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Studies on some pathogenic microorganisms and their toxins in some contaminated foods

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Abstract

One of the critical concerns about food safety is that various contaminants in food which affecting human health. Every year about 9.4 million foodborne diseases with about 56,000 hospitalizations and 1,300 deaths from major foodborne pathogens are reported in the United States of America. Many foodborne diseases are associated with consumption of dairy and meat products. Some of the meat carcasses on sale might be contaminated with one pathogen or more include *Salmonella spp*., *Escherichia coli* and other enterohaemorrhagic *E. coli* (EHEC), *Listeria monocytogenes*, *Staphylococcus aureus*, *Aspergillus niger*, etc. Toxins are chemical substances produced by diverse microbes (such as bacteria, fungi, and algae) that can pose diverse health threats to humans and animals. Plants produce a large and diverse array of organic compounds that appear to have antimicrobial effect that are named as secondary metabolites.

Keywords: *Foodborne diseases, Toxins*

Introduction

Consumption of dairy and meat products has been associated to a variety of foodborne illnesses. Among the pathogens detected on some of the bodies sold for eating include *Listeria monocytogenes*, *Staphylococcus aureus*, *Aspergillus niger*, and different enterohaemorrhagic *E. coli* (EHEC). While meat is an excellent source of protein, it is also susceptible to microbial contamination, which results in food spoilage and outbreaks of foodborne disease, with severe economic and health effects (Komba *et al*., 2012).

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While bacteria cannot infect the muscles of healthy animals, beef tissue can get contaminated at any time during the production process, from slaughter to transportation (Ercolini *et al*., 2006).

Often, these food-borne diseases can be prevented by the use of a number of natural or synthetic food preservatives. Organic acids, chitosan, bacteriocins, and lactoferrin are all natural preservatives with slow and limited antibacterial activity (Juneja *et al*., 2012). Consumers rarely seek chemical preservatives because to their well-documented detrimental consequences (Pawlowska *et al*., 2012). Although antibiotics have well-documented antibacterial qualities, they are not permitted in food.

Toxins are chemo chemical compounds produced by a range of microorganisms (including bacteria, fungi, and algae) that have the potential to cause harm to people and animals. The molecular structure of a toxin can range from simple single molecules to large macro-biomolecules (Janik *et al*., 2019; Fowler *et al*., 2017). Toxins have the ability to damage the plasma membrane of host cells by creating pores in the phospholipid bilayer. Mycotoxins are toxins produced by certain fungus, bacteria, and algae that can retain their pathogenicity even after the organisms have perished (Pohanka, 2019).

Plants are critical for human health and well-being. They are present in a wide range of items, including medications, cosmetics, dyes, and beverages (Khare, 2004). Secondary metabolites are organic compounds produced by plants that exhibit antimicrobial activity. Additionally, these chemicals' biological activity may be mediated by their ability to form complexes with specific enzymes or directly inhibit those enzymes, as well as their toxicity and effects on membrane structure and integrity, as well as their ability to quench free radicals.

The researchers intended to see how well numerous methanolic extracts of plants, including Cassia nodosa, delonix, melaleuca, casuarin, and magnolia, functioned against pathogenic bacteria using contaminated foods as test subjects. They were once marketed as safe natural preservatives for items like laboratory specimens and tainted food (Bai *et al*., 2016). We're interested in determining which pathogenic bacteria and their toxins are most abundant in contaminated foods in order to create strategies to inhibit them using wild plant components. This research focuses on characterizing harmful microorganisms in food, detecting their toxins, and controlling them with wild plants.

Aim and Objects

- isolate and identify the most predominant pathogenic bacteria and fungi.
- definition of pathogenic microorganisms in foods.
- detection of their toxins and use wild plant to inhibit them.

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Materials and Methods

Collection of food samples

There were a variety of food samples (dairy and meat products) collected in sterile plastic bags with defined weights from open markets and hyper markets and organized into groups. In aseptic circumstances and with defined weight and closed plastic bags, these goods were collected, tagged and transferred to the laboratory.

Isolation of microbial flora

Using Sabouraud's dextrose agar plates, food samples were diluted to 0.01 mL and incubated at 37°C for 24 hours. Isolation was then performed. To obtain a diluted community of their microbiota, liquid or soft goods were diluted in sterile water. Each food source's microbe-containing solution was cultivated on sterile media to see what kind of microbial growth could be predicted. Incubated at 32°C for 48 hours, cultures of nutrient agar showed bacterial growth; Sabouraud's dextrose agar showed fungal growth; both were incubated at 27°C for four days.

Identification of the isolated microflora

The isolated mushrooms were macro culturized and microscopically studied in accordance with Gilman, 1959, and Moubasher, 1993, to be identified as a species. API 20 C Aux method (API system, Montalieu, France) used 19 carbohydrate assimilation tests and a negative control to identify both isolated bacteria and yeast-like fungi. The kit was used as follows, following the manufacturer's instructions (Barnett *et al*., 1990):

API 20 C AUX identifiers provide for the following identification: In order to accurately identify the most commonly seen bacteria and yeasts, API 20 C AUX is a system employed as follows:

Preparation of the strip

Sterilized distilled water was poured into the honey combed wells of the tray to produce a humid environment before closing the incubation box. The reference code for the strain was written on the tray's lengthy flap. The strip was taken out of its packing and placed in the incubation tray.

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Preparation of inoculum

He popped open a 2 ml API suspension medium vial. A part of the yeast colony was suctioned up using a pipette. The employment of youthful cultures was advocated (18 - 24 hours old). It was necessary to prepare the suspension to meet the turbidity criterion of 2 MC Farmland. The previous suspension was gently homogenized with the pipette before the API C medium was added. This prevented bubbles from forming.

Inoculation of the strip

Using a pipette, the API C medium suspension was pipetted into the capsules, where bubble formation was minimized by resting the pipette tip against the side of the capsule. The capsules were filled with care (the surface should be flat or slightly convex, but never concave). The tray was covered and incubated for 24-72 hours at 29 2C with the lid on.

Reading and interpretation of strip

Growth in each capsule was compared to the 0 capsule, which was employed as a negative control, after 48 hours of incubation or 72 hours (if the tests, in particular glucose, are not clear cut after 48 hours). A capsule that was cloudier than the control showed a positive reaction on the results sheet. The lid was removed only when reading the strip and immediately returned afterward to reduce contamination hazards during re-incubation.

Molecular identification of most common isolates

These isolates were used for more conclusive identification techniques such as 16S rDNA differentiation for bacteria, and 18S rDNA differentiation for fungi, to better understand the phylogenetic tree of the discovered species: -

Chromosomal DNA preparation

modified technique of preparing genomic DNA (Sambrook *et al*, 1989). TEN buffers with 1 mg/ml lysozyme were used to resuspend cells centrifuged from overnight culture. The resuspended cells had been previously resuspended in TEN buffers without the lysozyme. After 30 minutes of incubation at 37°C, 75 L of 10% SDS was added and gently mixed until full lysis was achieved.

To this, we added 3 L proteinase K (20mg/ml), and then incubated the tubes at 37°C for 1 hour. In order to get rid of the protein, the mixture was extracted three times with phenol: chloroform: isoamyl alcohol (12:12:1) and once with chloroform. The DNA was precipitated with 0.7 volumes of isopropanol and washed with 70 percent ethanol after being added to 5 M NaCl. A little amount of dried and dissolved DNA was kept in Tris-HCl pH (8.0) at -20°C.

