ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Alternative Antibiotic Assessment Against Isolated Pathogenic Salmonella Typhi From Water Resources.

El Dougdoug[1], KA; Nahed [2], MEl Aiate ; Fatehi[3], A Khalifa and Nashwa[4], HA*.

[1]Microbiology Dept., [2]Agric .Fac., Ain Shams Univ. Egypt.
[3]Botany Dept., Sci .Fac., Benha Univ. Egypt.

ABSTRACT

This study aims to investigate the potency of plant extracts as alternative antibiotics against pathogenic Salmonella sp. isolated from different water resources. Seven Salmonella typhi out of twenty Salmonella isolates were detected and identified from domestic water, industrial water, Agricultural waste water and River Nile water according to serological characters , Bergy's manual of systematic bacteriology and confirmed by the API 20 E strips. Salmonella typhi isolates had different serological characteristics using infected serum with typhoid by slide agglutination test. As well as Salmonella sp isolates had different antibiotics sensitivity using disk diffusion test . Three different methanolic extracts of Cinnamon ; Rose mary and Grapefruit plants were assessed for antimicrobial activity to inhibit S. typhi . The methanolic extracts were revealed the inhibition potency at different dilutions against isolated Salmonella typhi for representing a good alternative to the use of traditional antimicrobial in treatments.

Keywords: Plant extracts , Antibiotics, Water resources , Pathogenic bacteria serological characteristics .

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Salmonella infection of humans and animals is a world wide health problem. A majority of salmonellosis outbreaks flow due to the consumption of contaminated food and/or water. The complex routes by which Salmonella are cycled through the environment suggest that water may play an important role in the transmission of this organism from waters. Salmonella species are responsible for such potentially water transmitted diseases such as typhoid fever (Salmonella typhosa), the less sever paratyphoid (Salmonella paratyphi). The increase of pollution in natural water has necessitated the detection frequency and persistence of pathogenic organism mainly Salmonella spp. The first reports on antibiotic resistant Salmonella had been indicated since 1960s and describe mainly case with monoresistance strain (1). In the late 1980s, the appearance multiple resistances against ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline were found in serovar Thyphimurium definitive type 104 (DT 104) (2). The main mechanism of bacteria exhibit resistance to antimicrobial agents can be due to many factors including drug inactivation, reduced drug accumulation, alteration of metabolic pathway and target site (3), (4).The present study was done to Determination of microbial pollution indicators in different types of water, Isolation and identification of Salmonella sp. in collected water samples, Multiple antibiotic resistant for bacterial isolates and antimicrobial activity for some plant extract against bacterial isolates.

MATERIALS AND METHODS

The water resources 40 samples , 10 of each of domestic water (DW) , industrial water (IW) , Agricultural waste water (AW) and River Nile water (NW) were collected from (Drains & Rosetta branch & chlorinated samples) at Qualybia Governorate according to methods of water and wastewater examination APHA (2005) (5). Water samples were collected from the outer most terminals before flow of the drainage to the municipal sewage . Polypropylen containers of two liters capacity were used for bacteriological analyses and delivered immediately to the laboratory in ice cooler box for analysis within through 6 hrs. from collection according to APHA (2005). Detection and isolation of S. typhi : According to standard method No. 9260 B (APHA, 2005) (5), Salmonella sp was isolated by enrichment concentrated water samples The concentrated sample using membrane filter technique to filter a sample volume of 1000 ml. was enriched in a growth medium.

Purification and identification of bacterial isolates:

Bacterial colonies developed from all previously mentioned media were chosen and picked up according to variation in culture characteristics and colony formation then purified by streak-plate method on Nutrient agar medium (Difco) of the following composition (g/liter): Bacto beef extract; 3.0, Bacto Peptone; 5.0, Bacto agar and 15.0, Distilled water; 1.0 liter. Pure isolates were maintained on slants of the same medium at 4^{o}C for subsequent identification were carried out according to (Collins and Lyne, 2004 and Cheesbrough, 2006) (6,7) . The Analytical Profile Index (API) 20E strips obtained from Biomerieux, France were used as biochemical system for identification of enterobacteriaceae and other non-fastidious gram-negative rod bacteria. .The API 20E strip consists of 20 micro-tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media. The strips were incubated for 18 - 24 h. at 37^{o}C.

