbance was read at 570 nm using ELISA reader (BMG LabTech, Germany) to estimate the percentage of cell viability. The safe dose (EC_{100}, 100% cell viability) was calculated from the relation between the cell viability and different extracts or dexamethasone using Graphpad Instat software.

2.6 Qualitative phytochemical analysis of *S. nigrum* leaves Water Extracts

The qualitative phytochemical analysis was performed according to (Mbatchou and Kosoono [46]. Among of these phytochemicals compounds are; Tannins, Flavonoids, Alkaloids, Reducing Sugars, Volatile Oils, Glycosides, Amino Acids and Proteins, Saponins (Foam Test), Terpenoids (Salkowki's Test) and Steroids.

2.7 Determination of Total Phenolic Contents in *S. nigrum* leaves Extract

The total phenolic content of the *S. nigrum* extract was determined by Folin-Ciocalteu spectrophotometric method according to [47]. The 0.1 mL of Folin-Ciocalteu reagent was added to 2 mL of the *S. nigrum* extract. The mixture was allowed to stand for 15 min. Then, 3 mL of saturated sodium carbonate 2% (Na_2CO3) was added. The mixture was allowed to stand for 30 min at room temperature and the total phenolic content was determined spectrophotometrically (Labo America, USA) at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of mg of Gallic acid equivalent per gram of the Sausage sample using the linear regression equation obtained from the standard Gallic acid calibration curve; y = 0.006x + 0.253. All samples were analyzed in triplicates [41].

2.8 Trace Element Analysis

About 2 g of ash was digested with a triple acid mixture comprising of nitric acid, sulfuric acid, and perchloric acid in the ratio of 11:6:3, respectively, for the complete removal of organic content. The digested sample was made up to 100 mL using deionized water and then used for assaying of the trace elements by atomic absorption spectroscopy (Analytik Jena-zeenit 700p-Germany) using hollow cathode lamps.

2.9 Antioxidant Activity

The antioxidant activity of *S. nigrum* plants extract was detected according to [48] adopted with suitable modifications performed by of [49]. The inhibition percentage was calculated using Equation (Inhibition % = [(A of control−A of Sausage) /A of control] x100). While the IC50 values were estimated from the percentage of inhibition versus concentration plot, using a nonlinear regression algorithm.

2.10 Statistical Analysis

All data in this study were subjected to one-way analysis of variance (one-way ANOVA) using the statistical analysis software "Co Stat 6.4" [50]. Differences between means were assessed by Duncan's multiple-range test and effects with a probability of $p<0.05$ were considered significant [51].

3. RESULTS

3.1 Bacterial Detection in the Selected Meat Products by Multiplex PCR

The multiplex PCR was approached on the extracted DNA of all the collected samples and the results presented in Table 2 revealed that, amplicons with different molecular sizes 366 and 361bp were observed. These amplicons indicated that the tested samples contaminated with *E. coli* (Beef 1-10, Sausage 2,4,5,7,8 and Pastirma 2, 4) and *E. coli* O157:*H7* (beef 3 and 9, Sausage, 2, 4, 5 and Pastirma 2, 4). Another amplicons with different molecular sizes (284, 404, and 510 bp) were obtained by using different primers for genes; *invA, stx* and *hlyA* which are specific for *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7, respectively.