Amplification of the 16S rDNA gene

To amplify a 1500-base pair segment of the 16S rDNA according to the *E. coli* genomic DNA sequence (a standard bacteria), we used polymerase chain reaction (PCR) and universal primers (F: 5' TGAGCCTTGTAAGCGTCCAC 3'; R: 5' TTCATGCCGTGCTTCCAG 3'). EZ-10 Spin Column DNA Gel Extraction Kit (BIO BASIC INC.) was used to purify the leftover mixture after the PCR reaction was completed. The remaining mixture was then sequenced by Sigma Company utilizing agarose gel electrophoresis. They compiled the data and then looked for similarities among it.

Polymerase chain Reaction

The PCR procedure was used to increase the concentration of a specific DNA fragment (Mullis *et al*., 1986). Table (1) lists the PCR components that were pipetted into a PCR test tube for conventional PCR. The cyclic reaction lasted for three minutes at 94°C, followed by 30 cycles of one minute at 94°C, one minute at 55°C, and two minutes at 72°C, with an extra ten minutes at 72°C in between each.

Table (1): Concentrations of different PCR components.

Component	Final concentration
10X polymerase buffer	1 X
dNTPs mix (each 2.5 mM)	0.2 mM
Primer F	1.0 pM
Primer R	1.0 pM
Template DNA	0.1 µg
DNA polymerase (Taq)	1-2 U
$MgC1_2$ 25 mM	25 mM

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Gel Electrophoresis

According to the approach provided by (Sambrook *et al*., 1989), the following gel electrophoresis was carried out: pH (8.0) was used to dissolve the Agarose gel electrophoresis range (0,8-1 percent) in 0.5 x TBE (0.9M Tris5 0.9 Boric acid and 20mM EDTA). In the DNA loading buffer, 30 percent glycerin (w/v), 0.2% bromophenol blue (w/v), and 25 mM EDTA were each added to the sample six times. For a 1% gel concentration, a constant voltage of 120V was used during the procedure. The gel was stained with ethidium bromide staining solution for 20 minutes after electrophoresis. Transilluminator (UVP Dual Intensity transilluminator) was used to see what was going on with DNA).

PCR product purification

An extraction kit called the EZ-10 Spin Column DNA Gel Extraction Kit (BIO BASIC INC.) was used to remove unincorporated nucleotides and excess primers from the PCR result).

Detection of microbial toxins

Toxins from bacteria and fungi isolated from various food sources were compared to real samples at Tanta University Central Lab's Fine Analysis Unit using chromatographic methods. Each kind was evaluated on both a qualitative and quantitative basis.

Collection of wild plants for antimicrobial survey

A total of five plant species were gathered in the summer of 2020 from the North West Coast region of Borg El-Arab City in Alexandria, Egypt. TANE's Herbarium of Botany Department, Faculty of Science houses the voucher specimens for future use in botanical research. Antimicrobial activity was tested on a variety of plant components that had been exposed to active ingredient extraction.

Antimicrobial activity of collected plants against isolated microbial pathogens from food

Electrical mixers ground up air-dried plant materials into powders. The Sohxlete was used to extract 100 ml of methanol from ten grammes of each leaf sample over the course of 48 hours. The extracts were dried in a rotatory evaporator at 35°C under reduced pressure, weighed, and then

stored at -80°C. The antibacterial activity of the selected plant extracts was tested using the cut plug method developed by Pridham, *et. al*. (1956). For each test microorganism, freshly prepared cell suspension was mixed with 9.5ml of melting sterile Sabouraud's dextrose medium (fungi) or nutrient agar medium (bacteria) at 45°C, then spooned out onto sterile Petri dishes and allowed to solidify at room temperature for at least an hour before further testing. A sterile cork borer with a diameter of 0.7 mm was used to make regular wells in the inoculated agar plates. Each tested powder was put into a separate well and weighed out to see how much went into each one. Each test had three replicates, and the fungi and bacteria were cultured at 27°C for three days and 32°C for twenty-four hours, respectively. The average inhibitory zone sizes in millimeters were then measured and compared across all plates.

Plant extract antimicrobial assay

All extracts were tested for antibacterial activity using the agar disc diffusion method established by Manandhar *et al* (2019). The test species were inoculated in nutritional broth and cultured at 37° C for 72 hours to reduce the turbidity to 0.6, resulting in a final bacterial inoculum concentration of 1.5 108 CFU/ml. They were inoculated into Sabouraud's dextrose agar, incubated for seven days at 27 degrees Celsius, and the turbidity was changed to 0.6, yielding 1.5x108 CFU/ml as the final fungal inoculum. 0.2 m Millipore filters were used to filter nutritional agar for bacteria (Sabouraud's dextrose for fungus) into Petri plates. For incubation, 50 l of each extract was injected aseptically into the discs, which were then inserted aseptically into the Petri dishes and heated to 50°C for solvent evaporation. Four plates were utilized for each treatment. The extraction antibacterial activity was established by creating a clean zone around discs in plates. A measurement of the IZ in mm was made after it was discovered.

MIC determination for the most efficient antimicrobial plant extract against most susceptible microbes

To prepare concentrations of 6.25, 12.5, 25, 50, and 100 mg/ml in distilled water, half-fold serial dilutions of a selected plant extract were made; zero concentration was used as a negative control. In order to determine the minimum inhibitory concentration (MIC), a pre-prepared pure spore suspension of each test microorganism was mixed with 9.5 ml of each concentration in sterile test tubes and incubated at 27°C for 3 days for fungi and at 32°C for 24 hours for bacteria. The optical

density of growth was then measured by spectrophotometer at 620 nm for each incubated mixture, results were represented graphically, and MIC was recorded for each tested).

Biochemical analysis of the most potent antimicrobial plant extract

As reported by Jindal and Singh (1975), they utilized Foline-reagent Ciocalteu's and Na2CO3 (20 percent) to estimate total phenolic content. Gallic acid standard curves were employed in the analysis to determine the total phenolic compounds' concentration (mg g-1). Aluminum chloride colorimetric technique (Chang *et al*., 2002) was used to quantify flavonoids, and the results were represented as mg g-1 for flavonoids such as quercetin, a standard flavonoid.

Following the method developed by Ajana *et al*., the total alkaloid content was determined (2012). Alkaloids react with bromocresol green in this approach (BCG). We used a spectrophotometer to find out how many alkaloids were in the sample at 470 nm. mg/g dry weight was used to measure the total alkaloid content. In order to assess the steroid concentration, 10 ml of chloroform was used to dissolve the extracted plant residue. Two milliliters of the solution were added to the test tube, followed by one milliliter of acetic anhydride and one milliliter of 1N H2SO4, all of which were carefully poured on the test tube's walls. The intersection of the two layers generated a reddish violet tint, and the solution turned green, suggesting that the samples contained unsaturated sterols (Wall *et al*., 1954). 5 ml of 5 percent iron ammonium citrate solution was added to the plant extract leftover before it was dissolved in 5 ml of ethanol/water and 1g sodium acetate. After boiling, cooling, and re-boiling, the mixture was perfect. The presence of tannins was confirmed by a dark purple-black ppt (Robinson, 1962). A persistent froth was generated after vigorously shaking the residue with water, showing that saponins were present (Clause and Tyler, 1967).

