Combined Efficacy of *Lawsonia inermis* and *Myrtus communis* Extract as a Potential Factor in Bacterial Treatment to Hospital Wastewater, Iraq

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Combined Efficacy of *Lawsonia inermis* and *Myrtus communis* Extract as a Potential Factor in Bacterial Treatment to Hospital Wastewater, Iraq

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Abstract. The current study aimed to use biological treatment using plant extracts; extracted from some locally available plants: *Lawsonia inermis* (Henna), and *Myrtus communis* (Yass), against pathogenic bacteria isolated from heavy water in hospitals that do not have treatment plants. The two studied plants were extracted using two different solvents (water and alcohol). Diagnosis of these extracted using Gas chromatography (GC -MS) showed that the alcoholic extraction led to a higher percentage of effective phenolic compounds than the aqueous extract. Sewage water was used to isolate bacteria and according to the morphological, and biochemical, confirmed by using the vitke II instrument; six types of pathogenic bacteria were identified (*Staphylococcus lentus, Staphylococcus xylosus Sphingomonas paucimobilis, Escherichia coli, klebsiella oxytoca, Serratia ficaria*).The two Alcoholic plant extracted showed excellent antibacterial activity against all pathogenic bacteria than the water extracted as revealed by the diameter of the inhibition zone.

Keywords. Bacterial treatment, *Lawsonia inermis* (Henna), *Myrtus communis* (Yass), Medical extracts, Private and government hospitals.

1. Introduction

Nature has always been and continues to be a source of foods and ingredients beneficial to human health and plant extracts are increasingly becoming important additives in the food industry due to their content of bioactive compounds such as polyphenols and carotenoids [1], which have antimicrobial and antioxidant activity, especially against Low-density lipoprotein [2]. *Lawsonia inermis* (Henna) is a shrub or small tree grown in many regions as a commercial ornamental dye crop [3]. They are mostly found in tropical, subtropical and semi-arid regions of Africa (tropical savanna and tropical arid regions), southern Asia and northern Australia [4], a wide range of chemical constituents have been isolated from henna which includes naphthoquinone derivatives (lawson which is the main component and coloring matter in leaves), phenol derivatives, coumarins, xanthones, flavonoids, aliphatic components, triterpene, sterols and other chemical components such as glucose and gallic acid. And amino acids, mannitol, trace elements and minerals [5]. While *Myrtus communis* (Yass), common myrtle or true myrtle, is a type of flowering plant in the myrtle family Myrtaceae. It

is an evergreen shrub native to southern Europe, northern Africa, western Asia, Micronesia and the Indian subcontinent, and is also cultivated. It is also sometimes known as Corsican pepper; the plant is an evergreen shrub or small tree up to 5 m (16 ft) high. Leaves are 2–5 cm (1–2 in) long with essential oil [6]. It grows outdoors in moist but well-drained soil, with tough conditions but can grow under glass in silt-based compost in filtered light with good aeration but generally requires a long hot summer to produce flowers. Water is one of the important elements for all forms of life on Earth, and therefore any continuous pollution may result from several factors that lead to the deterioration of the Earth's life systems, the most important of which are population inflation, the development of light and heavy industries, and the dependence of most of the population on modern consumer technologies at the expense of the environment and natural resources [7], therefore preserving this wealth is one of the most important basics of life, one of the things that pollute water is biological contamination with bacteria. Bacteria is one of the most important contaminants of hospital wastewater, on average, hospitals produce 750 liters of wastewater per day. This effluent is loaded with pathogenic microorganisms, partially metabolized pharmaceuticals, radioactive elements and other toxic chemicals. Where studies revealed several pollutants coming out of sewage and other substances such as anti-tumor agents, antibiotics, and organohalogen compounds (OHC) such as absorbable organically bound halogens, sewage treatment plants are left untreated, which often leads to the deterioration of the environment in general [8]. There are many studies that used plant extracts as antibiotics, such as: [9-14]. The aim of this study is to use an effective plant extract that eliminates and reduces bacterial contamination in hospital water, and this plant is available in the environment and has a simplified extraction process that can be used in the future as filters in hospital treatment plants.

2. Materials and Methods

2.1. Field Work

Multiple samples were collected from different places in the center of Basrah Governorate, based on the APHA method [15]. These samples are wastewater from various hospitals. The following is a presentation of those hospitals: Al-Sadr Teaching, Basrah oil company, Ibn Al-Bitar (private), and Al-Mawaddah (private) (Fig 1). Water samples were taken from the main treatment plants of the hospitals mentioned above before and after treatment using 1-liter autoclaved sterilized bottle sampling between December, 2021 and February, 2022.

