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Studying the effect of natural extracts to control bacterial contamination of drinking water.

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ABSTRACT : **Some biological parameter of raw water as the source and treated water in Abassa station,Abu hammad,Sharkia Governarate such as algal count,protozoa,total bacterial count,total coliform and fecal coliform .These results showed that the algal count of algae in treated water ranged between 9 to 36 algal count /1L ,no protozoa , no total coliform and no fecal coliform found in treated water while the count of total bacteria found in treated water ranged between 0 to 2 CFU/1ml.Different bacterial isolates which isolated from different stages in abassa water station (Raw , Filtrate , Treat, Net) and identified by using some biochemical tests such as Staphylococcus spp,E.coli spp,Proteus spp,Salmonella spp,Bacillus spp,Micrococcus spp,Klebsiella spp .Using natural extracts such as moringa olifera seed,okra seed , clove seed and ginger seed as antibacterial activity against some isolated bacteria and results showed that moringa seed extract was the most effective extract as antibacterial activity with concentration 1.0gm/50ml give 28 mm inhibition zone diameter against Stapylococcus spp.**

KEYWORDS: **Moringa oleifera, Okra seed, Clove seed, Ginger seed, antibacterial activity, Drinking water.**

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I. INTRODUCTION

Water is the most common liquid in the world, covering approximately 71% of the earth's surface. Nevertheless, only 2.8% is fresh water and the greater part of that is not available given that 75% is frozen and only 0.63% (8 million km3) is available in lakes, rivers and lagoons(Young and Yong, 2003).

Most of the parameters that selected for analysis are obligatory from the directive, comprising both physicochemical (such as pH, conductivity and total dissolved solids-TDS) and chemical properties which are related to the treatment of water itself and its hardness (Cl-, Na+, Ca2+ K+, and Mg2+), heavy metals (Cd, Pb, ,Cr, Cu and Ni), ions (F-, NO2-, PO43-, NO3-, Br-, SO42- and NH4+) as well as dissolved organic carbon (DOC).

Significant effect on human health either through deficiency or toxicity due to excessive intake. Nitrate (NO3) and nitrites (NO2) are found in water naturally (Jordao et al. 2002) and the toxicity of nitrate to humans is mainly lead to its reduction to nitrite.

The major biological effect of nitrite is the oxidation of normal hemoglobin to methaemoglobin, which is unable to transport oxygen to the tissues. Human health risk associated with nitrate consumption is considered to be of methaemoglobinaemia by nitrate-derived (Siddiqui et al., 2010) studied the microbiological quality of some samples of drinking water and found 36% samples contaminated with pathogenic bacteria.

The most bacterial diseases that caused by bacterial contamination of water are typhoid fever, cholera and bacillary dysentery (Joao and Cabral ,2010).

Moringa oleifera is the sole genus in the flowering plant family Moringaceae that spread in Asia and Africa. Among the 14 species, of Moringa oleifera most widely identified and utilized (Morton, 1991; Steinitz et al., 2009).

In medicine, leaves are rubbed against the temple to treatment headaches and poultice of fresh leaves are used to stop bleeding from a shallow cut. The leaf juice is used to control glycaemia and is applied for swollen glands. They are traditionally used in the treatment of fevers, inflammation of nose, throat, bronchitis, ear and eye infections and to combat vitamin C deficiency. They are also used to control hypertension and hypercholesterolemia (Asare et al., 2012).

M. oleifera leaves exhibiting antimicrobial activity against both Gram- negative and Gram positive bacteria. Because of the acetone extracts had bactericidal activity against E.coli which was tested in high concentrations. Gram-negative bacteria have been known to be generally less response to the activity of plant extracts because of permeability barrier provided with the cell wall or to the membrane accumulation mechanism (Abaliwan et al., 2008).

The ability of the extract of okra to inhibit bacterial growth was observed by broth micro dilution susceptibility tests and by diffusion of the compounds on agar plates (CLSI (2010).

Sofia, (2007) studied the antimicrobial activity of clove against many bacterial and fungal strains and also studied the antimicrobial activity of various Indian spice plants such as ginger, mint, garlic, mustard and cinnamon.