Table 1. Primers used in bacterial detection

Strain name	Tareget gene	Primer sequence 5`-`3	Amplicon size (bp)
E. coli	ST	TTTATTTCTGTATTGTCTTT ATTACAACACAGTTCACAG	366
Salmonella sp	*InvA* gene	TATCGCCACGTTCGGGCAA TCGCACCGTCAAAGGAACC	275
Staphylococcus aureus	nuc.	TCAGCAAATGCATCACAAACAG CGTAAATGCACTTGCTTCAGG	255
Salmonella typhimurium	fimA	CCTTTCTCCATCGTCCTGAA TGGTGTTATCTGCCCGACCA	85
Klebsiella *pneumonia*	fimA	GTTTAACATTCAGCTGAA TAGGACCAATTGCCGTACCT	85
Staphylococcus aureus	nuclease gene	GCGATTGATGGTGATACGGTT CAAGCCTTGACGAACTAAAGC	276
Bacillus cereus	hemolysin gene	CTGTAGCGAATCGTACGTATC TACTGCTCCAGCCACATTAC	185
Vibrio cholera	toxin gene	GGCAGATTCTAGACCTCCT TCGATGATCTTGGAGCATTC	563
Streptococcus pyogenes	*cpa* locus	GGATATGAGATTGCCGAACCTATTACTTTTAAAG GGAGCCTGTTTATCTTCCATTCGAATAATATCCAC	600
Shigellaspp	ipahgeneShi	CTTGACCGCCTTTCCGATAC CAGCCACCCTCTGAGAGTA	610
E.coli-O157:H7	hlyA gene	GTAGGGAAGCGAACAGAG AAGCTCCGTGTGCCTGAA	361
Helicobacter pylori	ureC gene	GAATAAGCTTTTAGGGGTGTTAGGGG GCTTACTTTCTAACACTAACGCGC	294

Table 2. Bacterial detection in some meat collected samples (Beef, Sausage and Pastirma) using multiplex PCR using specific primers

Sample source	*S. pyogenes*	*E. coli*	*E.coli-O157:H7*	*B.cereus*	*S. aureus*
Beef 1	-	+	-	-	-
Beef 2	-	+	-	-	-
Beef 3	-	+	+	-	-
Beef 4	-	+	-	-	-
Beef 5	-	+	-	-	-
Beef 6	-	+	-	-	-
Beef 7	-	+	-	-	-
Beef 8	-	+	-	-	-
Beef 9	-	+	+	-	-
Beef 10	-	+	-	-	-
Beef 11	-	-	-	-	-
Beef 12	-	-	-	-	-
Sausage 1	-	-	-	-	-
Sausage 2	-	+	+	-	-
Sausage 3	-		-	-	-
Sausage 4	-	+	+	-	-
Sausage 5	-	+	+	-	-
Sausage 6	-		-	-	-
Sausage 7	-	+	-	-	-
Sausage 8	-	+	-	-	-
Sausage 9-12	-				
Pastirma 1	-	-	-	-	-
Pastirma 2	-	+	+	-	-
Pastirma 3	-		-	-	-
Pastirma 4	-	+	-	-	-
Pastirma 5	-	-	+	-	-
Pastirma 6-12	-				

Notes: (+) positive isolation, (-) negative isolatio

3.2 Bacterial Detection in the Selected Meat Products by Selective Media

The selective media is one of the conventional methods used for detection the pathogenic bacteria existing in the biological sample. The results presented in Table 3, showed that different bacterial strains were detected in addition to the two *E. coli* strains which were previously detected by multiplex PCR. *Streptococcus pygony* strain was detected in beef sample 4 and pastirma sample 5, whenever, *E. coli* was detected in beef samples from 1 to 10, sausage samples; 3, 4, 5, 7, 8 and pastirma samples 2, 3*. E. coli* O157:H7 was isolated from beef samples 3, 9, sausage samples 2,3,4,5 and pastirma sample 5. However, *bacillus cereus* was isolated from beef sample 8, sausage sample 2 and pastirma sample 5. The *staphylococcus* isolates were found in beef samples 5, 10, sausage sample 3, where, *salmonella* was detected and isolated from beef sample 4, sausage sample 6 and pastirma sample 4.

3.3 Antibacterial Activity of *Solanum nigrum* Plant Leaves Extract

Due to the presence of six different food born pathogenic bacteria in the collected samples, water extract for herbal plant *Solanum nigrum* was used as an antibacterial agent. The antibacterial activity of the plant extract against the human pathogenic bacteria (*Salmonella sp, E.coli, E.coli* O157:*H7, Bacillus cereus, Staphylococcus areus,* and *streptococcus pyogenes*) were examined and the results tabulated Table 4. It was observed that the diameter of the inhibition zones obtained by the extract against the six examined pathogenic

Table 3. Isolation of pathogenic bacteria from different meat samples (Beef, Sausage and Pastirma) on selective media