Multidrug bacteria:

Antibiotic resistance (table 1) of bacteria isolates were tested using disk diffusion test according to National Committee for Clinical Laboratory Standards (NCCLS) (8). For the estimation of the multidrug bacteria (MDR), diluted bacteria isolate were spread over agar plates supplemented with antibiotic disk saturated with 10 to 30 mg (table 4).

Serological tests:

Salmonella sp isolates were detected and identified using infected serum provided from hospital (Central Qaluob hospital) by slide agglutination with polyvalent H and O group as a reference antiserums according to (Himedia Laboratories) .

Plant extract:

In the present study, S. typhi isolates were treated with Cinnamon ; Rose mary and Grapefruit methanolic extracts at different concentrations obtained from Horticulture Department , Faculty of Agriculture, Ain shams University. The inhibition zone was determined by standard Kirby-Bauer disk diffusion . It was found that plant extracts has antibacterial activity against Salmonella sp. isolates.

Mean growth inhibition percentage (broth microdilution method) by ELISA reader:

The bacterial suspension equivalent to the turbidity of 0.5 McFarland standard (10^8cfu/ml) prepared from a fresh subculture of tested bacteria in Mueller Hinton Broth (MHB) then the suspension was diluted to (10^6cfu/ml) using MHB. The adjusted microbial inoculums (100µL) were added to each well of sterile 96-well flat-bottomed micro titer plate containing the tested concentration 100 (mg/ml) of each plant extract 100(µL/well). As a result, last inoculum concentration of ($5x10^5$ CFU/ml) was obtained in each well. Three wells containing microbial suspension without tested extract used as (Growth control) and two wells containing only media as (background control) were included in this plate. Optical densities were measured at (620nm)after 24h at 37 ^{0}C using ELISA microplate reader at the Botany and Microbiology Department Faculty of science Al-Azhar University (Sun Rise–TECAN, Inc. ®,USA). Ampicillin and Gentamicin were used as standard antibiotics for Gram positive and Gram negative bacteria respectively. Finally, cell concentrations were transformed to a percentage of bacterial inhibition. The percentage of bacterial growth reduction (GR %) was estimated using as reference the control treatment (without extract) as:

$$GR\% = \frac{C - T}{Cx100}$$

Where, C is the cell concentrations under the control treatment and T is the cell concentrations under the extract treatment. Three replicates were considered. The results were recorded as means ±SE of the triplicate experiment (NCCLS/CLSI, 2007) (8).

RESULTS

Incidence of water borne Salmonella typhi:

Salmonella typhi was found in low level contamination percentages ranged from16% to 34% according to the type of water as well as, the number of positive samples was 3 ; 1 ; 2 and 1 samples with incidence percentages7.5 ; 2.5 ; 5 and 2.5 % of Domestic ; Industrial ; agriculture and Nile water samples respectively (table, 1)

Table 1: Assessment of Salmonella sp counts of the water resources.

water resources	Salmonella sp counts		
	Positive samples	Incidence percentage	Average log no.
Domestic water (DW)	3	7.5 %	7.58
Industrial water (IW)	1	2.5 %	2.31
Agricultural waste water (AW)	2	5 %	2.22
River Nile water (NW)	1	2.5%	3.7

Ten sample of each water resources.