Ginger have the ability to inhibit biofilm formation that consider essential part of antimicrobial resistance and infection so ginger inhibited the growth of a multidrug-resistant strain of Pseudomonas aeruginosa by inhibiting biofilm formation and affecting on membrane integrity of bacterial cell (.Chakotiya, et al., 2017).

. II.Materials and Methods

1. Isolation of different bacterial isolates:

Filtrations of different water sample within a membrane filter (M F) consists of a cellulose pore diameter of 0.45 micron that remained the bacteria on the surface of membrane filter paper for (clarified and filtrate stages).Take the membrane containing the bacteria on the surface of sterile a selective media (nutrient agar) media on petri plate and incubate at appropriate temperature (37°c) in which colonies of coliform counted directly. The method (protozoa, algae) is based on M.F and centrifugation at 4000 rpm for 10 min then examined pellet under microscope. (WHO , 2004 ; Gautam, 2014).

2. Identification of isolated bacteria:

Morphological and biochemical tests were done to identify the bacterial isolates according to (Bergey's Manual of Determinative Bacteriology 1994).

2.1. Gram staining technique:

Smear of about 200mm in diameter on grease free slide which was also fixed above a burning flame. A crystal violet dye was putted to cover the smear for 30 seconds , then washed with distilled water. Secondly lugol's iodine was putted to the surface for 30 seconds. Ethyl alcohol was used to decolorize the stain and finally, the safranin dye solution was applied to the surface of slide for one minute then washed with distilled water and remains slide to dry at room temperature. Then the stains have been scanned under microscope with oil immersion and Gram- negative bacteria is observed as red color and Gram- positive bacteria is observed as violet color (Cheesbrough, 2000).

3. Biochemical identification of isolated bacteria:

3.1. Lysine decarboxylase broth

Detected salmonella spp from group of enterobacteriaceae (Ewing et al, 1972).

Composition (g/l)

Peptic digest of animal tissue	5
Yeast extract	3
Dextrose	1
L-Lysine hydrochloride	5
Bromocresol purple	0.02
Final pH (at 25°C)	6.8±0.2

Procedure:

Dissolve 14.02 grams in 1000 ml distilled water. Heat until complete dissolves the medium. Put 5 ml amount into screw-closed test tubes. Sterilize the medium by autoclaving at 1.5 lbs pressure (121°C) for 20 minutes. Cool

the tubed medium at room temperature. Finally color appearance: Light yellow to greenish yellow homogeneous free flowing powder.

3.2. Tryptone Bile X-Glucuronide (TBX):

This media is used to the isolation and identification of E. coli in water treatment laboratory (Gross and Rowe , 1985).

Composition: (g/l)

Tryptone	20
Bile salts	1.5
X-glucuronide	0.075
Agar	15
Final pH(at 25°C)	7.2 ± 0.2

Procedure:

Suspend 36.6 g of medium in 1000 ml of distilled water, heat with frequent shaking and boil for one minute until dissolve the medium is completed, autoclave at 15 lbs pressure and 121°C for 15 minutes.

3.3. Citrate agar :

To identification and differentiate the members of Enterobacteriaceae on the basis of citrate utilization from clinical and non-clinical samples (Eaton et al., 2005).

Composition: (g/l)

Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Bromothymol blue	0.08
Sodium chloride	5
Sodium citrate	2
Agar	15
Final pH (at 25°C)	6.8± 0.2

Procedure:

Dissolve 24.28 grams in 1000 ml pure distilled water, heat and boiling to complete dissolve the medium, mix well and distribution tubes, sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes, finally before using water, ensure pH of water is 6.5 to 7.0.Initial color of the to medium may different from expected color, if the pH is ignored.

3.4. Triple sugar iron agar:

Identification of bacteria is according to dextrose, lactose and Sucrose fermentation and production of hydrogen sulfide (Eaton et al., 2005).

Composition: (g/l)

Beef extract	3
Gelatin/meat peptone	15
Lactose	10
Dextrose	1
Ferric ammonium citrate	0.2
Yeast extract	3
Casein/meat peptone	5
Sucrose	10
Sodium chloride	5
Sodium thiosulfate	0.3
Phenol red	24
Agar	12

Procedure:

By a straight sterilizing needle, select an isolated colony from the culture plate, put needle into the butt of the medium, pull needle to the slant and streak half way up the slant surface, exchange cap loosely on the tube, Incubate aerobically for (18–24 hours) at 35 ± 2°C, watch and record reactions, perfect growth must occur in the butt and slant.