Sample	*S. pyogenes*	*E. coli*	*E. coli-O157:H7*	*B.cereus*	*S. aureus*	*Salmonella sp*
Beef 1	-	+	-	-	-	-
Beef 2	-	+	-	-	-	-
Beef 3	-	+	+	-	-	-
Beef 4	+	+	-	-	-	+
Beef 5	-	+	-	-	+	-
Beef 6	-	+	-	-	-	-
Beef 7	-	+	-	-	-	-
Beef 8	-	+	-	+	-	-
Beef 9	-	+	+	-	-	-
Beef 10	-	+	-	-	+	-
Beef 11-12	-					
Sausage 1	-	-	-	-	-	-
Sausage 2	-	+	+	+	-	-
Sausage 3	-		+	-	+	-
Sausage 4	-	+	+	-	-	-
Sausage 5	-	+	+	-	-	-
Sausage 6	-		-	-	-	+
Sausage 7	-	+	-	-	-	-
Sausage 8	-	+	-	-	-	-
Sausage 9-12	-					
Pastirma 1	-	-	-	-	-	-
Pastirma 2	-	+	-	-	-	-
Pastirma 3	-		-	-	-	+
Pastirma 4	-	+			-	-
Pastirma 5	+	-	+	+	-	-
Pastirma 6-12	-					

Notes: (+) positive, (-) negative

bacteria was ranged from 15 to 26 mm. The highest antibacterial activity for the used extract was recorded against both *Bacillus cereus* and *E.coli* O157:H7. On the other hand, the lowest activity of the plant extract was observed (1.5 cm) against *Staphylococcus aureus*.

Table 4. Clear zones of the *S. nigrum* extract activities on isolation pathogenic bacteria

Strains	Diameter of inhibition zone (mm)
Salmonella sp	20±0.56
E. coli	18±0.26
Bacillus cereus	26±0.60
Staphylococcus aureus	15±0.28
streptococcus pyogenes	22±0.47
E. coli-O157:H7	26±0.51

3.4 Minimal Inhibitory Concentration (MIC) of *Solanum nigrum* Plant Extract and Cytotoxicity

The MIC of the *S. nigrum* extract against the food born pathogenic bacteria was detected and the results presented in Table 5 indicated that the MIC value was about 3.12% with *Salmonella* sp, 12.5% with *E. coli*, *Bacillus* cereus and *Streptococcus pyogenes* while it was about 25% with *Staphylococcus aureus* and *E. coli-O157:H7*. The MTT assay should that the *S. nigrum* leaves extract has no toxicity of the tested cell lines.

3.5 Effect of *Solanum nigrum* Extract as Antibacterial on Shelf-life of Minced Beef Meat as a Food Model

In this study minced beef meat was used as a food model to study shelf-life and the inhibitory

effect of *Solanm nigrum* extract against the spoilage pathogenic bacteria. The meat samples were inoculated with 10^4 CFU/g of each bacterium in separate manner. The inoculated meat samples were treated with three different concentration of *S. nigrum* extract (0.25%, o.5%,1%) and the experiment lasted for 15 days. Result in Table 6 revealed that the extract of *Solanm nigrum* shows high antimicrobial activity against the pathogenic bacteria which inoculated

Table 5. The MIC of the *S. nigrum* plant extract as antibacterial

Strains	Diameter of inhibition zone (mm)						
	Aqueous extract (mg/100mL)						
	100	**75**	**50**	**25**	**12.5**	**6.25**	**3.12**
Salmonella sp	24±0.48	19±0.63	13±0.37	9±0.07	5±0.06	3	2
E. coli	19±0.57	16±0.77	12±0.20	8±0.05	5±0.09	-	-
Bacillus cereus	21±0.36	17±0.78	11±0.17	8±0.06	6±0.16	-	-
Staphylococcus aureus	22±0.67	18±0.32	12±0.54	7±0.03	-	-	-
Streptococcus pyogenes	21±0.72	19±0.57	15±0.36	10±0.23	6±0.18	-	-
E. coli-O157:H7	20±0.59	18±0.43	13±0.49	5±0.05	-	-	-

Table 6. Shelf-life for effect *Solanm nigrum* extract as antibacterial on beef meat after treatment