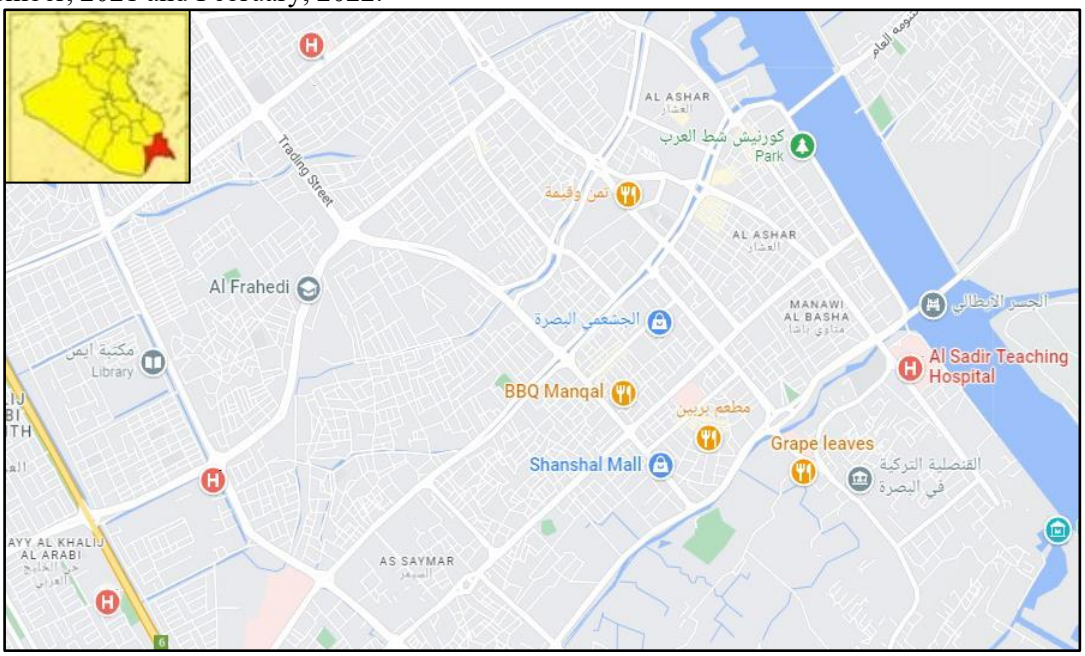


Figure 1. Google map image to the location of the studied hospitals, Basrah city, Iraq.

Table 1. Field work location.

No	Hospital	Latitude	Longitude
1	Al-Mawaddah	30º 30´ 1.41˝ N	47º 48´ 3.84˝ E
2	Ibn Al-Bitar	30º 29´ 37.56˝ N	47º 47´ 39.68˝ E
3	Basrah oil company	30º 3´ 31.75˝ N	47º 48´ 30.92˝ E
4	Al-Sadr Teaching	30º 30´ 21.43˝ N	47º 51´ 1.51˝ E

2.2. Plants Collection and Preparation

The survey included two types of plants which are *Myrtus communis* (Yass) and *Lawsonia inermis* (Henna), Plant materials were gathered once all the data had been compiled. The botanical names of the plants were ascertained [16,17], only leaves were used in this study. These plants were obtained from different houses in Basrah city, it was not collected from plant nurseries, even to ensure that it was not imported from abroad. After collecting, the leaves were cleaned from the dust, they were dried for two weeks in the shade areas, and then were ground by an electric mill and turned into powder for the purpose of preservation in special samples.

2.3. Plants Extraction

Fifteen g of either of the two powders of *Myrtus communis* and *Lawsonia inermis* was weighed and placed in a paper thimble tray which was fixed in the middle part of the Soxhlet. An amount of 150 mL of distilled water was added in the case of aqueous extraction or the same volume of ethyl alcohol in the case of ethanolic extraction into the circular flask of the Soxhlet . The aqueous or ethanolic solvent was heated to the boiling point to start the extraction process, which lasted for 8 hours in two days, four hours per day, where the green color of the solvent was observed as a result of the extraction process. After the time elapsed when the color of the extracted solvent was observed light yellow, the extraction process was stopped. The volume of the extracted solvent was reduced using a rotary evaporator to obtain a concentrated extract of about 20 mL volume, which was left at room temperature to dry [14,15]. The dry extract was weighed and the extraction yield was calculated according to equation 1:

$$Y\% = \left(\frac{We}{Wi}\right) * 100 \quad (1)$$

Where: Y% = extraction yield, We = weight of dry matter extracted, Wi = the initial weight of the powder used at the beginning of the extraction process, its constant value which equals 15 g. table (2) represents the weight of the dry extract and the extraction yield.