Ultrastructural effects of the most potent antimicrobial plant extract on the most susceptible microbial growth

Studying antibacterial agents on the ultrastructure of bacterial cells required growing the microorganisms to a cell suspension of 5x106 cells/ml in a liquid nutrition medium and then adding the tested antibacterial agent from a previously recorded MIC. The entire combination was incubated at the correct temperature for 12 hours in a shaking incubator running at 60 revolutions per minute. To collect the cell pellet, the treatment mixture was centrifuged at 3000 rpm for 20 minutes, rinsed with sterile saline solution, and then re-centrifuged (Richards and Cavill, 1976).

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With order to fix the cell protein content and stop all metabolic reactions, the collected cell pellet was buffered in PBS of pH=7.4 and kept at 4°C for two hours after being fixed with glutaraldehyde (2.5% glutaraldehyde). Fixation of the lipid cell content was achieved by washing the fixed sample with 1 percent osmic acid for 30 minutes, then washing the sample three times with PBS for ten minutes each time. The sample was then dehydrated in ethanol concentrations of 30 minutes each for 50, 70, 90, and absolute alcohol, and filtered with acetone for one hour. Sample was embedded in araldite 502 resin for TEM analysis (to fix all cell contents completely) and cut into semi-thin sections in the ultra-cut microtome (LEICA ultracut UCT, Japan), stained with 1 percent toluidine blue, examined to confirm the success of sample preparation, then ultra-thin sections were prepared, stained with uranyl acetate, and counter stained with lead citrate (Ardenne and Beischer, 1940). The transmission electron microscope (JEOL-JEM-100SX, Japan) was used to study and photograph full-stained ultra-thin sections under magnifications of 4000, 5000, 6000, 8000, and 10000X for bigger fungus cells. The beam current was set to 60 A and the high voltage was 80 KV.

Results

It was discovered that pathogenic and non-pathogenic microorganisms coexisted in high numbers in frozen food (dairy and meat products) in the current study.

Table (2): Log number of most common microbes isolated from dairy and meat products in the open market.

No.	Food stuff	Log number of most common isolated microbes			
		Aerobic bacterial count.	Aerobic fungi and yeast count.	Pathogenic bacteria count.	Pathogenic fungi and yeast count.
1	Cow milk	3.02 ± 0.029	2.01± 0.005	1.37 ± 0.019	nil
2	Buffalo milk	4.06 ± 0.033	2.13 ± 0.008	1.67 ± 0.013	nil
3	Peasant cheese	4.11 ± 0.089	2.09 ± 0.007	1.73 ± 0.048	nil
4	Cow butter	2.03 ± 0.006	1.15 ± 0.003	0.97 ± 0.023	nil
5	Buffalo butter	2.33 ± 0.145	1.07 ± 0.002	1.13 ± 0.088	nil
6	Whole chicken	4.03 ± 0.033	3.03 ± 0.031	2.07 ± 0.022	nil
7	Chicken Pane	6.54 ± 0.088	3.14 ± 0.32	2.33 ± 0.031	1.80 ± 0.00
8	Chicken hips	5.30 ± 0.057	2.46 ± 0.014	2.62 ± 0.033	1.67 ± 0.088

9	Cow beef pieces	3.04 ± 0.031	1.08 ± 0.002	1.09 ± 0.086	Nil
10	Buffalo beef pieces	3.10 ± 0.034	1.27 ± 0.005	1.29 ± 0.029	Nil
11	Beef fillet pieces	4.33 ± 0.073	1.19 ± 0.004	1.44 ± 0.032	1.80 ± 0.057
12	Minced cow beef	6.22 ± 0.096	1.03 ± 0.002	2.37 ± 0.033	1.06 ± 0.028
13	Tilapia whole fish	4.09 ± 0.036	2.1 ± 0.023	1.35 ± 0.00	Nil
14	Puri whole fish	5.30 ± 0.057	1.75 ± 0.111	1.17 ± 0.003	1.13 ± 0.023
15	Sardine whole fish	3.07 ± 0.031	1.05 ± 0.006	1.01 ± 0.004	Nil
16	Smoked herring	3.06 ± 0.031	Nil	1.43 ± 0.013	Nil
17	Mackerel whole fish	2.22 ± 0.027	Nil	Nil	Nil
18	Whole shrimp	3.25 ± 0.032	1.02 ± 0.005	Nil	Nil

Table 2 shows that minced beef and chicken pane had the highest count, followed by chicken hips. Whole tilapia fish, beef fillet pieces, whole chicken, peasant cheese, and buffalo milk all have the same calorie count as other foods. Mackerel whole fish, buffalo butter, and cow butter had the lowest combined total count. Milk products had no fungal count, yet the data showed a bacterial count instead. Fungal counts were not found in the beef pieces from cows or buffaloes, but they were found in the same numbers in the beef fillets and the minced cow beef from cows.

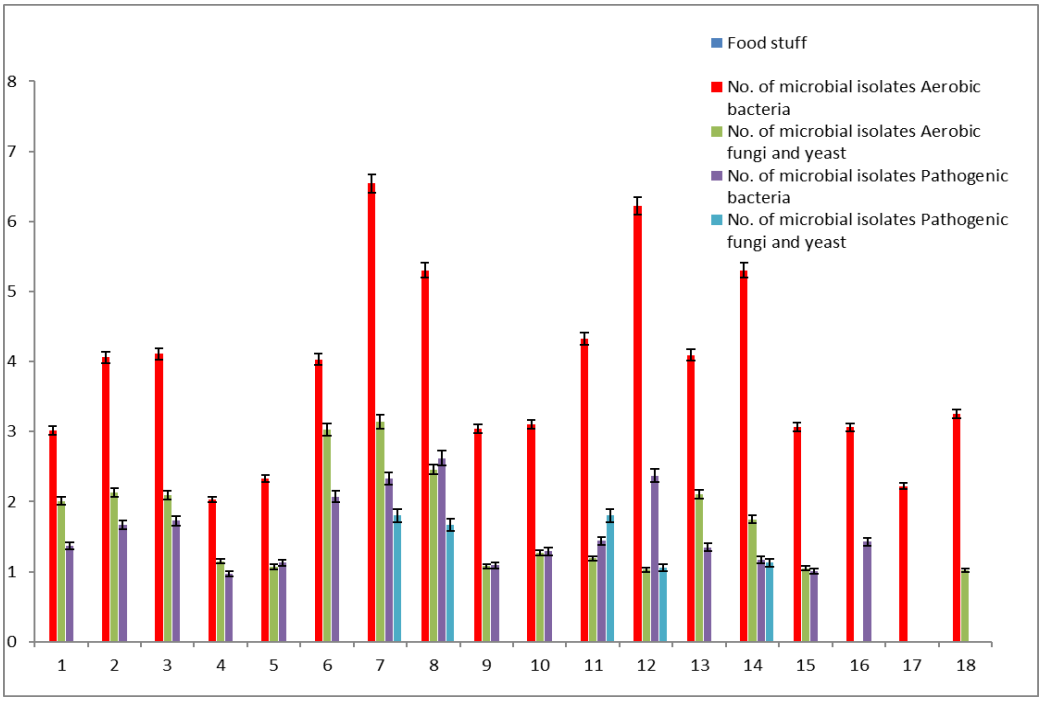

Figure (1): Survey of Aerobic and pathogenic microorganisms inhabited the tested dairy and meat products.