3.5. Sulfide indole motility medium (SIM)

Differentiation of enteric bacteria according to their ability to produce indole and hydrogen sulfide and display motility (MacFaddin , 2000).

Composition: (g/l)

Pancreatic digest of casein	20

Peptic digest of animal tissue	6.1
Ferrous ammonium sulfate	0.2
Sodium thiosulfate	0.2
Agar	3.5
pH	7.3 ± 0.2

Procedure:

Pure isolated colony from a pure culture plate were transferred by using a straight inoculating sterile needle, inoculate by transfixion the middle of the tube the depth of the medium, incubate tubes aerobically at 35°C for 18 to 24 hours, observe tubes and explain results after 18 to24 hours of incubation, for the indole test, add few drops of Kovac'sReagent and read results during 1 minute.

3.6. Urease-berthelot method:

Mix and incubate for 10 min. at 37ºC, measure the absorbance of the sample (A Sample) and of the standard (A Standard) against the blank at 550 nm,(530 – 570 nm). color is stable for 5 hours, linearity up to 200 mg / dl (33.3 m mol/L) inserum or plasma and 4 g / dl (665 mmol/L) in urine. (Eaton et al., 2005) .

. 3.7. Indole test:

This test is performed to identify the E .coli, Shigellae and Salmonellae from tested colonies. A sterile loop was used to inoculate overnight growth cultures into a test tube of 5ml peptone water then incubated at 35 - 37 ºC for 24 hours then add 5 drops of Kovac'sindole reagent and shaken gently. Appearance of red color on the top layer. E. coli and Shigella were indole positive while Salmonella was indole negative (Cheesbrough, 2000).

3.8. Catalase test:

Rapid air bubbling or effervescence indicates positive result by adding few drops of hydrogen peroxide to colony stand on clean slide by platinum loop. The lack of bubbles indicates the absence of catalase (Forbes et al., 1998b).

3.9. Macconky agar media:

This media used for identification of some isolated bacteria .

Composition: (g/l)

Pepton	20
Lactose	10
Bile salt no31	5
Sodium chloride	5
Neutral red	0.03
Crystal violet	0.001
Agar	15
Distilled water	1000 ml

Procedure:

Suspend 55.03 g in 1 litre of distilled water, bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minute(Sonnenwirth, 1980).

3.10. Enterococcus media:

Used for isolating and identification enterococci in water and other materials by filter membrane or pour plate technique (Eaton, et al,2005).

Composition: (g/l)

Tryptose	20
Yeast extract	5
Dextrose	2
Dipotassium phosphate	4
Sodium azide	0.4
2,3,5-Triphenyl tetrazolium chloride	0.1
Agar	10

Procedure:

Follow the filter membrane procedure as showed in standard methods for the examination of water and wastewater, choose a sample size so that 20-60 colonies will result, transfer the filter to agar medium in a Petri dish, with avoiding air bubbles under the membrane, keep plates stand for 30 minutes, invert plates and incubate at 35 ± 0.5°C for 48 hours.

4. Antibacterial activity of different plant extract (moringa , okra,clove and ginger)against some isolated bacteria:

The sensitivity test was applied by using disc diffusion method. Serial dilution from seed extracts 0.5gm/50 ml, 0.1gm/50ml, and the sterilized filter paper discs were immerged with different concentration of seed extracts. The nutrient agar plates were inoculated by streaking the test organisms separately within the entire surface of the

plates. Discs immerged with extracts putted above the surface of the inoculated nutrient agar plates while it was wet with the sterilized forceps. The inoculated plates were incubated at 370 C. After24 hours, plates having clear zone of inhibition were showed and zone diameter were measured using a ruler in millimeters. (Kalpana et al., 2013 ; Abdallah, 2014).

Discs of about 6mm diameter were made from Whatman's No.1 filter paper and sterilized in the autoclave at 1210C for15minutes (Abdallah, 2014).