Table 2. Extraction yield to the studied extraction of Henna and Yass.

Extraction	We (g)	Y%
Ethanol Henna	6	40
Water Henna	3	20
Ethanol Yass	2.9	19
Water Yass	4	26

2.4. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The studied samples are analyzed by GC-MS followed to the Nahr Bin Umar laboratories, GC-MS analysis was carried out at the Basrah Oil Company Laboratory, by using an Agilent Technologies, 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector , using HP-5ms 5% phenyl , 95% methyl siloxane (30m*250um*0.25) , the oven temperature was set at 40 °C hold for 4 min then raised to 10 °C/min to 300 °C for 20 min, Helium carrier gas flow rate was 1 mL/min and purge flow 0f 3 mL/min. The injection mode was split with injection temperature 290 C and the injection sample volume was 0.5 micro letter. The mass spectrometer used Ion Source Temperature 230 °C, the mass range of 44-650 m/z, Data was run through the NIST 2020 and 2014, data base as an additional tool to confirm the identity of a compound.

2.5. Biological Test

Bacterial contamination in samples collected from treatment plants before and after treatment was examined, as follows:

2.5.1. Total Coliform and Feacal Coliform Examination

This examination was carried out by using selective media to count and identify fecal coliform bacteria. Both mediums (Endn agar and M.Fc agar base) were used, which were prepared according to the manufacturer's instructions (Hi media). The prepared media were kept in the refrigerator until used for testing. The method of decimal dilutions was followed in their preparation, which used the following dilutions (10^{-1} - 10^{-5}) by adding of sterile distilled water. The filtration method was used to culture the media with diluted samples, where the last dilution (SM and 9222G 9222D) was used. The bacterial numbers were calculated in the equation:

$$\text{Colony formed units}\frac{(\text{CFU})}{10\ mL} = \frac{\text{calculated number of colonies}}{\text{sample volume (mL)}} * \text{dilution factor} * 100 \quad (2)$$

2.5.2. Total Count of Bacteria

To find out the total number of bacteria in water samples diluted with decimal dilutions (10^{-1}-10^{-5}), a plate count agar culture medium was used, which was prepared according to the manufacturer's instructions (Hi media). 0.1 mL of the last dilution was taken and sown on the plant medium by the flatted method. After culture, the dishes were incubated at 37°C for 24 hours.

2.5.3. Isolation and Identification of Bacteria

To isolate the bacteria, 0.1 mL of the sample was taken and planted by diffusion method on the nutrient medium prepared according to the manufacturer's instructions (Hi Media). The dishes were then incubated in the incubator at 30°C for 24 hours. The growing cultures were purified on the plant medium. The incubation period is considered by doing several repeating the transplantation process until reaching the pure culture, whose colonies are characterized by the same shape, color and size, which was relied on in the subsequent diagnostics [18-20]. To diagnose the isolated bacteria, some phenotypic characteristics and some biochemical properties (gram stain) were studied. To confirm the diagnosis, samples were sent for diagnosis with the Phytek.

2.6. Antibacterial Activity Test

Different concentration from *Myrtus communis* (Yass) and *Lawsonia inermis* (Henna), was prepared (12.5, 25 and 50 mg/mL), using DMSO as a solvent agent. The antibacterial activity of these two-plant extracts was confirmed using the disc diffusion method against pathogenic bacteria isolated from the water sample; where bacteria was cultured on the surface of the Mueller Hinton agar plate (Himedia). Disk saturated with the suspension of plant extract with different concentrations put on the surface of plate and incubated for 24 h at 37°C.

3. Results

3.1. . GC-Mass Results

The results of GC-MS are illustrated in the Figs 2, 3, 4, and 5 and the data represented by Tables 3, 4, 5, and 6.