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Table 3: Biochemical characterization of B1

Reaction	Result	Reaction	Result
Morphological characters			
Gram staining	-ve		
Motility	+ve		
Cell shape	Straight rods	**Fermentation of sugars**	
Endospore formation	-ve	Glucose	+ve
Biochemical characters		Sucrose	+ve
Enzyme profile		Mannitol	-ve
β-galactosidase	+ve	Inositole	-ve
Arginine dihydrolase	-ve	Sorbitol	-ve
Lysine decarbolase	-ve	Rhamnose	-ve
Orenthine decarbolase	-ve	Melibiose	-ve
Urease	-ve	Amygdalin	-ve
Tryptophane deaminase	-ve	Arabinose	+ve
Gelatenase	-ve	Starch	-ve
Catalase	+ve	Citrate utilization	-ve
Amylase	-ve	**Other tests**	
Lipase	-ve	H_2S production	-ve
Cytochrome xidase	-ve	Acetoin production	-ve
Nitrate reduction		Indole production	
-to nitrite	-ve		
-to N_2 gas	+**ve**		

Table 4: Biochemical characterization of B2

Reaction	Result	Reaction	Result
Morphological characters			
Gram staining	-ve		
Motility	+ve		
Cell shape	(unipolar)	**Fermentation of sugars**	
Endospore formation	Rod	Glucose	-ve
Biochemical characters	-ve	Sucrose	-ve
Enzyme profile		Mannitol	+ve
β-galactosidase		Inositole	–
Arginine dehydrolase	+ve	Sorbitol	-ve
Lysine decarboxylase	+ve	Rhamnose	–
Ornithine decarboxylase	-ve	Melibiose	–
Urease	-ve	Amygdalin	–
Tryptophane deaminase	-ve	Arabinose	–
Gelatenase	-ve	Starch	+ve
Catalase	-ve	Citrate utilization	
Amylase	+ve	**Other tests**	-ve
Lipase	-ve	H_2S production	-ve
Cytochrome xidase	+ve	Acetoin production	-ve
Nitrate reduction	-ve	Indole production	
-to nitrite			
-to N_2 gas	+ve		
	+**ve**		

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Table 5: Biochemical characterization of B3

Reaction	Result	Reaction	Result
Morphological characters			
Gram staining	+ve		
Motility	-ve		
Cell shape	Rod/club	**Fermentation of sugars**	+ve
Endospore formation	-ve	Glucose	-ve
Biochemical characters		Sucrose	-ve
Enzyme profile		Mannitol	–
β-galactosidase	+ve	Inositole	-ve
Arginine dehydrolase	-ve	Sorbitol	_ve
Lysine decarboxylase	-ve	Rhamnose	–
Ornithine decarboxylase	-ve	Melibiose	–
Urease	-ve	Amygdalin	+ve
Tryptophane deaminase	–	Arabinose	-ve
Gelatenase	-ve	Starch	-ve
Catalase	+ve	Citrate utilization	
Amylase	-ve	**Other tests**	+ve
Lipase	-ve	H_2S production	-ve
Cytochrome xidase	–	Acetoin production	-ve
Nitrate reduction		Indole production	
-to nitrite	+ve		
-to N_2 gas	+**ve**		

Table 6: Biochemical characterization of B4

Reaction	Result	Reaction	Result
Morphological characters			
Gram staining	-ve		
Motility	+ve		
Cell shape	Straight rods	**Fermentation of sugars**	+ve
Endospore formation	-ve	Glucose	+ve
Biochemical characters		Sucrose	-ve
Enzyme profile		Mannitol	–
β-galactosidase	+ve	Inositole	+ve
Arginine dehydrolase	-ve	Sorbitol	–
Lysine decarboxylase	-ve	Rhamnose	–
Ornithine decarboxylase	-ve	Melibiose	–
Urease	-ve	Amygdalin	+ve
Tryptophane deaminase	–	Arabinose	+ve
Gelatenase	+ve	Starch	-ve
Catalase	+ve	Citrate utilization	
Amylase	-ve	**Other tests**	+ve
Lipase	-ve	H_2S production	-ve
Cytochrome xidase	–	Acetoin production	+ve
Nitrate reduction		Indole production	
-to nitrite	-ve		
-to N_2 gas	**-ve**		

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Morphological characterization of Fungi and Yeast:

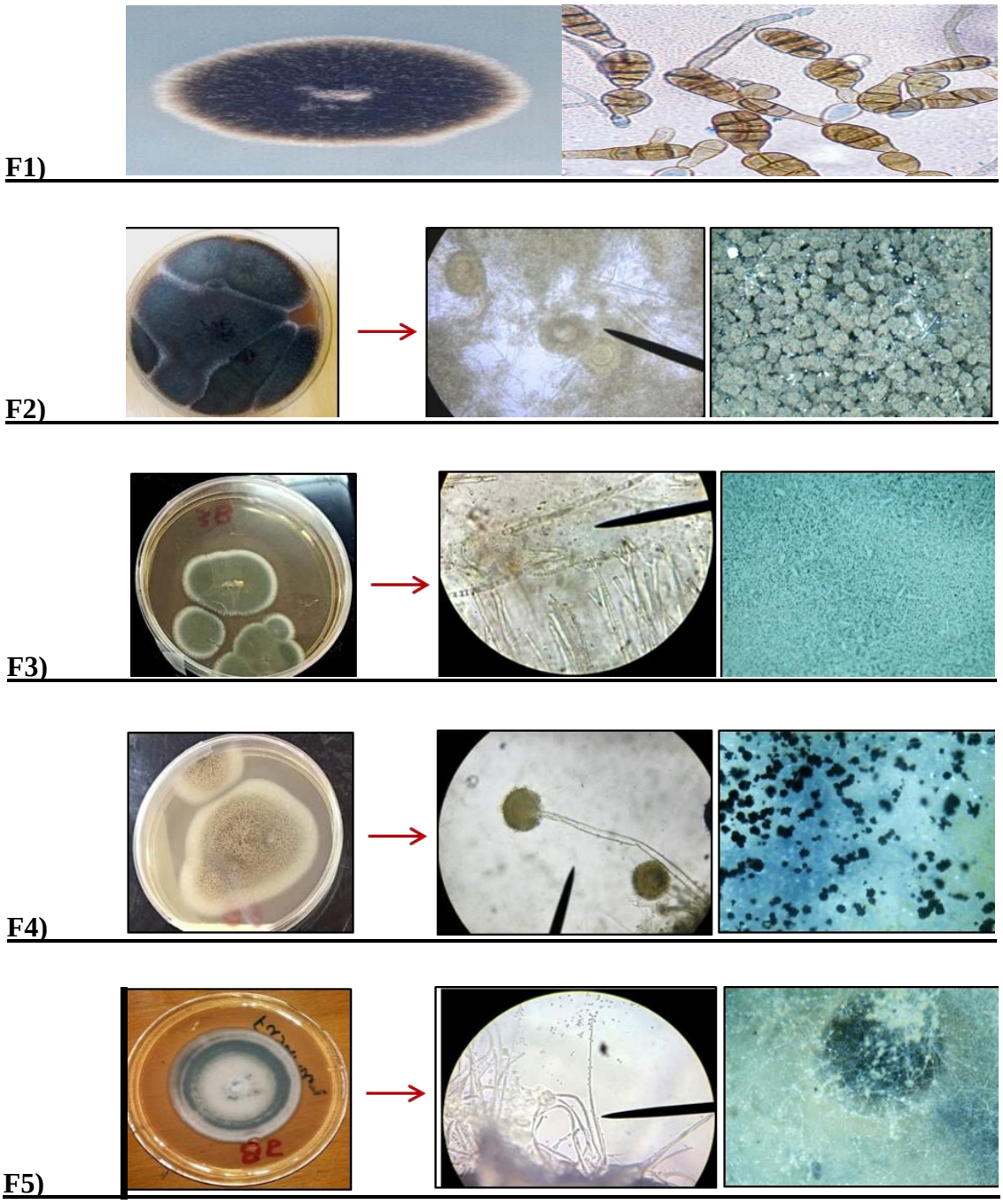

Figure2: Morphological characterization of F1, F2, F3, F4, and F5.