III.RESLUTS And DISCUSSIONS

1-Biological analysis of water samples of abassa water station:

Samples from water station abassa water station were collected and analyzed for main parameters:

The obtain results in tables (1-4) clearly showed the result of biological analysis of raw water as the source and final stage in the water treatment in abassa station in the period from 1/1/2018 to 24/12/2018 and these results showed the count of total bacteria ranged between no bacterial growth to 2 CFU/1ml and no protozoa found in treated water also results showed that no total coliforms found in water treatment at different season's .The obtained results also indicated that there is no fecal coliforms in treated water and algal count was 9 to 36 algal count/1L in treated water at abassa station. On the other hand the total bacterial counts in raw water ranged from 118to 238 CFU/1ml and present of protozoa .The obtained resultsin raw water showed also that total coliform ranged from 540 to 1600 MPN/100 ml ,fecal coliform ranged from 70 to 280 MPN/100 ml and algal count ranged from 1820 to 4216 algal count/1L.

Table (1): Biological analysis for raw water and treated water at abassa water station at winter season2018 (january, february, march).

Tested parameter	1/1/2018		24/1/2018		1/2/2018		24/2/2018		1/3/2018		24/3/2018	
	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat
Bacterial count CFU/1ml	136	2	142	-ve	128	-ve	132	-ve	118	1	143	2
Total coliforms MPN/100 ml	1600	-ve	920	-ve	540	-ve	1600	-ve	920	-ve	540	-ve
Fecal coliforms MPN/100 ml	70	-ve	94	-ve	220	-ve	110	-ve	120	-ve	94	-ve
Algal count Algal count/1L	4200	24	3880	32	4216	36	2882	28	2218	18	1822	14
Protozoa	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Table (2): Biological analysis for raw and treated water at abassa water station at spring season2018 (april, may, june).

Tested parameter	1/4/2018		24/42018		1/5/2018		24/5/2018		1/6/2018		24/6/2018	
	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat
Bacterial count CFU/1ml	124	-ve	238	-ve	124	1	184	2	128	-ve	132	1
Total coliforms MPN/100 ml	920	-ve	540	-ve	1600	-ve	920	-ve	540	-ve	1600	-ve
Fecal coliforms MPN/100 ml	280	-ve	94	-ve	120	-ve	70	-ve	130	-ve	120	-ve
Algal count Algal count/1L	2100	18	2218	24	1820	16	1820	14	2224	22	2418	32
Protozoa	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Table (3): Biological analysis for raw and treated water at abassa water station at summer season2018 (july, august, september).

Tested parameter	1/7/2018		24/7/2018		1/8/2018		24/8/2018		1/9/2018		24/9/2018	
	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat
Bacterial count CFU/1ml	148	-ve	142	-ve	118	-ve	108	-ve	138	2	124	-ve
Total coliforms MPN/100 ml	540	-ve	1600	-ve	920	-ve	1600	-ve	920	-ve	540	-ve
Fecal coliforms MPN/100 ml	220	-ve	70	-ve	110	-ve	230	-ve	94	-ve	70	-ve
Algal count Algal count/1L	2200	18	1820	32	2218	28	2418	32	2416	22	2218	24
Protozoa	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Table (4): Biological analysis for raw and treated water at abassa water station at autumn season2018 (october, november, december).

Tested parameter	1/10/2018		24/10/2018		1/11/2018		24/11/2018		1/12/2018		24/12/2018	
	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat	Raw	Treat
Bacterial count CFU/1ml	150	2	142	-ve	182	1	136	-ve	120	-ve	138	-ve
Total coliforms MPN/100 ml	1600	-ve	920	-ve	540	-ve	540	-ve	1600	-ve	920	-ve
Fecal coliforms MPN/100 ml	280	-ve	160	-ve	230	-ve	110	-ve	170	-ve	220	-ve
Algal count Algal count/1L	4218	32	4012	34	3818	32	4216	36	3218	34	4216	28
Protozoa	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

2-Isolation, charactraization and counting of different bacterial isolates from different sources in the period from january to december 2018.

Collecting samples from raw water and different treatment stages and filtrate it through specific cellulose membrane (M.F) that have specific pore diameter 45 micron that have ability to remain bacteria on surface of filter membrane .

Samples were collected during the period January to December 2018from Abassa station water, from treatment stages, net water, raw water and treated water. Inoculated isolated bacteria on surface of media then incubated for 24 hrs at 37^0C.

Examine incubated bacteria that growth by eye and showing shape of colony on media, high and color on media.