ISSN: 0975-8585

Identification of Salmonella typhi:

The obtained results from morphological, cultural characteristics and biochemical identification indicated that, there are 40 different isolates of water borne Salmonella isolates. The results of identification were as the following:

The morphological and biochemical characteristics of Salmonella isolates were detected in water samples were belonging to bacterial family Enterobactericeae. Morphological and biochemical characteristics of bacterial isolates: Morphological characteristics: Shape of colony (Low convex, entire) ; Texture(Smooth) ; Pigmentation (-) ; Motility (+) ; O2 requirements (facultitative anaerobic) . Microscopic examination: Gram reaction (-) ; Sporulation (-) ; Capsule (-) , Cell shape (Rods singly or in pairs) . Biochemical characteristics : Catalase(+) , Coagulase(-) , Oxidase(-) , Urease (-) , Gelatin liquefaction (-) , Starch hydrolysis (-) , Phenyl alanine deaminase (-) , H_2S production (-) ; Hemolysis on blood agar (Alpha) ; Nitrate reduction (+) , Indole formation (-) , Methyl red (+) , Voges-Proskauer (-) , Citrate utilization (-) . Fermentation of sugar : D-glucose (A/-) , Sucrose(-/-) , Mannose (A/-) , Lactose (-/-) , Mannitol (-/-) .

Identification of S. typhi isolates according to API strips system (fig. 1) , the results of Tests showed that , Ortho Nitro Phenyl-BD-Galactopyranosidase (ONPG -),Arginine dihydrolase(-),Lysine decarboxylase (+), Ornithinedecarboxylase (-), Citrate utilization(-),H_2S production(+),Urea hydrolysis(-),Tryptophan deaminase(-), Indole production(-),Voges-proskauer(-),Gelatinase(-),D-Glucose(+),D-Mannitol(+),Inositol(-),D-Sorbitol(+),L-Rhamnose(-),D-Sucrose(-),D- Melibiose (+), Amygdalin (-), L-Arabinose(-) and Oxidase(-).

Fig 1: API strips system Showing the results of tested S. typhi .

Serological test:

Pathogenic potency of Salmonella isolates were detected and identified using infected serum of typhoid patient by slide agglutination . It was found that , seven isolates , 3,1,2,and 1 of (DW) ; (IW) , (AW) and (RN) detected as Salmonella typhi out of 40 Salmonella isolates respectively which serological reacted with antibodies containing infected typhoid serum .

Antibiotic sensitivity of food borne Salmonella isolates:

The antibiotic sensitivity of seven tested Salmonella isolates showed different susceptibilities ranging from sensitive, intermediate and resistant against different tested antibiotics as shown in Table (2).

Table 2: Antibiotics sensitivity profiles of food borne nine tested Salmonella isolates.

Antibiotics	Salmonella isolates						
	S1	S2	S3	S4	S5	S7	S9
Amoxicillin(10mcg)	10*	8	22	2	2	2	5
Ampicillin (10mcg)	12	10	18	1	3	1	8
Corbenicillin(10mcg)	3	17	20	3	4	0	12
Ceproflaxin (10 mcg)	5	15	20	1	3	0	3
Tetracycline (30 mcg)	5	12	13	4	0	4	5
Streptomycin (10mcg)	3	15	21	3	1	3	5
Kanamycin (10 mcg)	4	16	20	0	3	2	10
Erythromycin (15mcg)	5	13	22	1	2	2	15
Penicillin (10 mcg)	2	16	22	2	5	0	5
Cephalosporin(30mc)	3	15	22	3	3	1	3