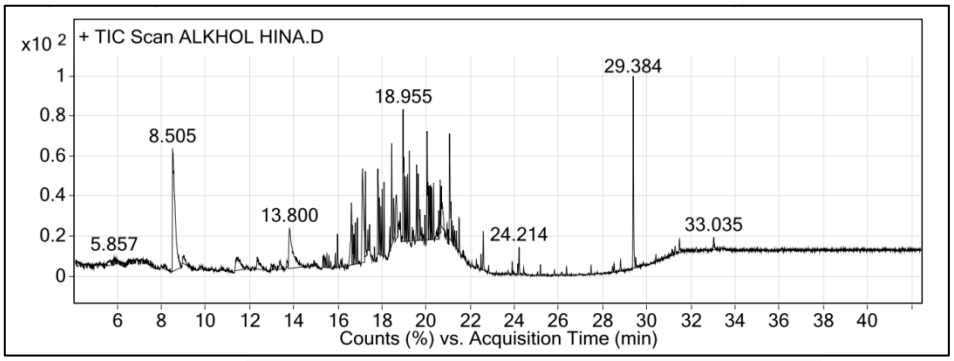
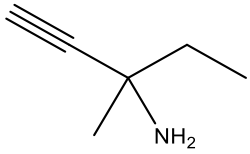
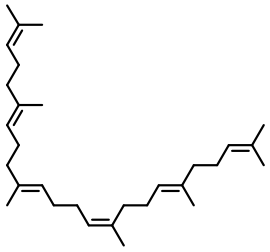
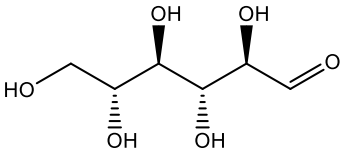
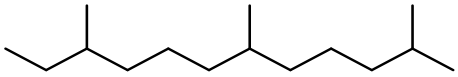
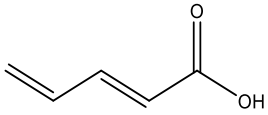
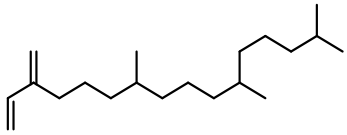
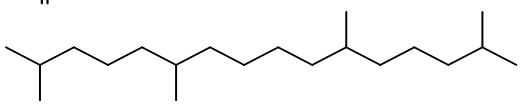
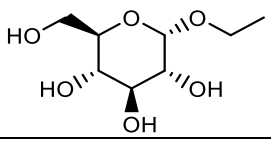


Figure 2. GC-MS chromatogram of ethanol Henna extract.

Table 3. Phytoconstituents of Ethanol Henna extract identified by GC/Mass. Pk: the global serial, Retention time (R.T).

PK	RT	Area %	Library/ID	Molecular formula	Chemical structure
1	8.5046	14.9022	1-Pentyn-3-amine, 3-methyl-	$C_6H_{11}N$	
58	29.3841	6.1789	Supraene	$C_{30}H_5O$	
13	17.119	6.096	D-Allose	$C_6H_{12}O_6$	
29	18.9555	4.955	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	
4	13.8001	4.3847	1,3-Butadiene-1-carboxylic acid	$C_5H_6O_2$	
48	21.0574	3.7914	Neophytadiene	$C_{20}H_{38}$	
18	17.8123	3.4879	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	
24	18.4392	3.4444	Hexadecane	$C_{16}H_{34}$	
26	18.6457	3.0497	Ethyl. alpha. -d-glucopyranoside	$C_8H_{16}O_6$	

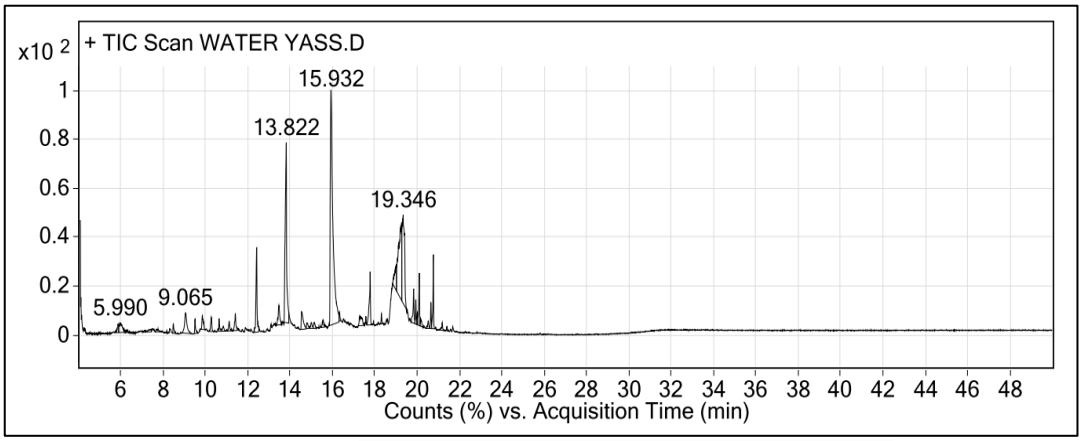
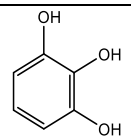
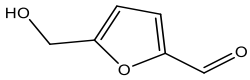
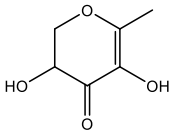
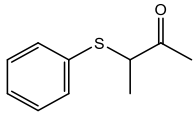
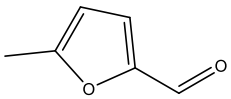
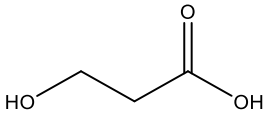
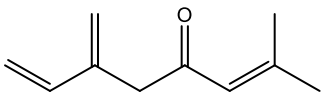
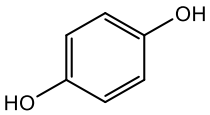
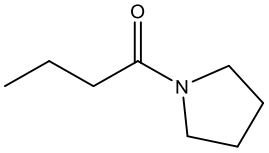