Strains / Conc. extract	Inhibition (CFU/g) in day							
	0	**1**	**2**	**3**	**4**	**7**	**10**	**15**
Salmonella sp								
Control	$3.9x10^3$	$3.5x10^4$	$2.8x10^5$	$3.7x10^5$	$2.4x10^6$	$1.3x10^8$	$1.7x10^9$	$3.5x10^9$
0.25 mg/g	$3.9x10^3$	$2.6x10^3$	$2.1x10^3$	$3.7x10^2$	$2.6x10^2$	$0.9x10^2$	0.0	0.0
0.5 mg/ g	$3.9x10^3$	$1.5x10^3$	$1.6x10^3$	$3x10^2$	$1.8x10^2$	$0.5x10^2$	0.0	0.0
1 mg/ g	$3.9x10^3$	$0.9x10^3$	$1.2x10^3$	$2.6x10^2$	$0.8x10^2$	$0.3x10^2$	0.0	0.0
E. coli								
Control	$3.8x10^3$	$3.6x10^4$	$2.9x10^5$	$3.9x10^5$	$2.6x10^6$	$1.4x10^8$	$1.6x10^9$	$3.6x10^9$
0.25 mg/ g	$3.8x10^3$	$3.2x10^3$	$2.7x10^3$	$3.4x10^2$	$2.4x10^2$	$0.7x10^2$	0.0	0.0
0.5 mg/ g	$3.8x10^3$	$2.8x10^3$	$2.2x10^3$	$2.9x10^2$	$1.3x10^2$	$0.3x10^2$	0.0	0.0
1 mg/ g	$3.8x10^3$	$2x10^3$	$0.9x10^3$	$2.1x10^2$	$0.7x10^2$	$0.2x10^2$	0.0	0.0
Bacillus cereus								
Control	$3.9x10^3$	$3.4x10^4$	$2.7x10^5$	$3.5x10^5$	$2.4x10^6$	$1.5x10^8$	$1.7x10^9$	$3.3x10^9$
0.25 mg/ g	$3.9x10^3$	$3x10^3$	$2.8x10^3$	$3.9x10^2$	$2.5x10^2$	$0.8x10^2$	0.0	0.0
0.5 mg/ g	$3.9x10^3$	$2.5x10^3$	$2.1x10^3$	$3.3x10^2$	$1.4x10^2$	$0.6x10^2$	0.0	0.0
1 mg/ g	$3.9x10^3$	$2x10^3$	$1.4x10^3$	$1.7x10^2$	$0.9x10^2$	$0.4x10^2$	0.0	0.0
Staphylococcus aureus								
Control	$1x10^4$	$3.3x10^4$	$2.6x10^5$	$3.7x10^5$	$2.5x10^6$	$1.6x10^8$	$1.8x10^9$	$3.6x10^9$
0.25 mg/ g	$1x10^4$	$3.2x10^3$	$3x10^3$	$3.6x10^2$	$2.6x10^2$	$1.2x10^2$	0.0	0.0
0.5 mg/ g	$1x10^4$	$2.4x10^3$	$2.6x10^3$	$3.1x10^2$	$1.9x10^2$	$0.9x10^2$	0.0	0.0
1 mg/ g	$1 x10^4$	$1.8x10^3$	$1.7x10^3$	$2.7x10^2$	$0.8x10^2$	$0.5x10^2$	0.0	0.0
Streptococcus pyogenes								
Control	$3.9x10^3$	$3.6x10^4$	$2.8x10^5$	$3.6x10^5$	$2.2x10^6$	$1.2x10^8$	$1.6x10^9$	$3.4x10^9$
0.25 mg/ g	$3.9x10^3$	$3.3x10^3$	$2.9x10^3$	$3.6x10^2$	$2.3x10^2$	$0.6x10^2$	0.0	0.0
0.5 mg/ g	$3.9x10^3$	$2.6x10^3$	$2.2x10^3$	$3.1x10^2$	$1.3x10^2$	$0.3x10^2$	0.0	0.0
1 mg/ g	$3.9x10^3$	$2x10^3$	$1.6x10^3$	$2.8x10^2$	$0.7x10^2$	$0.2x10^2$	0.0	0.0
E. coli-O157:H7								
Control	$3.9x10^3$	$3.7x10^4$	$2.9x10^5$	$3.5x10^5$	$2.4x10^6$	$1.4x10^8$	$1.8x10^9$	$3.2x10^9$
0.25 mg/ g	$3.9x10^3$	$2.9x10^3$	$2.3x10^3$	$3.2x10^2$	$2.5x10^2$	$0.7x10^2$	0.0	0.0
0.5 mg/ g	$3.9x10^3$	$2.4x10^3$	$1.9x10^3$	$2.9x10^2$	$1.4x10^2$	$0.5x10^2$	0.0	0.0
1 mg/ g	$3.9x10^3$	$1.9x10^3$	$1.2x10^3$	$2.3x10^2$	$0.9x10^2$	$0.3x10^2$	0.0	0.0