Finally microscopic examination to differentiate between Gram positive and Gram negative as illustrated in table (5).

Table (5) Isolation, charactraization and counting of different bacterial isolates from different sources in the period from january to december 2018.

Isolates	Source	Gram stain	Bacterialshape cell	Color on media	High on media	Colony on media
1	Raw	Positive	Cocci mono	White	Under	Irregular undulate numerous
2	Raw	Positive	Cocci compact	Yellow	Under	Smooth entire
3	Raw	Positive	Cocci mono	White	On	Irregular undulate tree
4	Floc	Positive	Many Rod	White	Under	Irregular undulate

5	Raw	Positive	Cocci mono	White dark	Under	Irregular undulat
6	Raw	Negative	Cocci + rod	Slightly White	Under	Smooth entire
7	Floc	Positive	Cocci mono	Slightly White	Under	Filamentous lobate
8	Floc	Positive	Cocci strep	White	Under	Filamentous lobate
9	Raw	Negative	Cocci mono	Dark	Under	Irregular lobate
10	Floc	Positive	Cocci compact	Slightly White	On	Irregular undulate
11	Floc	Negative	Cocci strep	White dark	Convex	Irregular undulate numerous
12	Raw	Positive	Cocci 1&2&3	White medium	On	Irregular undulate numerous
13	Raw	Positive	Cocci mono	dark	Flat	Smooth round
14	Floc	Negative	Cocci 1&2&3	Slightly White	Convex	Smooth entire
15	Raw	Positive	Strep rod	Slightly White	On	Smooth entire
16	Raw	Positive	Cocci mono	Yellow	Flat	Irregular undulate numerous
17	Raw	Negative	Cocci + rod	White	On	Irregular undulate numerous
18	Raw	Positive	Cocci 3+ strep	Slightly White	Flat	Irregular dots
19	Raw	Positive	Cocci mono	Very slightly White	Under	Smooth entire
20	Raw	Positive	Cocci strep	White dark	Umbonate	Irregular lobate
1	Floc	Positive	Cocci strep	Yellow	On	Irregular undulate
22	Raw	Positive	Cocci mono	Slightly White	Flat	Irregular undulate
23	Raw	Negative	Rod 2&3	Slightly White	On	Irregular undulate
24	Floc	Negative	Medium rod	High white	On	Irregular undulate
25	Raw + Treat	Positive	Long rod	Yellow	On	Irregular undulate
26	Raw + Treat	Positive	Long + medium rod	Ice white	Flat	Smooth entire
27	Raw	Negative	Medium rod	White	Under	Irregular pyramids
28	Raw	Positive	Cocci strep	White medium	Flat	Irregular pen
29	Floc	Negative	Strep rod	Slightly White	On	Irregular undulate numerous

30	Raw	Negative	Medium rod	White	On	Irregular tree
31	Raw	Negative	Cocci	Yellow	On	Irregular hand
32	Treat	Positive	Cocci mono	Slightly yellow	Flat	Irregular pen
33	Raw + Treat	Negative	Cocci strep	White medium	On	Irregular pyramids
34	Raw	Positive	Cocci mono	High white	On	Irregular undulate
35	Floc	Negative	Rod mono	White	On	Irregular undulate numerous
36	Floc	Negative	Cocci strep	White medium	Convex	Filamentous lobate
37	Raw	Negative	Cocci strep	White	On	Irregular pen
38	Floc	Negative	Cocci 1&2&3	Yellow	Convex	Irregular tree
39	Floc	Negative	Cocci strep	White	On	Irregular
40	Raw	Positive	Strep rod	High white	On	Irregular undulate numerous
41	Raw	Positive	Cocci 1&2&3	High white	On	Filamentous lobate
42	Raw	Negative	Strep rod	High white	On	Irregular undulate numerous
43	Raw	Negative	Cocci mono	White	Flat	Irregular pen
44	Raw	Positive	Cocci strep	High white	Flat	Irregular undulate
45	Raw	Negative	Cocci strep	White	On	Irregular undulate numerous
46	Raw	Positive	Strep rod	High white	On	Filamentous lobate
47	Raw	Positive	Strep rod	White	On	Irregular tree
48	Floc	Negative	Cocci strep	Yellow	On	Irregular undulate numerous
49	Raw	Positive	Cocci mono	White medium	Under	Irregular undulate
50	Floc	Negative	Cocci strep	White	On	Irregular undulate numerous
51	Floc	Positive	Cocci strep	White	On	Irregular undulate numerous
52	Floc	Positive	Cocci mono	Slightly White	On	Smooth entire
53	Floc	Negative	Many rod	High white	On	Filamentous lobate
54	Raw	Negative	Cocci	White medium	Under	Irregular undulate numerous
55	Floc	Negative	Many rod	White	On	Regular