Figure 3. The GC-MS chromatogram of water Yass extract.

Table 4. Phytoconstituents of water Yass extract identified by GC/Mass. Pk: the global serial, Retention time (R.T).

PK	RT	Area %	Library/ID	Molecular formula	Chemical structure
38	15.9316	39.1388	1,2,3-Benzenetriol	$C_6H_6O_3$	
31	13.8223	19.3397	5-Hydroxymethylfurfural	$C_6H_6O_3$	
25	12.4284	4.8714	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	
41	17.7828	3.6382	2-Butanone, 3-(phenylthio)-	$C_{10}H_{12}OS$	
9	9.0652	3.3683	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	
45	18.9924	3.2829	Propanoic acid, 3-hydroxy-	$C_3H_6O_3$	
53	20.7698	2.766	2-Methyl-6-methyleneocta-2,7-dien-4-one	$C_{10}H_{14}O$	
32	14.5451	2.2533	Hydroquinone	$C_6H_6O_2$	
46	19.8406	2.0796	Pyrrolidine, 1-(1-oxobutyl)-	$C_8H_{15}ON$	

The results showed in table 4. that $C_6H_6O_3$ presents the maximum concentration (39.13%) with compared with the other compounds.

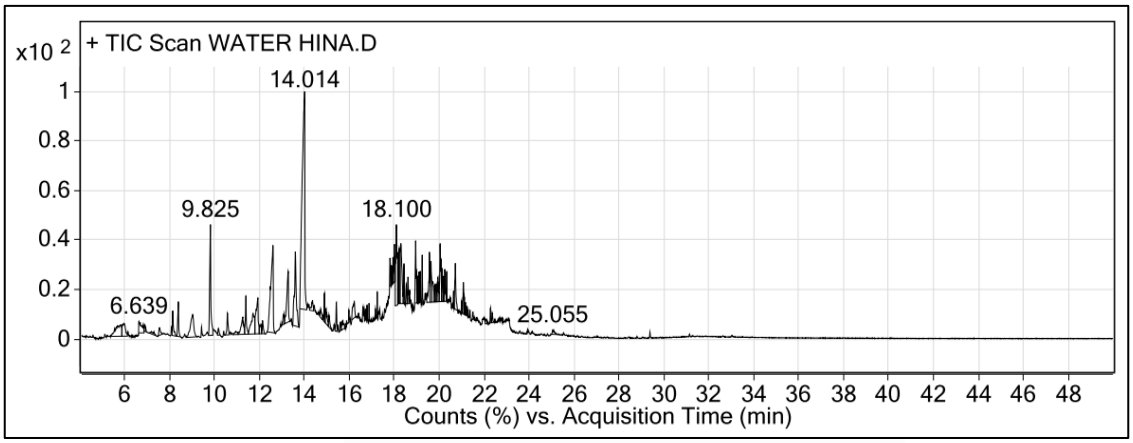
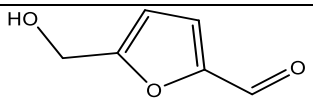
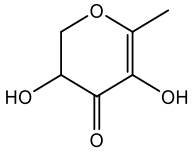
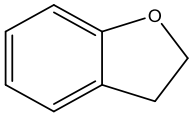
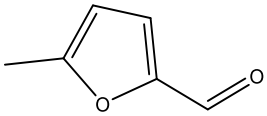
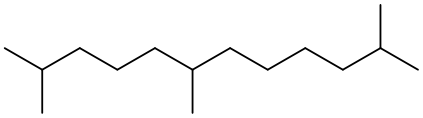
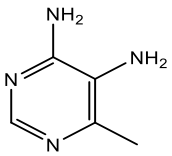
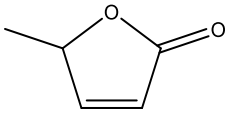


Figure 4. GC-MS chromatogram of Water Henna extract.