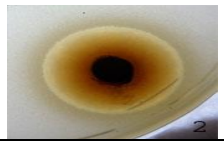
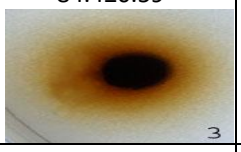
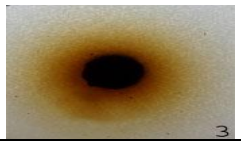
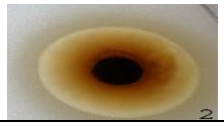
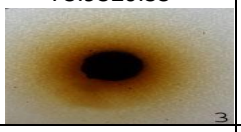
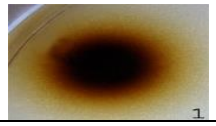
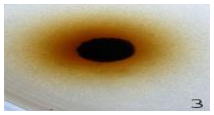
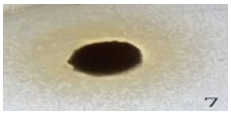
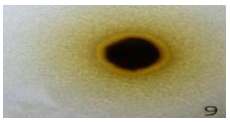
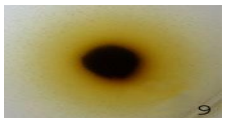
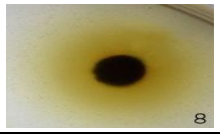
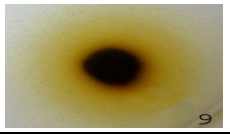
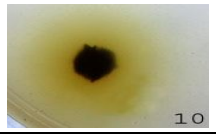
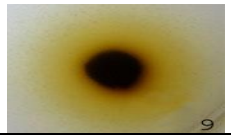
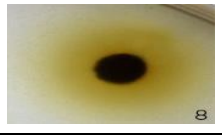
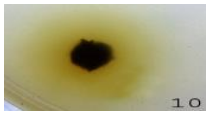
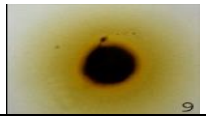
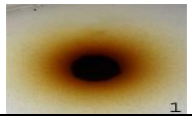
ISSN: 0975-8585

Gentamycin (20 mcg)	5	13	22	5	1	2	8
Chloramphenicol(30 mcg)	8	7	25	7	3	7	4

R: Resistant (less than 1) , IS: Intermediate sensitive (I – 10) and S: Sensitive(high than 10 mm inhabitation zone .
* Mean diameter of inhibition zone (mm) included antibiotic disk for tested isolates.

Bioactivity of used aqueous plants extracts : Three different methanolic extracts of Cinnamon ; Rose mary and Grapefruit plants were assessed for antimicrobial activity to inhibit S. typhi for representing a good alternative to the use of traditional antimicrobials in treatments . The results of the clear zones diameter of inhibition in Table (3) and mean growth inhibition percentages in Table (3) showed the antibacterial activity of methanolic plants extracts against S. typhi isolate.

Table 3: The antibacterial activity of methanolic extracts (100 mg/ml)/well) based on the mean growth inhibition of S. typhi isolates using (broth microdilution method) by ELISA plate reader..

S.typhi isolates	MGI (%) (O.D.620) of different plant extract (conc.100mg/ml)		
	Cinnamon	Grapefruit	Rose mary
1 S.typhi	86.4±0.33 	84.4±0.59 	71.8±0.51 
2 S.typhi	83.1±0.63 	73.93±0.33 	81.56±0.39 
3 S.typhi	82.43±0.63 	50.2±0.36 	63.76±0.44 
4 S.typhi	77.9±0.97 	69±0.51 	46.03±0.62 
5 S.typhi	80.6±0.26 	72±0.51 	71.5±0.48 
7 S.typhi	74.43±0.46 	70.2±0.45 	72.6±0.50 
9 S.typhi	88.16±0.49 	46.06±0.53 	00±00 

Each value is mean of 3 replicates ± standard error

Susceptibility of S. typhi : The data obtained from Tables (4) showed that, S. typhi, revealed sensitivity against the methanolic extracts of Cinnamon ; Rose mary and Grapefruit methanolic extracts . The inhibition percentage was 55.4±0.45 and 50.0±0.49 of Rose mary and Grapefruit respectively . While, Cinnamon methanolic extract showed higher activity with inhibition zone diameter 100 % (mm) and 100±00% mean growth inhibition percentage. respectively.

Table 4: The antibacterial activity of plants aqueous extracts (100 mg/ml)/well)based on the mean growth inhibition of bacterial isolates using(broth microdilution method) by ELISA plate reader.