in minced beef meat by decreasing their count number along the experiment. The relation between the plant extract concentration and its antibacterial activity against all the examined strains was positive. In addition, after 10 days, none of the inoculated bacteria were detected.

3.6 Phytochemical Analysis and Mineral Composition of *S. nigrum* leaves Extract

The results shown in Table 7 indicated the phytochemical components in the *S. nigrum* plant extract are founded in high concentration. Moreover,the mineral concentration in the *S. nigrum* plant extract with (µg/g); Calcium: 240, Magnesium: 95, Sodium: 115, Potassium: 56, Zinc: 12, Iron: 141 and phosphorus: 762 the results presented in Table 8.

3.7 Antioxidant Activity and Total Phenolic Content

The total phenolic compounds of the *S. nigrum* leaves extract showed an acceptable level of antioxidant capacity with IC_{50} (66.59 µg/ml) compared to ascorbic acid (as standard) (IC_{50}=19.94 µg/ml) Table 9. The total Phenolic content in the *S. nigrum* leaves extract was 439.66 mg/ml. The most determined phytochemical compounds in the *S. nigrum* extract are; Tannins Glycosides, Alkaloids, Flavonoids, Terpens and Steroids in different concentration.

3.8 Cytotoxicity and Anti-inflammatory Assay

Table 10 shows EC_{100} dose of *Solanum nigrum* extract toward human WBCs was seven fold higher than EC_{100} of currently used anti-inflammatory drug (hydrocortison) that reflects the high safety of the investigated extract. Both extract and hydrocortisoson were able to reverse the abnormal stimulation index (abnormal proliferation of LPS-stimulated cells WBCs) to normal immune response at 363.29 and 57.32 µg/ml, respectively Table 10.

4. DISCUSSION

When we used Multiplex PCR in detecting the food born bacteria in the collected meat samples, the used primers succeeded to detect only two strains of *E.coli* but failed to detect the others. Multiplex PCR could be used for detect the food borne bacteria but it is not effective more than the selective media method. Selective media method could be used to characterize the possible culturable bacteria founded in the contaminated food. But the PCR may gave false positive results because its results depend on DNA, that is mean the dead cells will be counted. We assume that this could have happened because the food could be a barrier which inhibits the PCR reaction especially the *Taq* DNA Polymerase to amplify the gene and detect the most of the existed bacteria in the sample DNA. The same observation was demonstrated by [52-56] and they assumed that different food constituents may block the PCR amplification. In contrary, (Lee and other [57] postulated that multiplex PCR for detection of food borne bacteria in ready to eat Korean food was effective and informative. Most of the used multiplex PCR in detection of the food borne bacteria depend on primers designed for specific genes such as; (rfbE) gene for *E. coli* O157:H7, (hly) for *L. monocytogenes*, (gyrB) for *B. cereus*, (invA) for *Salmonella spp*., (toxR) for *V. parahaemolyticus*, and the (*nuc*) gene for *S. aureus*, we assumed that may these genes was not present in our strains or may be mutated. The study performed by Valderrama based on the PCR results (especially multiplex PCR) postulated that a commercial rapid detection method for the food borne pathogens was developed and could be approached and generalized [58].