Table 5. Phytoconstituents of water Henna extract identified by GC/Mass. Pk: the global serial, Retention time (R.T).

PK	RT	Area %	Library/ID	Molecular formula	Chemical structure
23	14.014	38.5178	5-Hydroxymethylfurfural	$C_6H_6O_3$	
21	12.598	12.4326	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	
15	9.8249	8.0272	2H-Pyran, 3,4-dihydro-	C_5H_8O	
22	13.601	7.9356	Benzofuran, 2,3-dihydro-	C_8H_8O	
13	9.0357	4.0198	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	
34	18.9555	3.3236	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	
39	20.7256	3.1042	4,5-Pyrimidinediamine, 6-methyl-	$C_5H_8N_4$	
11	8.3941	1.956	2(5H)-Furanone, 5-methyl-	$C_5H_6O_2$	

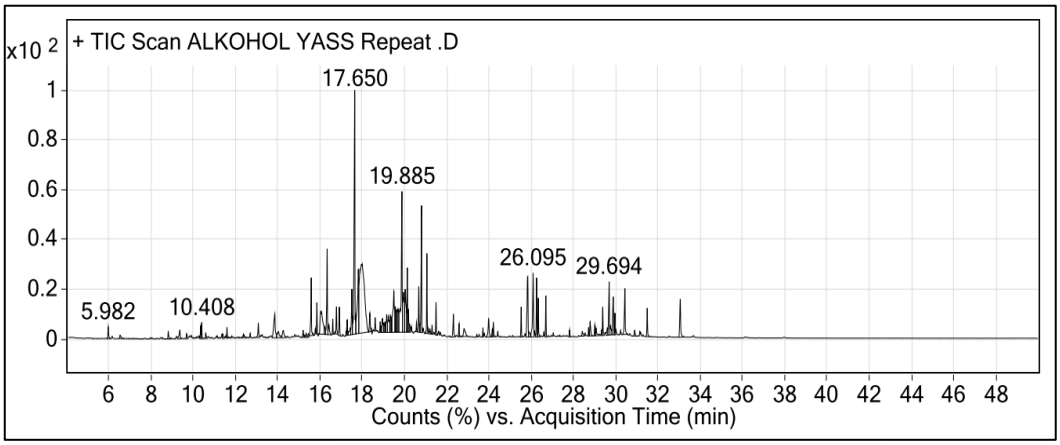
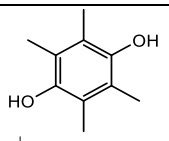
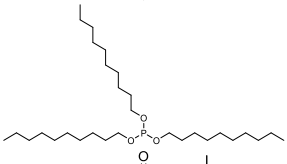
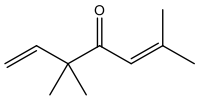
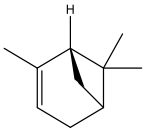
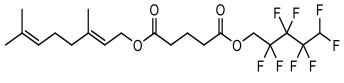
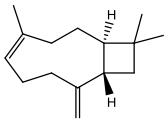
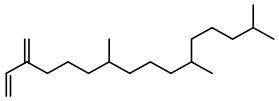
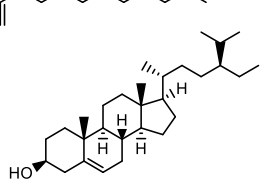
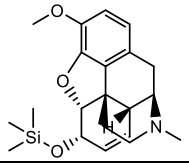


Figure 5. The GC-MS chromatogram of ethanol Yass extract.

Table 6. Phytoconstituents of Ethanol Yass extract identified by GC/Mass. Pk: the global serial, Retention time (R.T).