56	Raw	Positive	Cocci strep	White	On	Regular smooth
57	Raw	Negative	Cocci 1&2&3	Yellow	Flat	Irregular tree
58	Raw	Negative	Cocci compact	White	On	Regular
59	Raw	Negative	Short rod	White medium	On	Irregular undulate numerous
60	Raw	Negative	Cocci compact	Slightly White	Flat	Irregular undulate numerous
61	Raw	Positive	Cocci mono	White	On	Irregular undulate numerous
62	Raw	Negative	Cocci 1&2&3	High white	On	Irregular tree
63	Net	Negative	Strep cocci rod	White	Convex	Irregular undulate
64	Net	Positive	Many rod	Yellow	On	Irregular undulate numerous
65	Net	Negative	Strep rod	White	On	Irregular tree
66	Raw	Positive	Cocci compact	Slightly White	On	Regular
67	Raw	Negative	Cocci mono	Slightly White	On	Irregular flower
68	Raw	Negative	Cocci strep	High white	Flat	Irregular undulate
69	Raw + Net	Positive	Many cocci	White medium	On	Regular smooth
70	Net	Positive	Cocci 1&2	High white	On	Irregular undulate numerous
71	Raw + Net	Negative	Cocci 1&2	Slightly White	On	Irregular undulate numerous
72	Raw + Net	Negative	Cocci strep	White medium	On	Irregular tree
73	Raw + Net	Negative	Short rod	White	Under	Irregular undulate
74	Net	Positive	Cocci 1&2	Yellow	Under	Irregular undulate numerous
75	Raw	Positive	Cocci 1&2&3	White medium	Flat	Regular
76	Raw	Negative	Cocci mono	White	On	Irregular undulate
77	Net	Negative	Cocci 1&2	Red	On	Regular
78	Net	Negative	Cocci strep	White	On	Irregular undulate numerous

Table (6) Percent of G+ve and G-ve bacteria that isolated from different sources:

Source	Total count	G+ve count	%	G-ve count	%
Raw	44	24	54.5	20	45.5
Floc	19	7	36.8	12	63.2

Raw+treat	3	2	66.6	1	33.4
Treat	1	1	100	_	0
Raw+net	7	3	42.8	4	57.2
Net	4	1	25	3	75

The obtained resut in table (6) classified the bacteria that isolated from different sources according to gram stain and the result in case of raw water source showed that the percent of G+ve bacteria 54.5%,G-ve bacteria 45.5%,in case of flocculation source the percent of G+ve bacteria 36.8%,G-ve bacteria 63.2%,in case of raw+treated source the precent of G+ve bacteria 66.6%,G-ve bacteria 33.4%,in case of treated water source only G+ve bacteria that present as apercent 100% ,in case of raw +net source the percent of G+ve bacteria 4.28%,G-ve bacteria 57.2% and finally in case of network source the percent of G+ve bacteria 25%,G-ve bacteria 75% .

3-dentification of selected bacterial isolates from Abassa water station.

3.1. Gram staining for isolated bacteria:

Table (7) showing that bacteria can classify into two types

1- Gram negative (red color) bacteria number 41,6,30,55
2- Gram positive (violet color) bacteria number 69,4,44,19

3.2-Biochemical analysis for bacteria.

In Table (7) the obtain results clearly showed the results of biochemical analysis to identified selected isolated bacteria as:

3.2.1. Sulfide indole motility media test (SIM):

Used to identification of enteric bacteria based on their availability to produce hydrogen sulfide and indole ring and showing motility.SIM media is a semisolid medium designed to aid in the identification of *Enterobacteriaceae* particularly *Shigella* and *Salmonella* based on their availability to produce hydrogen sulfide, to break indole from tryptophan, and to showing motility and data in table table (19) showed the results of this experiment as bacteria number 44 ,69,55 , 19 showed negative and bacteria number 41,4, 6 , 30 give positive results.