According to result obtained in this study and for more extra mining about the food borne bacteria in contaminated meat samples, selective media method succeeded to detect more strains than the multiplex PCR method, but this not quite good for many reasons. The same observation was obtained by [15] but them considered this method is time consuming. Likewise, the same opinion was presupposed by [59] but [57] add that this method based only one the living cells and the culturable avoiding the dead and non culturable bacteria. Here in, we assume that the selective media method is not quantitative but it is qualitative and the preventative organism is needed either as living or culturable for selecting the suitable antibiotics to control. So, there is no doughty that selective media method is important even the nucleic acid based method was approached. Finally, the selective medium method is, expensive, time consuming, only not effective in microbial counting and time consuming but the PCR is more effective and readable.

Table 7. Qualitative Phytochemical analysis of the *S. Nigrum*

Tannins	Reducing sugar	Glycosides	ALkaloids	Flavonoids	Voltile oils	Amino acids and proteins	Terpens	Saponines	Steroids
+	-	+	+	+	-	+	+	+	+

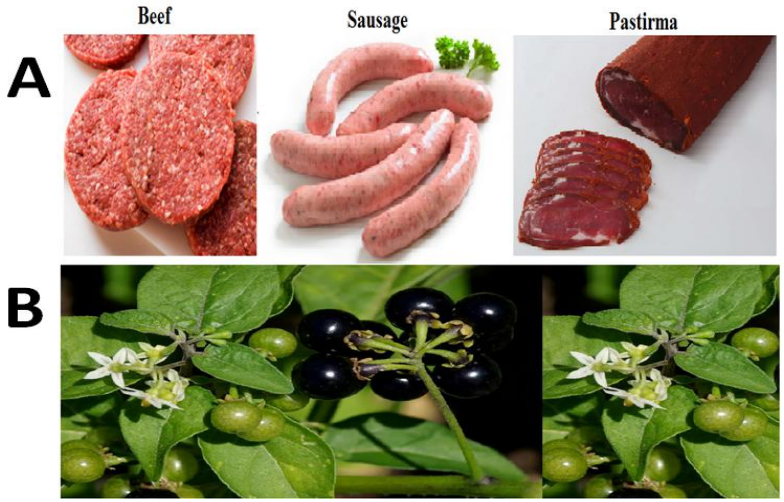

Fig. 1. A: A presentitive meat samples collected in this study. B: picture of *Solanum nigram* plants wild plant showing its leaves, flowers and fruits.

Table 8. Mineral composition of *S. nigrum* leaves

Mineral Element in *S.nigrum* leave extract	Concentration (µg/g)
Calcium (Ca)	240
Magnesium (Mg)	95
Sodium (Na)	115
Potassium (K)	56
Zinc (Zn)	12
Iron (Fe)	141
Phosphorus (P)	762

Based on our findings, *S. nigrum* leaves extract shows high antbacterial activity against the detected strains in this study. These results are in agree with the results obtained by [60] who used the ethanol leaves extract of the *Rhodomyrtus tomentosa* plant and they reported that the extract showed high bactericidal activity against 22 isolates of *Listeria monocytogenes* bacteria in comparing with 15 different biogenic antibiotics. The inhibition zones obtained by the *S. nigrum* extract were in range of 14 to 16 mm and the MIC was ranged from 128 to 512 µg/ml. It was reported that *S. nigrum* leave extract has high antibacterial activity against number of human pathogenic bacteria [61]. In addition, Zubair and his colleges demonstrated the activity of *S. nigrum* leaves extract as antibacterial against different human pathogens such as; *S. aureus*, *Pasteurella multocida*, *E. coli* and *B. subtilis* [62]. The same observation was reported by [63] that *S. nigrum* extract of the whole plant showed antibacterial activity against *B. subtilis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Abbas and other [64] revealed that the extract of the fruit of *S. nigrum* showed high antibacterial activity against both gram negative and gram positive bacteria, and the high inhibition zone were 14 mm and the good dose used from the fruit extract was 15 mg/ml.

It was observed that *S. nigrum* extract contains different chemical constituents which are naturally occurring as well as secondary metabolites such as; alkaloids, flavonoids, saponins, steroids and sterols [65,66]. Results obtained in this study agree with the finding of Modilal and other [67] that *S. nigrum* extract contains many phytochemicals such; alkaloids, terpenoids, flavonoids, saponins, steroids and phenols.