Bacterial isolates	MGI (%) (O.D.620) produced from methanolic extracts (conc.100mg/ml)		
	Rose mary	Cinnamon	Grapefruit
1 S.typhi	41.2±0.88	100±00	52.4±0.45
2 S.typhi	62.4±0.45	100±00	43.4±0.66
3 S.typhi	48.4±0.45	100±00	33.4±0.34
4 S.typhi	70.1±0.58	100±00	64.9±0.62
5 S.typhi	52.4±0.45	100±00	52.6±0.31
6 S.typhi	62.4±0.45	100±00	42.4±0.45
9 S.typhi	52.4±0.45	100±00	64.4±0.45

Each value is mean growth inhibition of 3 replicates ± standard error

DISCUSSION

Water contamination are spoiled or tainted because they either contain microorganisms or toxic substances that make them unfit for consumption. The water carry microbial associations, whose composition depends upon which organisms gain access and how they grow, survive and interact in the water over time. As well as, the west water considered as rich medium for the growth of different microorganisms, among these, the most important group are bacteria (9),(10),(11). The obtained results indicated that the values of standard plate count (SPC) determined at 22ºC was higher than those determined at 37ºC. The optimum temperature for the bacterial population which can grow at the human and animal bodies is 37ºC. Whereas, at 22ºC most of air and soil bacteria can grow well. This may explain why the numbers of bacteria counted at 22ºC were much higher than those determined at 37ºC. These results are in agreement with those reported by Sabae and Rabeh, 2007 and Ezzat, 2008, reported that indicator organisms are used almost universally as a measure of sanitary quality of water (12),(13) . In general, the bacteria identified in our study were reported to be potential human pathogens of public health risk (7),(14).

In this study, the most widespread bacteria obtained were Salmonella typhi. This indicates that the water in drains and Rosetta branch is subjected to sewage pollution from drains. On the other hand, the high incidence of Salmonella typhi recovered from drains and Rosetta branch was correlated with the fecal contamination represented by fecal coliforms present in the samples. Our findings were in accordance with those reported by Edberg et al., 2000 (15). The presence of P.aeruginosa an opportunistic pathogen of humans and S.aureus in considerable densities in water resources is a matter of concern because these organisms cause a wide range of infections. Moreover, P.aeruginosa has been reported as a multi-drug resistant organism (16). Salmonella sp. was obtained from drains in two sites of our study and this could be attributed to contamination of these drains by feces of infected humans or animals and especially from poultry farms. On the light of the previously mentioned data obtained from antibiotic sensitivity tests and calculations of MAR index values, it was clear that the pollution levels indicated by the physico-chemical and bacteriological analyses play an important role in the incidence of antibiotic resistant bacteria (ARB) and that dissemination of these bacteria depends on pollution extents. The same conclusion was reported by Abo-State et al., 2012 (17).

Our results showed that uncontrolled pollution discharged into aquatic environments may be a source of antibiotic resistance (AR). Such conclusion was in accordance with those reported by Graham et al., (2011), who found that unregulated pollution possible sources include pharmaceutical wastes, inadequate domestic treatment and a large landfill- has the potential of affecting antibiotic resistance(18). Baquero et al.,

(2008) reported that in contaminated aquatic environments, resistant strains were transferred from human and animal sources to environment and these bacteria are able to spread their genes into native bacteria (19). Moreover, contaminated rivers become reservoir of antibiotic resistance genes and under normal conditions, transferred to water borne pathogens as concluded by McArthur et al., 2011 (20).

The obtained results of the multi-resistant patterns of our isolates showed high related percentages to tested antibiotics, this emphasizes the transfer of antibiotic resistant genes between different bacterial groups and this investigation indicate that the origin of antibiotic resistance is one and come from pollution discharged in River Nile. On the other hand, the abundance of similarity in AR related to emergent and opportunistic pathogens in the resistant strains highlights the health risk that sewage-contaminated rivers represent as a reactor of AR and pathogenesis evolution as reported by Garcia-Armisen et al., 2010 (21).