3.2.2. Tryptone bile x-guconride agar test (TPX):

Used to identification of *E. coli* in water in a laboratory setting. The result in table (7) showing bacteria number 41, 4 , 30 , 55 , 69 , 19 , 44 give negative results and bacteria number 6 showed positive results.

3.2.3. Indole test:

This test identifies *E .coli, Salmonellae and Shigellae* from suspected colonies, the results in table (7) showing bacteria number 41, 4, 30, 55, 69 , 19 , 44 give negative results and bacteria number 6 showed positive results.

3.2.4. Citrate test:

Citrate agar is performed to identify the members of *Enterobacteriaceae* on the basis utilization of citrate from clinical and nonclinical samples. the results in table (7) showing bacteria number 30 , 41 , 6 give negative resultsand bacteria number 55,19,44,4,69,41 showed positive results.

3.2.5. Catalase test:

The results in table (7) showing that all bacteria number gives positive results.

3.2.6. Lysine decarboxylase test (LIA):

Used to identification of Salmonella from the group of *Enterobacteriaceae* and the rsults in table (7) showed bacteria number 4, 19, 44, 69, 55 give negative results and bacteria number 30, 41, 6 showed positive results.

3.2.7. Mcconky agar media:

This media used to identification of some bacteria in which pale colonies indicate the presence of Salmonella &Shigella and pink color indicate the presence of E.coli and the results in table (7) showing bacteria number 69,4,44,19 give negative results and bacteria number41,6,30,55 showed positive results.

3.2.8 .M-enterococcus media for isolated bacteria:

This media used to identification, counting and isolating enterococci by pour plate or membrane filtration technique and the results in table (7) showing bacteria number 69,4,44,19 give negative results and bacteria number 41,6,30,55 showed positive results .

3.2.9. Triple sugar iron agar (TSI):

This test used to differentiates between bacteria that have ability to formed fermentation to glucose, sucrose, lactose and producing hydrogen sulfide and the results in table (7) showing that all bacteria number gives positive results in case of glucose, in case of sucrose bacteria number 6, 69, 44,41give negative results and bacteria number 19, 4, 30, 55 showed positive results,in case of lactose bacteria number 19,69,4,44,30,41give negative results and bacteria number 6,55 showed positive results.

Table (7) Identification of selected isolated bacteria by using biochemical tests.

Bacteria number	SIM	TPX	Indole	Citrate	Catalase	LIA	Gram stain	Macconky	M- entero	Glucose	Sucrose	Lactose	Urea	Suspected bacteria
6	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	***E.coli spp***
19	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	***Micrococcus spp***
69	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	***Staph-aurus spp***
4	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	***Bacillus spp***
44	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	***Micrococcus spp***
30	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	***Proteus spp***

41	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	***Salmonella spp***
55	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	***Klebsiella spp***

4-Antibacterial activity of different natural extract of moringa, okra,ginger and clove seeds at different concentrations on selected isolated bacteria:

The obtained results in table (8) clearly showed that that moringa seed extract at concentration 1.0gm/50ml showed the highest antibacterial activity compared with all plant extracts in which moringa seed at concentration 1.0gm/50ml give 28 mm inhibition zone diameter and at 0.50gm/50ml give 24 mm inhibition zone diameter against *staph-aureus spp*,okra seed extract give the highest antibacterial activity 10mm at concentration 1.0 gm/50ml and 6mm at concentration0.50 gm/50ml against *salmonella spp* ,ginger seed extract give the highest antibacterial activity 9 mm at concentration 1.0 gm/50ml and 3mm at concentration 0.50gm/50ml against *micrococcus spp*, clove seed extract give the highest antibacterial activity 12 mm at concentration 1.0 gm/50ml and 4mm at concentration 0.50gm/50ml against *micrococcus spp*.In generally moringa seed extract was the most effect extract used as antibacterial activity especially with concentration 1.0gm/50ml give 28 mm inhibition zone diameter against *staph-aureus spp*.