Minimum Inhibitory Concentration (MIC) is a crucial method to determine the lowest concentration of the tested substances that capable of absolutely inhibit and stop the bacterial growth [68]. As it become noted formerly, the prepared *S. nigrum* plant extract with different concentrations of mg/100 ml. The examined concentrations showed realistic antibacterial activity with clear zones in diameters ranged from 2.4 to 0.2 cm with *Salmonella sp* strain (Table 5 and Fig. 2) while with *E. coli* were 1.9 to 0.5 but in case of *Bacillus cereus* clear zones were ranged between 2.1 and 0.6. Also *Staphylococcus aureus* growth was inhibited and the size of the inhibition zones ranged from 2.2 to 0.7 and *E. coli*-O157:H7 growth was inhibited and the inhibition zones ranged from 2 to 0.5. On the other hand, the growth of *Streptococcus pyogenes* was inhibited in range of 2.1 to 0.6 cm. The recorded *S. nigrum* plant extract MIC value was about 3% with *Salmonella sp*, 13% with *E. coli*, *Bacillus cereus* and *Streptococcus pyogenes* and it was about 25% with both of *Staphylococcus aureus* and *E. coli-O157:H7*. Sen and Batra (2012) [69] reported that the importance of MIC to confirm the susceptibility limit of special microorganism to antimicrobial agent and also to monitor the activity of new antimicrobial agents.

Table 9. Antioxdant activity of *S. nigrum* compard with Ascorbic acid standard

Ascorbic acid (µg /mL)	Inhibition (%)	IC_{50} (µg/mL)	Extract conc. (µg /mL)	Inhibition (%)	IC_{50} (µg/mL)
10	43.04	19.94	10	000	66.59
20	50.14		20	000	
30	54.24		30	9.17	
40	58.68		40	14.07	
50	61.28		50	41.89	
60	66.35		60	47.48	
70	71.60		70	52.56	
80	76.63		80	58.79	
90	80.84		90	60.12	
100	87.36		100	68.09	