The purpose of this study is to evaluate the microbiological safety of water and detection of S typhi. In Egypt there is a lack of information about the occurrence of water borne diseases in general and tap water especially or in particular. S. typhi was found in low level contamination ranged from 2.5 % to 7.5 % according to the type of water ; Domestic ; Industrial ; agriculture and Nile water samples . Egypt has been suffering from severe water scarcity in recent years. Uneven water distribution, misuse of water resources and inefficient irrigation techniques are some of the major factors playing havoc with water security in the country. River Nile is the main water resource in Egypt. It services the country's demands for drinking water as well as both industrial and agricultural activities. Rising population and rapid economic development and the dramatic increase in pollution and environmental degradation are decreasing water availability for Egypt. Nowadays, Egypt is facing an annual water deficit of around 7 billion m^3. United Nations is already warning that Egypt could run out of water by the year 2025 (22).

There are many sources of water pollution, but two main general categories exist: direct and indirect contaminant sources. Direct sources include effluent outfalls from industries, refineries and waste treatment plants; whereas, indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. Otherwise, contaminants come under two broad classes: organic and inorganic. Some organic water pollutants include industrial solvents, volatile organic compounds, insecticides, pesticides and food processing wastes, etc. Inorganic water pollutants include metals, fertilizers and acidity caused by industrial discharges (23).

In this study most of tested plants extracts Cinnamon ; Rose mary and Grapefruit showed antibacterial activities against S.typhi tested water borne bacterial isolates, however only 3 plants methanolic extracts had broad spectrum antibacterial activities (T. vulgaris, Co. sativum, and N. Sativum) while, R. officinalis showed no activity against most tested bacteria. Indeed, Gram negative bacteria known to be more resistant to antibiotics than Gram positives ones. For instance, the resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (24) . In particularly, aqueous extract of T. vulgaris have greatly effective inhibition on growth of ten tested foodborne bacterial isolates, with inhibition zones diameter ranging from 17 to 24.8 (mm)and 100% mean growth inhibition percentage against the range of tested bacteria. This trend of results is in agreement with (Ayachiet al., 2009) who found that, the antibacterial activity of T. vulgaris against S. typhimurium isolated from poultry chain and E. coli ATCC 25922 showed a high antibacterial activity with inhibition zones 19.9 and 33 (mm) respectively (25).

This antimicrobial activity attributed to similarity in the mechanism of action of this plant extract and antibiotics. This plant extract was active even against organisms that have become resistant to antibiotics. The antimicrobial activity of the crude extract might be due to the presence of active compounds including alkaloid, quinines, tannins, flavonoides, saponins and iridoids (26). The main target for these compound might be some important enzymes, bacterial cell wall or membrane . It has been shown that, alkaloids are able to intercalate DNA, lipophilic compounds that might bind within or internal to the cytoplasmic membrane (27). Active compounds of plants extract have been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of several microorganisms such as E. coli, E. coliO157:H7, L. monocytogenes, Lactob. sakei, Ps. aeruginosa, S. enteritidis, and Staph. Aureus. Moreno et al., 2006 and Raybaudi-Massiliaet al., 2009, reported that, the antimicrobial action of plant extract compounds was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes. Increased membrane permeability is a major factor in the mechanism of antimicrobial action, whereas compounds may disrupt membranes and

cause a loss of cellular integrity and eventual cell death (28),(29). Generally, this plant extract possess activity against food spoilage and pathogenic bacteria thus considered as a promising natural antimicrobial agent for food preservation instead of hazardous chemical ones. As well as, generally recognized as safe (GRAS). "GRAS" is an acronym for the phrase Generally Recognized As Safe, which is an American Food and Drug Administration (FDA) designation that, a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements.

The (MIC) is defined as the lowest concentration of a drug/compound that will inhibit the visible growth of an organism in vitro after overnight incubation. Minimum inhibitory concentrations (MICs) are considered the "gold standard" for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of micro-organisms to an antimicrobial agent, to give a definitive answer when a borderline result is obtained by other methods of testing, and also to monitor the activity of new antimicrobial agents (30). An (MIC) is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (31). In recent years, (MICs) have been used in Phytotherapy, the use of plants for medical purposes, which is one of the oldest practices in the world. The demands for natural remedies along with the natural food additives combine phytotherapy with scientific studies. Thus, determination of the MICs has become the main factor for the scientific studies regarding the feasibility of bioactive components of the plants in industry (32).