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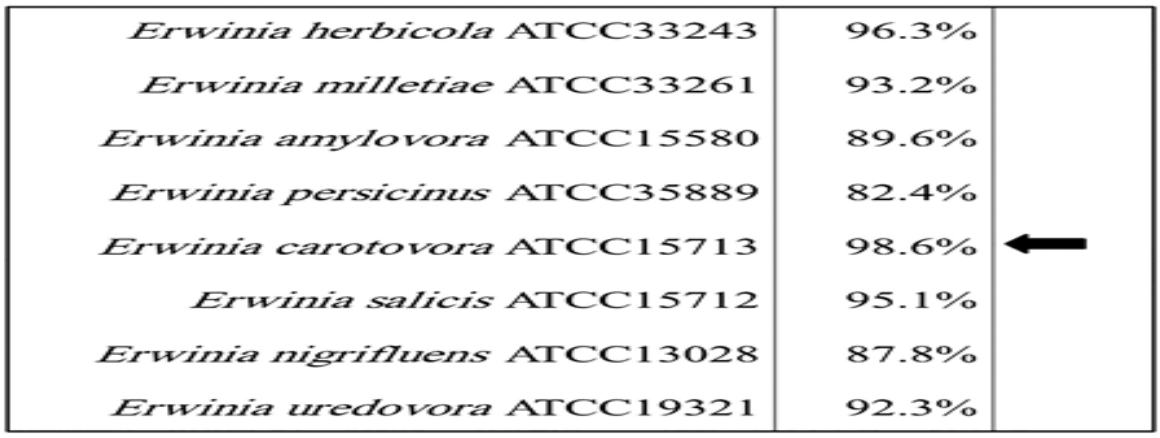

Figure 3. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA sequences, showing the position of isolate *Erwinia carotovora* among phylogenetic neighbors. The black arrow indicates the position of B1 strain.

- **The 16S sequence of (B1) (*Erwinia*): -**

1 gctcagattg aacgctggcg gcaggcctaa cacatgcaag tcgagcggta gcacagagga
61 gcttgctcct tgggtgacga gcggcggacg ggtgagtaat gtctgggaaa ctgcctgatg
121 gaggggggata actactggaa acggtagcta ataccgcata acctcgcaag agcaaagagg
181 gggaccttag ggcctctcgc catcagatgt gcccagatgg gattagctag taggtgaggt
241 aatggctcac ctaggcgacg atccctagct ggtctgagag gatgaccagc cacactggaa
301 ctgagacacg gtccagactc ctacgggagg cagcagtggg gaatattgca caatgggcgc
361 aagcctgatg cagccatgcc gcgtgtgtga agaaggcctt cgggttgtaa agcactttca
421 gcgaggagga aggcggtaag gttaataacc ttatcgattg acgttactcg cagaagaagc
481 accggctaac tccgtgccag cagccgcggt aatacggagg gtgcaagcgt taatcggaat
541 gactgggcgt aaagcgcacg caggcggtct gttaagttgg atgtgaaatc cccgggctta
601 acctgggaac tgcattcaaa actgacaggc tagagtcttg tagaggggg tagaattcca
661 ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg gtggcgaagg cggccccctg
721 gacaaagact gacgctcagg tgcgaaagcg tggggagcaa acaggattag ataccctggt
781 agtccacgct gtaaacgatg tcgatttgga ggttgtgccc ttgaggcgtg gcttccggag
841 ctaacgcgtt aaatcgaccg cctggggagt acggccgcaa ggttaaaact caaatgaatt

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Figure 4. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA sequences, showing the position of isolate *Salmonella enterica* among phylogenetic neighbors. The black arrow indicates the position of B2 strain.

- **THE 16S sequence of (B2) (*Salmonella*): -**

1 cctggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgaac ggtaacagga
61 agcagcttgc ctcttcgctg acgagtggcg gacgggtgag taatgtctgg gaaactgcct
121 gatggagggg gataactact ggaaacggta gctaataccg cataatgtcg caggaccaaa
181 gaggggggacc ttcgggcctc ttgccatcag atgtgcccag atgggattag cttgttggtg
241 aggtaacggc tcaccaaggc gacgatccct agctggtctg agaggatgac cagccacact
301 ggaactgaga cacggtccag actcctacgg gaggcagcag tggggaatat tgcacaatgg
361 gcgcaagcct gatgcagcca tgccgcgtgt atgaagaagg ccttcgggtt gtaaagtact
421 ttcagcgggg aggaagggga taaggctaat aaccttgttc attgacgtta cccgcagaag
481 aagcaccggc taactccgtg ccagcagccg cggtaatacg gagggtgcaa gcgttaatcg
541 gaattactgg gcgtaaagcg cacgcaggcg gtctgtcaag tcggatgtga aatccccggg
601 ctcaacctgg gaactgcatt cgaaactggc aggctggagt cttgtagagg ggggtagaat
661 tccaggtgta gcggtgaaat gcgtagagat ctggaggaat accggtggcg aaggcggccc
721 cctggacaaa gactgacgct caggtgcgaa agcgtgggga gcaaacagga ttagataccc
781 tggtagtcca cgccgtaaac gatgtctact tggaggttgt gcccttgagg cgtggcttcc
841 ggagctaacg cgttaagtag accgcctggg gagtacggcc gcaaggttaa aactcaaatg

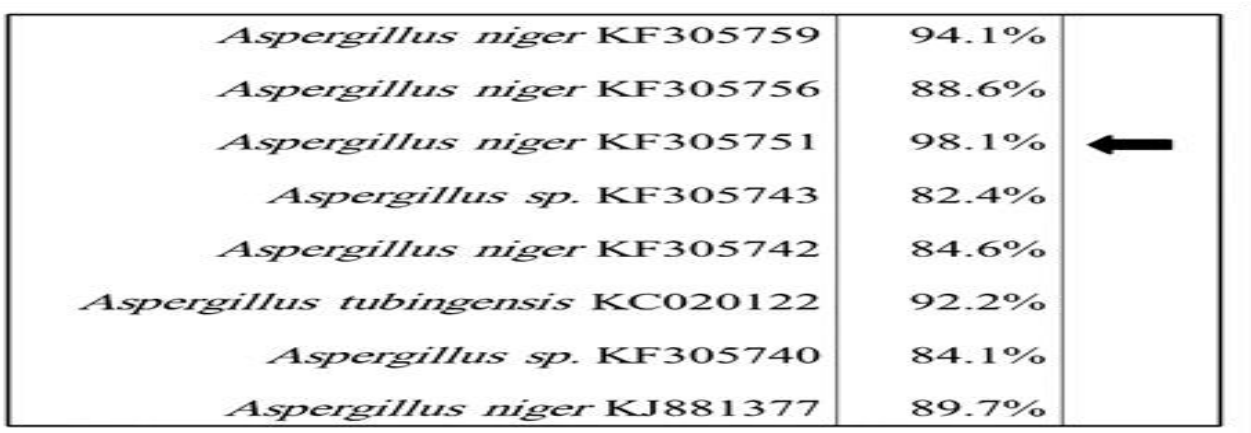

Figure 5. Phylogenetic dendrogram obtained by distance matrix analysis of 18S rRNA sequences, showing the position of isolate *Aspergillus niger* among phylogenetic neighbors. The black arrow indicates the position of F1 strain.