PK	RT	Area %	Library/ID	molecular formula	Chemical structure
22	17.6501	14.8852	Durohydroquinone	$C_{10}H_{14}O_2$	
28	19.8848	5.5967	Phosphorous acid, tris(decyl) ester	$C_{30}H_{63}O_3P$	
34	20.8141	5.5194	1,5-Heptadien-4-one, 3,3,6-trimethyl-	$C_{10}H_{16}O$	
44	25.8293	4.6565	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	
45	26.0948	3.8996	Glutaric acid, 2,2,3,3,4,4,5,5-octafluoropentyl geranyl ester	$C_{20}H_{26}F_8O_4$	
15	16.3447	3.8387	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	
35	21.0649	3.2762	Neophytadiene	$C_{20}H_{38}$	
60	33.0571	2.982	beta-Sitosterol	$C_{29}H_{50}O$	
57	30.4389	2.974	Codeine, TMS derivative	$C_{21}H_{29}NO_3Si$	

3.2. Bacterial Results

3.2.1. Fecal Contamination Bacteria with Their Total Number

The tables 7 shows the results of fecal contamination bacteria with their total count in water samples before and after the treatment process. The result indicated that the most contamination was found in the sample collected from Al-Sadr Teaching hospital, while they were minimum in the Ibn Al-Bitar hospital, as appeared by the number of bacteria.

Table 7. The total account (CFU) of bacteria before and after the treatment in the studied stations.

Hospitals	Fecal coliform		Total coliform		Total plate count	
	Before	After	Before	After	Before	After
Ibn Al-Bitar	0	0	uncountable	0.002	0.006	0.002
Al-Mawaddah	0.006	0.003	0	0	uncountable	0
Al-Sadr Teaching	0.024	0.003	0.038	0.026	0.062	0.013
Oil	0.010	0	0.006	0	uncountable	0

3.2.2. Identification of Bacteria

Table 8 illustrated the important bacteria that were identified during the current study from hospital station, as we can see from the results there are different pathogenic bacteria were identified and these bacteria are related to different phyla. The dominant bacterial phylum was *Pseudomonadota,* while the most occurrence bacteria was *(Staphylococcus lentus).*

3.2.3. Antibacterial Activity of Studied Plants

To evaluate the antibacterial activity of studied plants extracts, against pathogenic bacteria, it was tested against all isolated types of bacteria, two of which were Gram-positive (*Staphelococcus lentus, Staphylococcus Xylosus,)* and the others were negative *(sphingomonus paucemobilus, Serratia ficaria, Escherichia coli,and and klebsiella oxytoca)).* The concentrations from the plant's extracts used (12.5, 25, and 50 mg/mL) showed good anti-bacterial activity, but the most effective was to the highest concentration (50 mg/mL), as indicated by the measurements of the diameter of inhibition zone (table 9), (Figure 6). The results showed that the antibacterial efficiency of the AL-Yass plant was higher than that of the Al- Henna plant for both types of extraction.

Table 8. Identified bacteria that recorded in the studied hospitals with their phylum.

Phylum	Bactria	Shape and color	Bactria	Shape and color	Bactria	Shape and color
Bacillota	*Staphylococcus lentus*	blue spherical	*Staphylococcus Xylosus*	Blue rod		
Pseudomonadota	*Sphingomonas paucimobilis*	Red Rod				
Pseudomonadota	*Staphylococcus lentus*	Blue rod	*Escherichia coli*	blue spherical	*Staphylococcus lentus*	Red Rod
Pseudomonadota	*Staphylococcus lentus*	Blue rod	*klebsiella oxytoca*	Red Rod	*Serratia ficaria*	Red Rod

Table 9. The diameter of inhibition zone(mm), resulted from antibacterial activity of studied plant extracts in the different concentration(mg/mL), X=5 mm.

Abstract	Conc. mg/mL	*Staphy. Lentus blue*	*Staphy. Lentus red*	*Esch. coli*	*klebsiella oxytoca*	*Serratia ficaria*	*Staphylococcus xylosus*	*Sphingomonas paucimobilis*
Ethanol Yass	12.5	6	7	7	x	x	x	x
	25	8	8	8	x	x	x	7
	50	9	9	9	x	x	x	7
Water Yass	12.5	x	6	x	9	8	10	11
	25	x	x	x	9	8	10	10
	50	x	7	x	7	10	8	8
Ethanol Henna	12.5	x	x	6	6	x	6	x
	25	x	x	6	6	x	6	x
	50	x	x	x	6	x	6	x
Water Henna	12.5	x	x	x	7	11	6	x
	25	x	x	x	11	8	8	x
	50	x	x	x	x	x	x	x