Table (8) Antibacterial activityof different natural extract of moringa, okra,ginger and clove seeds at different concentrations on selected isolated bacteria:

Isolated bacteria	Diameter of inhibition zone (mm)							
	Moringa seed		Okra seed		Ginger seed		Clove seed	
	0.5 gm/50ml	1.0 gm/50ml	0.5 gm/50ml	1.0 gm/50ml	0.5 gm/50ml	1.0 gm/50ml	0.5 gm/50ml	1.0 gm/50ml
E.coli spp (6)	16	18	5	8	2	8	3	9
Micrococcus spp (19)	18	22	-ve	-ve	3	9	4	12
Staph-aureus (69)	24	30	3	4	-ve	2	-ve	5
Bacillus spp (4)	18	20	4	5	2	3	3	8

Micrococcus spp(44)	24	25	2	4	-ve	-ve	2	8
Proteus spp(30)	14	20	3	5	-ve	5	-ve	-ve
Salmonella spp(41)	14	22	6	10	-ve	3	-ve	7
Klebsiella spp(55)	16	22	-ve	7	-ve	-ve	3	11

IV.Discussion

Understanding susceptible factors related to microbial water safety at PoU may support to plan intervention for decreasing health vulnerability due to unsafe water **(Baidya *et al.*, 2018).**

The Millennium Development Goal (MDG) target called for the proportion the population without possible, access to safe drinking water to be halved between 1990 and 2015.During the MDG time it is estimated that, globally, use of enhanced drinking water source evaluated from 76 to 91per cent. The MDG target of 88 per cent was exceed in 2010 and 2015; 6.6 billion people use an inhanced drinking water source. Now only three countries with less than 50 per cent coverage, compared with 23 in 1990 **(WHO /UNISEF , 2015).**

The latter service level is determined by criteria including the absence of *E.coli*. Bottled water is grouped as improved if the household has increase to an improved source for cooking and washing (**WHO/UNISEF, 2006).** To determine exposition to faecal pollution through drinking water as showed by levels of *E.coli* or thermo tolerant coliform (TTC) in water source **(Bain *et al.*, 2014).** Similarly to our result, showed that untreated water source were more heavily polluted with both coliforms and total faecal than treated water source **(Hafez , 2013) .**

The obtain result clearly indicated that isolate *E. coli ,Salmonella spp, Proteus spp ,Staph.aurus spp ,Baccillus spp , Klebsilla spp and Micrococcus spp* from various stages in water treatment in Abassa station , net water Abassa water plants. All these and other were confirmed from various stages (Raw , Filtrate , Treat, Net).The results clearly indicated that chlorination is efficient against the bacteria in water , drinking water must be free some components which may affect the human health . Such component include minerals, organic substances and pathogenic microorganisms **(Haydar *et al.* , 2009).**

Microbiological parameter for Abassa water station showed that no present for protozoa ,no total coliforms found in treated water at different season's .The obtained results also indicated that there is no fecal coliforms in treated water and algal count was 9 to 36 algal count/1L in treated water at abassa station. On that is agree to standard limit for drinking water in egypt quality according to low 458 of 2007. **(Bain *et al.* , 2014).**

Isolation of bacteria at abassa water station showing that number bacteria reach to (78) and identification these isolates by using various method like gram stain, growth on enterococcus media , on maconky agar media and biochemical media **(Holt , 1994).**

Using different natural seed extracts as antibacterial activity against some isolated bacteria and the results showed that that moringa seed extract at concentration 1.0gm/50ml showed the highest antibacterial activity compared with all plant extracts in which moringa seed at concentration 1.0gm/50ml give 28 mm inhibition zone diameter and at 0.50gm/50ml give 24 mm inhibition zone diameter against *staph-aureus spp*,okra seed extract give the highest antibacterial activity 10mm at concentration 1.0 gm/50ml and 6mm at concentration0.50 gm/50ml against *salmonella spp* ,ginger seed extract give the highest antibacterial activity 9 mm at concentration 1.0 gm/50ml and 3mm at concentration 0.50gm/50ml against *micrococcus spp*, clove seed extract give the highest antibacterial activity 12 mm at concentration 1.0 gm/50ml and 4mm at concentration 0.50gm/50ml against *micrococcus spp*.In

generally moringa seed extract was the most effect extract used as antibacterial activity especially with concentration 1.0gm/50ml give 28 mm inhibition zone diameter against *staph-aureus spp*.

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