- **The 18S Sequence of (F1) (*Aspergillus niger*): -**

1 gacccgtgcc aggggtctta gtataagcac tttatactgt gaaactgcga atggctcatt

61 aaatcagtta tcgtttattt gatagtacct tactacatgg atacctgtgg taattctaga

121 gctaatacat gctgaaaacc tcgacttcgg aaggggtgta tttattagat aaaaaaccaa

181 tgcccttcgg ggctccttgg tgaatcataa taacttaacg aatcgcatgg ccttgcgccg

241 gcgatggttc attcaaattt ctgccctatc aactttcgat ggtaggatag tggcctacca

301 tggtggcaac gggtaacggg gaattagggt tcgattccgg agagggagcc tgagaaacgg

361 ctaccacatc caaggaaggc agcaggcgcg caaattaccc aatcccgaca cggggaggta

421 gtgacaataa atactgatac ggggctcttt tgggtctcgt aattggaatg agtacaatct

481 aaatccctta acgaggaaca attggagggc aagtctggtg ccagcagccg cggtaattcc

541 agctccaata gcgtatatta aagttgttgc agttaaaaag ctcgtagttg aaccttgggt

601 ctggctggcc ggtccgcctc accgcgagta ctggtccggc tggacctttc cttctgggga

661 atctcatggc cttcactggc tgtgggggga accaggactt ttactgtgaa aaaattagag

721 tgttcaaagc aggcctttgc tcgaatacat tagcatggaa taatagaata ggacgtgcgg

781 ttctattttg ttggtttcta ggaccgccgt aatgattaat agggatagtc gggggcgtca

841 gtattcagct gtcagaggtg aaattcttgg atttgctgaa gactaactac tgcgaaagca

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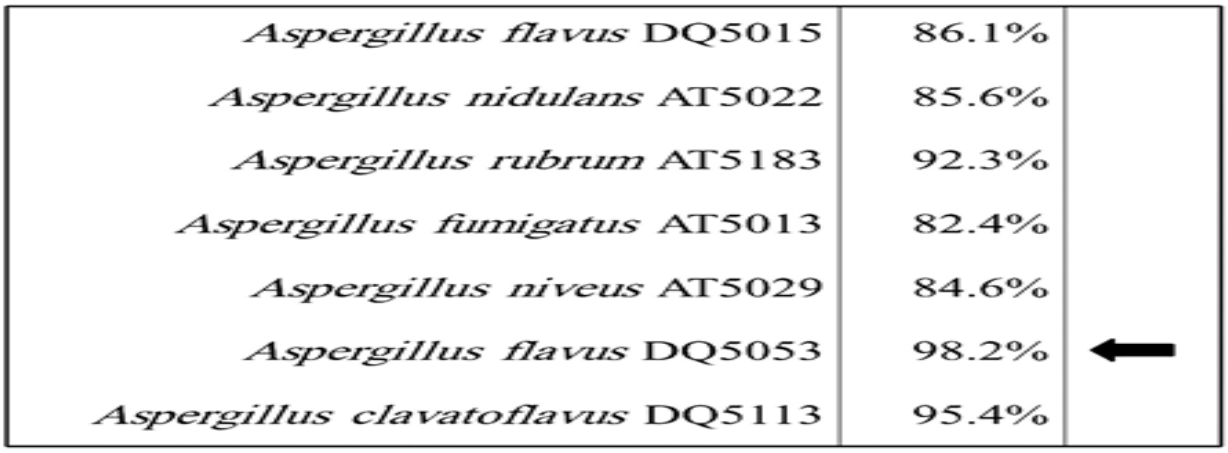

Figure 6. Phylogenetic dendrogram obtained by distance matrix analysis of 18S rRNA sequences, showing the position of isolate *Aspergillus flavus* among phylogenetic neighbors. The black arrow indicates the position of F2 strain.

- **The 18S Sequence of (F1) (*Aspergillus Flavus*): -**

1 cgcggtaatt ccagctccaa tagcgtatat taaagttgtt gcagttaaaa agctcgttag

61 ttgaaccttg ggtctggctg gccggtccgc ctcaccgcga gtactggtcc ggctggacct

121 ttccttctgg ggaacctcat ggccttcact ggctgtgggg ggaaccagga cttttactgt

181 gaaaaaatta gagtgttcaa agcaggcctt tgctcgaata cattagcatg gaataataga

241 ataggacgtg cggttctatt ttgttggttt ctaggaccgc cgtaatgatt aatagggata

301 gtcgggggcg tcagtattca gctgtcagag gtgaaattct tggatttgct gaagactaac

361 tactgcgaaa gcattcgcca aggatgtttt cattaatcag ggaacgaaag ttaggggatc

421 gaagacgatc agataccgtc gtagtcttaa ccataaacta tgccgactag ggatcgggcg

481 gtgtttctat gatgacccgc tcggcacctt acgagaaatc aaagtttttg ggttctgggg

541 ggagtatggt cgcaaggctg aaacttaaag aaattgacgg aagggcacca caaggcgtgg

601 agcctgcggc ttaatttgac tcaacacggg gaaactcacc aggtccagac aaaataagga

661 ttgacagatt gagagctctt tcttgatctt ttggatggtg gtgcatggcc gttcttagtt

721 ggtggagtga tttgtctgct taattgcgat aacgaacgag acctcggccc ttaaatagcc

781 cggtccgcgt ttgcgggccg ctggcttctt agggggacta tcggctcaag ccgatggaag

841 tgcgcggcaa taacaggtc

Table (7): Estimation of microbial toxins secreted by the most common microbial isolates within the most polluted food sources in the present study

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The most common microorganism	The most detectable toxin	Average concentration of the detected toxin (µg/Kg d. t.)
Erwinia carotovora ATCC15713	non	0.0
Salmonella enterica KX302887	C-enterotoxin	16.0
Aspergillus niger KF305751	Ochratoxin	4.0
Aspergillus flavus DQ5053	Aflatoxin	23.0