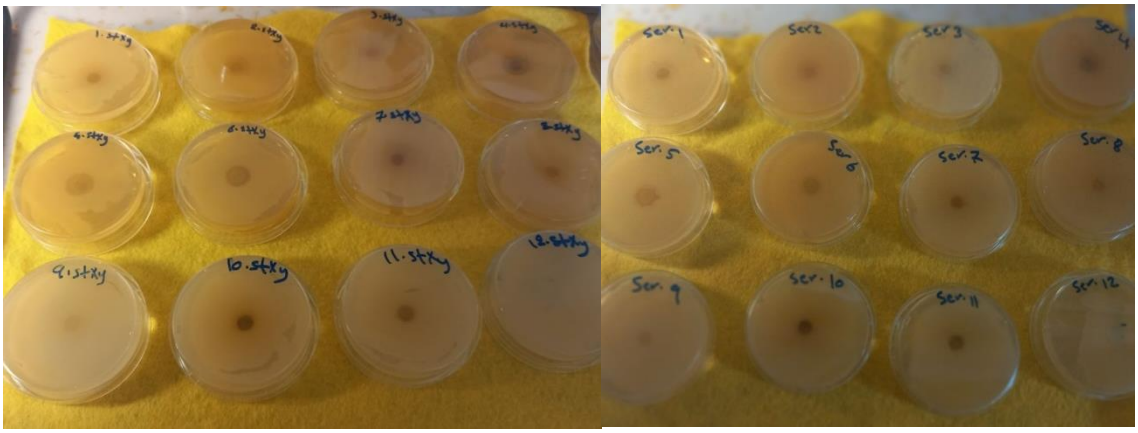


Figure 6. The halos of the identified bacteria in Al-Sadr Teaching hospital, as example.

4. Discussion

The results extracted from the GC-mass revealed that the concentrations of the studied extracts have different effect on the effectiveness of bacteria, but in general they did not eliminate all bacteria, but for each type and a specific concentration of the extract has the ability to reduce the effectiveness of a particular type of bacteria, the study shows some details for each concentration of the studied extracts, many studies have confirmed the existence of special compounds for these medicinal extracts that have a direct effect on the elimination of bacteria.

table 2 contains a number of compounds, recorded in Ethanol Henna, where: Pk: is the global serial, Rt present retention time, A: area under the curve, library: is the data stored in GC-mass, Chemical formula: for the identified compounds and chemical structure for identified compounds. The results showed many types of Aromatic and Aliphatic compounds. For example, $C_6H_{11}N$ presents the maximum concentration (14. 9002%) with compared with the other compounds. The effect of this compound is weak to the antibacterial activity [21]. While $C_{30}H_5O$ shows good activity, especially against *Klebsiella pneumonia* [22]. It corresponds with the current study; this compounds a positive effect against *Klebsiella pneumonia* and *Staphylococcus xylosus.* Table 3 represents the most important chemical compounds found in the water Yass extract, and the results were the best results extracted from the activity against bacteria (table 10), as this extract was able to reduce most of the bacterial activity. $C_6H_6O_3$ has an area of 39.1388% It represents the highest recorded area, Phenol compounds are the most effective against bacteria, especially with accompanying $C_6H_8O_4$ [23]. The study revealed that most phenolic compounds are present in water Yass, such as $C_3H_6O_3$, and $C_6H_6O_2$, these compounds have a high ability to oxidize. Table 4. Illustrate the chemical compounds in water

Henna, this extract had the weakest effect on reducing the effect of bacteria compared to the rest of the extracts, perhaps because it carries the least number of phenolic compounds. Nevertheless, the extract showed a good anti-bacterial ability against *klebsiella oxytoca, Serratia ficaria* and *Staphylococcus xylosus*. $C_6H_6O_3$ presents the maximum construction (38.5178%), which could be the factor affecting these bacteria [24], [25]. Table 5 presents the Ethanol Yass abstract; it is very effective against Staphylococcus lentus (Red and blue) as well as Escherichia coli. The results showed that $C_{10}H_{14}O_2$ presents the maximum concentration (14.88%) with compared with the other compounds, some studies emphasize these results, such as Liu et al., (2020) used $C_{10}H_{14}O_2$ to eliminate *Escherichia coli*, also [26] used it and noted the activity against *Staphylococcus*.

Conclusion

This study dealt with the most important polluting bacteria emerging from hospital water, where it identified the most important harmful bacteria present in it, for this treatment, ethanol Henna, water Henna, ethanol Yass, and water Yass were made as extracts. The most significant chemicals that proved extremely successful in reducing the activity of bacteria were identified after the key compounds for these abstracts were analyzed using a GC-MS equipment. The findings revealed that compounds containing phenolic compounds were the most effective in eradicating bacterial species. The results showed that the compounds containing phenolic compounds were the most effective in eliminating bacterial species, as the diameter of those halos around the diagnosed bacteria increases, which leads to inhibition of their effectiveness. In the present study, the alcohloic yass and henna extracts were the most successful against those bacteria because they contain the phenolic compounds.

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