"NCCLS/CLSI" National Committee for Clinical Laboratory Standards/ Clinical and Laboratory Standards Institute (2007): Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement, M2-A9 and M7-A7. Wayne, P. A., U. S. A.

CONCLUSION

The purpose of this study is to evaluate the microbiological safety of water, detection of Salmonella typhi and aims to investigate the potency of plant extracts as alternative antibiotics against pathogenic Salmonella isolated from different water resources. Salmonella sp isolates had different antibiotics sensitivity using disk diffusion test . Three different methanolic extracts of Cinnamon ; Rose marry and Grapefruit plants were revealed the inhibition potency at different dilutions against isolated Salmonella typhi for representing a good alternative to the use of traditional antimicrobial in treatments.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Ain shams faculty of Agriculture, hort culture department staff for providing Cinnamon ; Rose mary and Grapefruit plant extracts .Most sincere thanks to two of the authors, Prof. Khalid El Dougdoug and Prof. Nahed El Aiate.

REFERENCES

[1] Helmuth R .(2000): Antibiotic resistance in Salmonella . In: Wray C., Wary A (eds.) . Salmonella in domestic animals . CAB international ,pp 89-106.

[2] Montville TJ, Matthews KR (2005): Rapid and Automated Microbial Methods. In: Food Microbiology: An Introduction. Eds. Montville and Matthews. 1st ed. pages 53- 64. 2nd ed. pages 65-76. ASM Press

[3] Barbosa TM1 and Levy SB (2000): The impact of antibiotic use on resistance development and persistence. Drug Resist Updat. 2000 Oct;3(5):303-311.

[4] Schwarz, S., and Chaslus-Dancla, E. (2001): Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet. Res. 32, 201–225.

[5] APHA (2005): Standard Methods for the Examination of Water and Wastewater (21st Edition). Washington: American Public Health Association, American Water Works Association, Water Environment Federation.

[6] Collins, C. H. and Lyne, P. M. (2004). Microbiological Methods, (8th ed.). Arnold, London, England , 2004.

[7] Cheesbrough M, (2006). District laboratory practice in Tropical countries. Part nd edition. Cambridge University Press, U.K. p434. 2006. ISBN 0521676312, pp. 28-30.

[8] NCCLS/CLSI" National Committee for Clinical Laboratory Standards/ Clinical and Laboratory Standards Institute (2007): Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement, M2-A9 and M7-A7. Wayne, P. A., U. S. A.

[9] Beck, R.W. (2000) . A Chronology of Microbiology in Historical Context. ASM Press Washington, DC, 2000. [10] Azzam, M. I. (2010). Application of Enteric Viruses in the Detection of Water Pollution, Cairo, Egypt, Fac. of Agri., Ain Shams Univ., M. Sci. Thesis.

[10] Durán, A.E., Muniesa, M., Mocé-Llivina, L., Campos, C., Jofre, J. & Lucena, F (2003) Usefulness of different groups of bacteriophages as model microorganisms for evaluating chlorination. J. Appl. Microbiol. 95: 29-37.

[11] Sabae, S.Z. and Rabeh, S.A. (2007). Evaluation of microbial quality of the Nile River water at Damietta branch, Egypt. National institute of Oceanography and fisheries, Egyptian Journal of Aquatic Research, 33:301-311.

[12] Ezzat, S.M. (2008). Role of certain botanical extracts against bacteria isolated from Nile River and drainage water. Ph. D. In Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt. .

[13] Sneath, P.H.A., (1986). Bergeys Manual of Systematic Bacteriology. Vol. 2, Williams and Wilkins, Baltimore, pp: 965-1599.

[14] Edberg, S.C., E.W. Rice, M.J. Karlin and J. Allen.(2000). Escherichia coli: The best biological drinking water indicator for public health protection. J. Applied Microbiol., 88: 106S-116S.

[15] Tsoraeva, A and Martinez, C.R.(2000): Comparison of Two Culture Media for Selective Isolation and Membrane Filter Enumeration of Pseudomonas aeruginosa in water. Revista Latinoamericana de Microbiología (2000) 42:149-154.