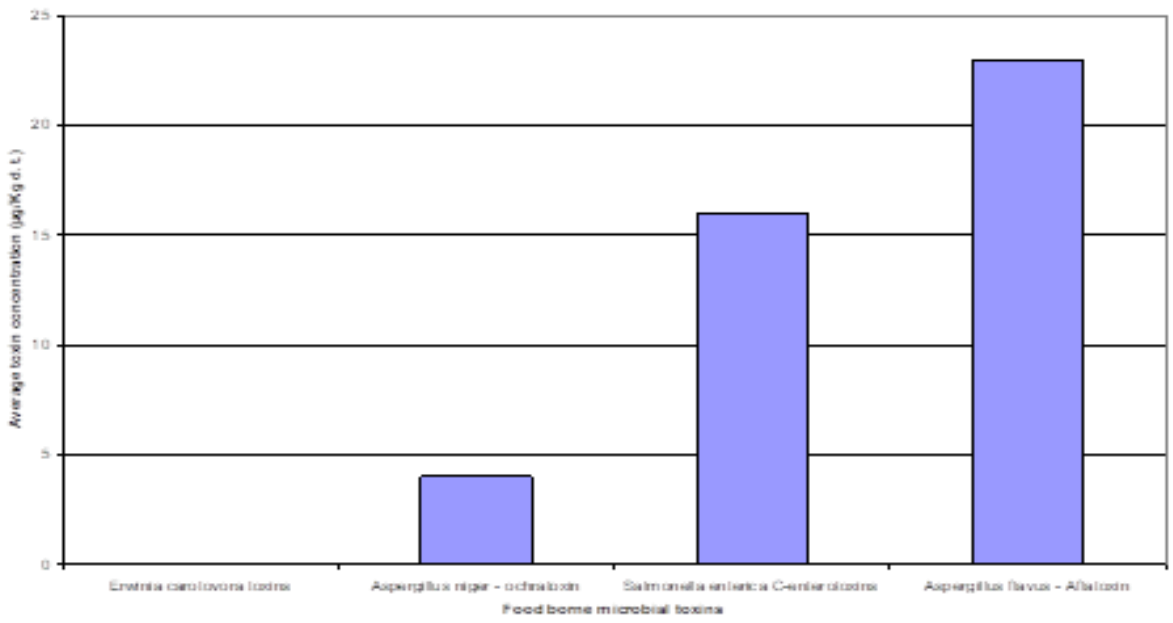

Figure (7): Estimation of microbial toxins secreted by the most common microbial isolates within the most polluted food sources in the present study.

Table (8): The tested Egyptian wild plants and used parts

No.	Latin name	Family	Used part
1	*Cassia nodosa*	Fabaceae	Leaves
2	*Delonix regia*	Fabaceae	Leaves
3	*Melaleuca quinquenervia*	Myrtaceae	Leaves
4	*Casuarina equisetifolia*	Casuarinaceae	Lateral branches
5	*Magnolia grandiflora*	Magnoliaceae	Leaves

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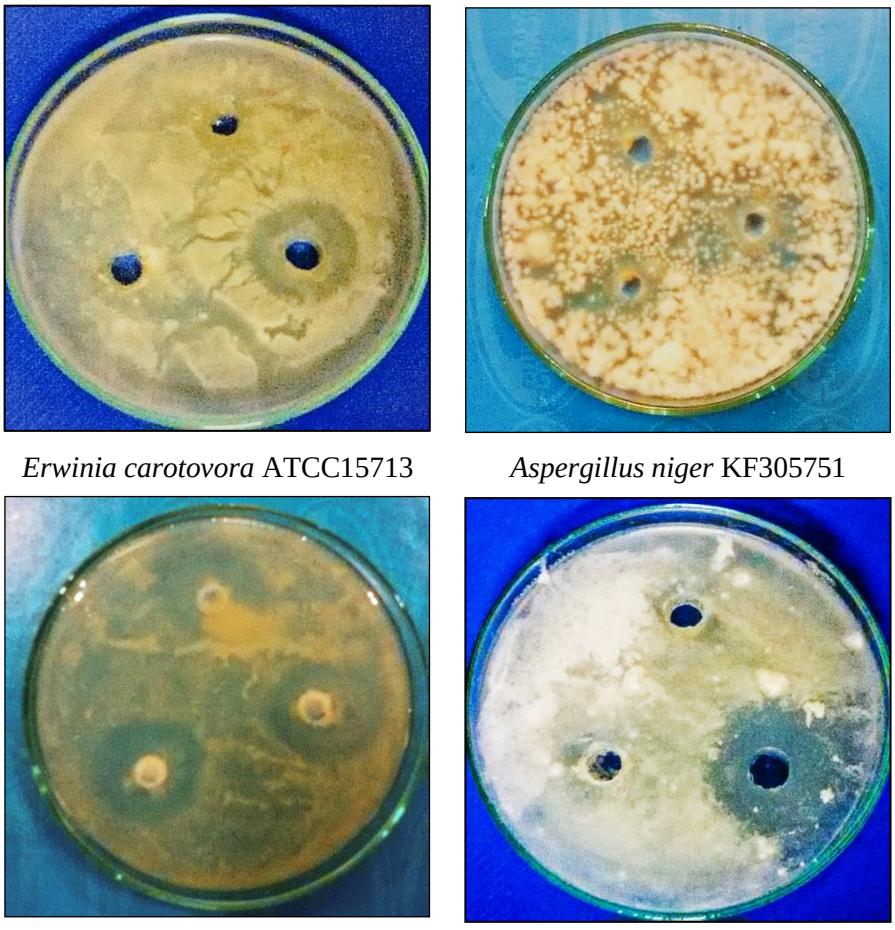
Table (9): The antimicrobial activity of the selected wild plant extracts against the most common isolated microbial.

No.	The tested wild plant	Inhibition zone diameter (mm) in the tested cultures of different isolates			
		Erwinia carotovora ATCC15713	*Salmonella enterica* KX302887	*Aspergillus niger* KF305751	*Aspergillus flavus* DQ5053
1	*Cassia nodosa*	0.0	1.3	1.0	0.0
2	*Delonix regia*	1.6	1.7	1.3	1.5
3	*Melaleuca quinquenervia*	0.0	1.2	1.0	0.0
4	*Casuarina equisetifolia*	0.0	0.9	0.9	0.9
5	*Magnolia grandiflora*	0.0	0.9	0.0	0.9



Erwinia carotovora ATCC15713 *Aspergillus niger* KF305751

Salmonella enterica KX302887 *Aspergillus flavus* DQ5053

Figure (9): Representative plates for the survey of antimicrobial activity of the selected Egyptian wild plants.

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Table (10): Estimation of MIC for the most potent plant extract against the most common microbial isolates in the present survey

Microbial isolate	Percentage of surviving cells (% Optical density)					
	Concentration of *Delonix regia* leaf extract (mg/ml)					
	0.0	6.25	12.5	25	50	100
Erwinia carotovora ATCC15713	100	68	48	29	27	28
Salmonella enterica KX302887	100	66	29	30	29	28
Aspergillus niger KF305751	100	71	28	27	26	26
Aspergillus flavus DQ5053	100	72	56	28	27	27