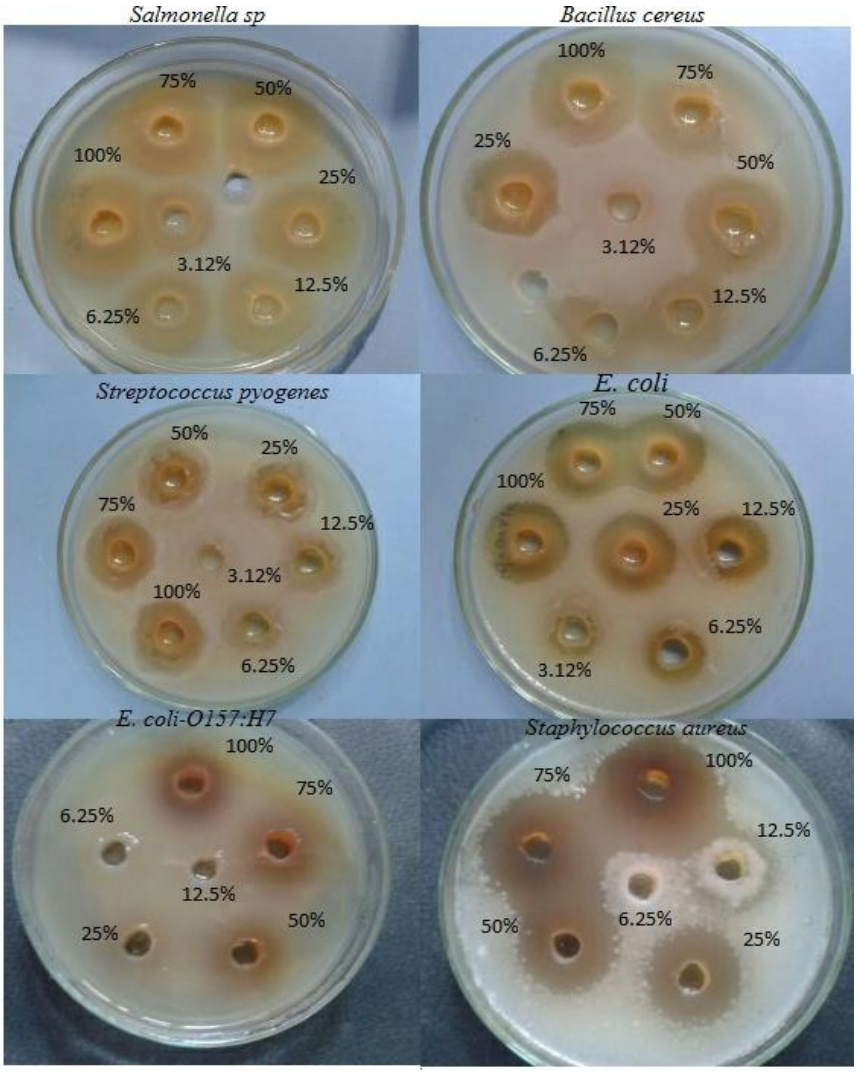

Fig. 2. The Minimum Inhibitory Concentration (MIC) of used plant exract against the pathogenic bacterial strains

The scavenging activity against DPPH free radical using different concentration of *Solanum nigrum* extract was compared with ascorbic acid as standard. *Solanum nigrum* extract concentrations range from 10 to 100 µg/ml showed percentage of inhibition ranged from 9.17 to 68.9%. While, lowest concentrations of 10 and 20 µg/ml were out of inhibition percentage range but the same concentrations of ascorbic acid showed sensible percentage of inhibition 43.04 and 50.14% respectively. Nevertheless, the *Solanum nigrum* extract was able to record IC50 of 66.59 µg/ml compared with 19.94 µg/ml for ascorbic acid. The antioxidant activity of most of the phyto-constituents like flavonoids, catechins, carotenoids, beta carotene and total phenolics have been proved by preventing cellular damage caused by free radicals [70,71].

Both extract and hydrocortisoson were able to reverse the abnormal stimulation index (abnormal proliferation of LPS-stimulated cells WBCs) to normal immune response at 363.29 and 57.32 µg/ml, respectively Table 10. Son et al. [72] postulated that the extraction of *Solanum nigrum* is antioxidant and anticancer especially for the breast cancer MCF-7. The same observation was demonstrated by Heo other (38) when they extrcated the glycoprotein from the *solanum nigrum* fruits and used the compound as antioxidant. On the other hand, the *solanum*

nigrum fruit extract (13 fractions) and leaves extract (17 fractions) showed high antioxidant and anticancer.The shelf life of the used plant extract and its activity as inhibitor for the existed bacteria in the contaminated food were examined and it was observed that antibacterial activity of the *S. nigrum* extract could be prolonged for 15 days in shelf after the production process. It has been reported that plant extract activity on the bacterial contaminated food may be lasts for 13 days after treatment by Rosmarey. (Jałosińska and Wilczak, 2009. Grohs et al.) [73,74]. reported that bacterial growth in fresh pork and beef may be inhibited by using spice mixtures. We assume that the phytochemical and the secondary metabolites existed in *S. nigrum* extract capable to inhibit the growth of the foodborne b acteria and could be used as food additives.

Table 10. Safe doses (EC_{100}, µg/ml) and effective dose (EC_{50}, µg/ml) of the investigated extract and standard antiinflammatory drug

	EC_{100} (µg/ml)	EC_{50} (µg/ml)
Solanum nigrum	773.75±1.15	363.29±4.7
Hydrocortison	104.16±4.6	57.32±5.12

All values were expressed as mean±SEM

5. CONCLUSION

It can conclude that the leave extract of *S.nigrum* could be used as antibacterial for the most common foodborne pathogens. In addition, the *S. nigrum* leave extract is safe and have no toxicity on human WBCs. The extract contains different active ingredient which could be very important for human health in addition to their activity as antibacterial. Due to this valuable activities *S. nigrum* leaves extract could be used as food additive to preservation and control the foodborne pathogenic Bacteria in meat products and its activity lass more than 15 days post production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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