[16] Abo-State MAM, Helimish FA, Husseiny ShM, Zickry ARA.(2012). Reduction of Health Hazard of Bacillus Species Contaminating Solution Lenses and Baby Powder by Imipenem and Gamma Radiation. World Appl Sci J. 2012;19:856–866.

[17] Graham DW , Knapp CW, Lázaro Lima , Werner D (2011): Antibiotic Resistance Gene Abundances Associated with Waste Discharges to the Almendares River near Havana, Cuba. Environ. Sci. Technol. 2011, 45, 418–42.

[18] Baquero F, Martínez JL, Cantón R.(2008). Antibiotics and antibiotic resistance in water environments. Current Opinion in Biotechnology. 19:260-265.

[19] McArthur JM, Nath B, Banerjee DM, Purohit R, Grassineau N (2011): Palaeosol control on groundwater flow and pollutant distribution: the example of arsenic. Environ Sci Technol 2011;45:1376–83.

[20] Garcia-Armisen T, Papalexandratou Z, Hendryckx H, Camu N, Vrancken G, De Vuyst L, Cornelis P (2010): Diversity of the total bacterial community associated with Ghanaian and Brazilian cocoa bean fermentation samples as revealed by a 16 S rRNA gene clone library. Appl. Microbiol. Biotechnol. 2010;87:2281–2292.

[21] Dakkak, Amir."Egypt's Water Crisis – Recipe for Disaster." EcoMena, 23 Dec. 2014. Web. 9 Sept. 2015. http://www.ecomena.org/tag/water-scarcity-in-egypt.

[22] Davise, E.G.R. and S.P. Simonovic (2011). Water Resources and Integrated Assessment Modeling – the ANEMI Model", Advances in Water Resources (available online: 18 February 2011), 34:684-700.

[23] Ceylan E and Fung DYC (2004): Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology 12:1-55.

[24] Ayachi, A., Alloui, N., Beneoune, O., Yakhlef, G., Amiour, S., Bouzid, W., Zoughlache, S., Boudjellal, K. & Abdessemed, H. (2009). Antibacterial activity of some fruits, berries and medicinal herb extracts against poultry strains of Salmonella. American- Eurasian Journal of Agriculture and Environmental Sciences, Volume 6, No 1, (February 2009), pp. 12-15.

[25] Nilforoushzadeha M, Shirani-Bidabadia L, Zolfaghari-Baghbaderania A, Saberia S, Mahmoudib M (2008). Comparison of Thymus vulgaris (Thyme), Achillea millefolium (Yarrow) and propolis hydroalcoholic extracts versus systemic glucantime in the treatment of cutaneous leishmaniasis in balb/c mice. J Vector Borne Dis. 2008; 45:301-306.

[26] Boyd L, Beveridge EG. (1981) : Antimicrobial activity of some alkyl esters of gallic acid (3,4,5,-trihydroxybenzoic acid) against Escherichia coli NCTC 5933 with particular reference to n-propyl gallate . Microbios. 1981;30(120):73-85.

[27] Moreno, S.; Scheyer, T.; Romano, C. and Vojnov, A .(2006): Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radic. Res., 40(2):223-231.

[28] Raybaudi-Massilia, R.; Mosqueda-Melgar, J.; Soliva-Fortuny, R. and Martin-Belloso, O. (2009). Control of Pathogenic and Spoilage Microorganisms in Fresh-cut Fruits and Fruit Juices by Traditional and Alternative Natural Antimicrobials. Compr. Rev. Food Sci. Food Saf., 8(3):157-180.

[29] Barros L, Baptista P, Estevinho LM, Ferreira IC(2007): Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of Lactarius sp. mushrooms. J Agric Food Chem. 2007;55:8766–71.

[30] European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). 2000: Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin. Microbiol. Infect. 6:509-515.

[31] Watase Renee A and Reppun Thomas s.(2011): Antimicrobial susceptibility testing. Avaliabe from < http://www.dlslab.com.