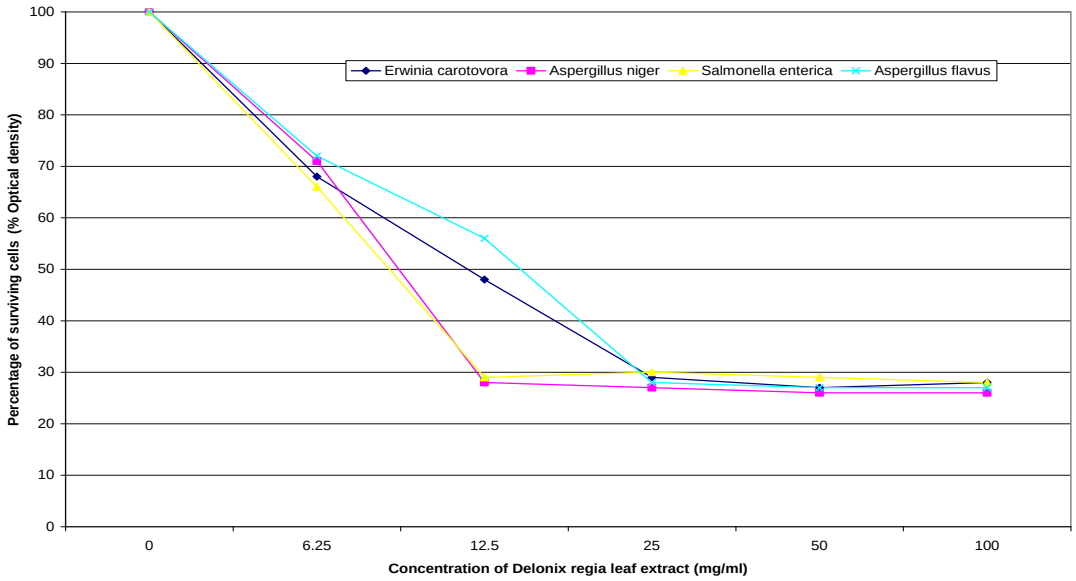

Figure (10): Estimation of MIC for the most potent plant extract against the most common microbial isolates in the present survey.

Table (11): Phytochemical analysis of Delonix regia leaf extract.

Phytochemical component	Concentration (mg/g d. wt.)
Phenolics	58.4
Flavonoids	3.92
Alkaloids	108.4
Saponins	24.9
Steroids	1.28
Tannins	3.14

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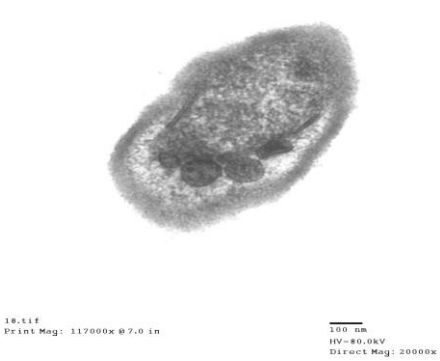
The inhibitory cellular effect of plant extract using TEM

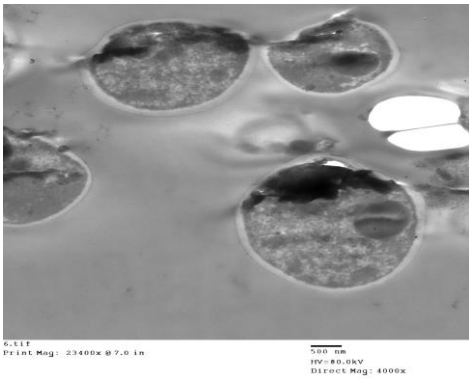
Salmonella enterica and Aspergillus flavus are the bacteria most adversely impacted by plant extract; hence they were chosen for TEM cellular study (13). Salmonella treated cells showed dissolution of the peptidoglycan wall, lighter cytoplasm from nutritional component leaking, and coagulation of nucleic material. A damaged chitin wall and lighter cytoplasm due to nutritional component leakage were seen in treated Aspergillus cells, whereas nucleic material was coagulated.



Salmonella enterica cells, affected by *Delonix regia* leaf extract.

Control (non-treated) *Salmonella enterica* cells

Figure (12): Transmission electron micrographs of inhibitory effect of *Delonix regia* leaf extract on *Salmonella enterica* cells, compared to control growth.



Aspergillus flavus spores, affected by *Delonix regia* leaf extract.

Control (non-treated) *Aspergillus flavus* spores

Figure (13): Transmission electron micrographs of inhibitory effect of *Delonix regia* leaf extract on *Aspergillus flavus* spores, compared to control growth.

Discussion

Microbes were isolated from food (dairy and meat products) in order to determine the prevalence of harmful and non-pathogenic microorganisms in this survey. We identified the most prevalent harmful

microorganisms in our study. 16S rRNA gene sequencing of the B1 isolate identified it as *Erwinia carotovora* strain ATCC 15713. 16S rRNA gene sequencing of the B2 isolate revealed it to be *Salmonella* enterica strain KX302887. 18S rRNA gene sequencing of the F1 isolation identified it as *Aspergillus niger* strain KF305751. It was shown to share the most sequence similarities with *Aspergillus niger* among the *Aspergillus* species. 18S rRNA gene sequencing of the F2 isolate identified it as *Aspergillus flavus* strain DQ5053. Findings from this study suggest that the most commonly isolated microbes in polluted food sources release a wide range of toxic compounds. *Erwinia carotovora* ATCC15713 had no toxins reported in the HPLC toxin assay, while *Salmonella* enterica KX302887 had a concentration of C-enterotoxin (16.0 ug/kg d.t.) that could be detected. Ochratoxin is found in *Aspergillus niger* KF305751, a mould strain. Aflatoxin has been found in *Aspergillus flavus* DQ5053.

Antibiotic resistance is a problem that affects the healthcare systems of both developing and developed countries. Traditional antibiotic therapy is in peril because of the emergence and spread of drug-resistant microorganisms. Many new antimicrobial compounds have been discovered as a result of this, including a wide spectrum of bioactive compounds with well-established antimicrobial effects found in plants like garlic and onions. A variety of medicinal plant extracts were tested for their antibacterial effectiveness against human disease-causing microorganisms as part of this study. As shown by their MIC values, many plant extracts displayed negligible antibacterial activity against the test bacterial isolates. This was in spite of certain extracts exhibiting significant antibacterial activity against different test bacterial strains. New research reveals antibacterial activity in plant leaf extracts used to treat bacterial infections addressed in this study. Since synthetic antimicrobials have negative effects, lantbased antimicrobials have a lot of therapeutic potential. Recently, antibacterial activity and overall toxicity of Delonix regia extract were investigated (Jahan *et al*., 2010). Further research is needed in order to completely understand the mechanisms behind D. regia methanol extract's liver-protective and cytotoxic effects (El-Sayed *et al*., 2011; Ahmed and colleagues, 2011). The presence of flavanoids in this extract is thought to be responsible for its theorised action. Due to their impact on signal transduction in cell proliferation and angiogenesis, flavanoids have a chemopreventive role in cancer (Jahan *et al*., 2010). Flavonoids are powerful in suppressing tumour and/or cancer development and promotion, but they are also non-toxic to normal cells, which is a crucial point to remember. Their ability to trigger apoptotic pathways to delay cancer in abnormal cancer cells and to regulate levels of metabolising enzymes and stimulate detoxifying enzymes, making them non-toxic to regular cells, is what truly distinguishes their effects in abnormal cancer cells from normal ones (Kwon *et al*., 2007). As a result, a natural substance that has both hepatoprotective and cancer-cytotoxic properties might be an effective novel treatment for liver cancer. As a result, the current study

focused on four distinct objectives relating to D. regia extract: phytochemical inquiry, in vitro cytotoxicity assessment, antioxidant evaluation, and lastly hepatoprotective activity evaluation.

Extracts of Delonix Regia methanol were tested for antibacterial properties (*Erwinia carotovora*, *Aspergillus falvus*, *Aspergillus niger* and *Salmonella*). The extracts from Delonix regia leaves were highly effective against all of the bacteria and fungus that were tested. Delonix regia leaf extracts (methanol) demonstrated a substantially better inhibitory impact against *Aspergillus* and *Salmonella* bacteria, according to our research.

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