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RESEARCH ARTICLE

Evaluation of the Bacterial Contamination of Well Waters and the Effects of Leaf Plant Extract of *Melia Azedarach* on Such Contamination

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Abstract

This work has been conducted to examine bioactivity of certain species of bacteria in well water samples collected from eight wells in Yusufiyah area southern Baghdad and to assess the effects of plant leaf extraction of Melia azedarach on these bacteria. Bacteriological properties such as total plate count cfu/ml, MPN/1.ml, Fecal Coliform, Fecal Streptococci, E. coli and Pseudomonas aeruginosa of well water samples have been evaluated in July and January 2017. Total numbers of bacteria in July was varied from 210 to 6070 CFU / ml while the total numbers of bacteria in January was ranged from 115 to 1300 CFU / ml. The local and international specifications MPN reached the upper limit for the preparation of the total coliform count in a manner most likely CFU / ml> 1600 (MPN) and have a minimum CFU / 900ml in July but in January, it has reached the upper limit of the number of total coliform manner that count the most likely CFU / 100 ml> 1550 (MPN) and minimum CFU / 100 ml 100 hence conclude that 87.5% of water wells in July and January. Well waters indicators fecal coliform bacteria and E. coli and fecal Streptococci contamination. However, the contamination with fecal coliform bacteria was considered as an acceptable because they are not exposed to any type of chlorination before consumption by users. The dried plant leaves of the Melia azedarach were extracted by using methanol alcohol and the bioactivity of this extract was evaluated with isolated bacteria. The results have showed that the effects of the extract depend on bacteria species and the extraction concentration used. However, it was found that the extract had significant bioactivity on bacterial contaminated water and such ability of methanol alcoholic extract may be due to the content of steroidal, phenols and tannins compounds in the examined plant leaves being very effective against microbes.

Keywords: *Bioactivity, Melia azedarach, Extraction, Bacterial contamination, Well water.*

Introduction

The different circumstances by which Iraq affected the quantity and quality of in land waters with the climate changes, and high temperature levels, and lack of rain, low surface water levels in rivers and marshes, lakes, and a result, drilling hundreds of wells by residents distributed throughout the country randomly without solicitude [1].The underground water content heavy elements such as iron, cadmium, nickel, chromium and content calcium, sodium, and the level of salinity and the rest of the water chemical properties physical and biological properties all affect well water quality and limit their use such as drinking or irrigation or industry and it's assumed that the wells best water is from surface water in terms of quality but that the wells subjected to contamination, such as agricultural waste and leakage of sewage water network in the city and poultry waste and excessive irrigation sources in addition to the fertilized soil feces of animals leads to the leakage of bacteria, including that nurse to underground and by following spoil the water quality of the wells [2,3]. The water was considered as a source for many of the pathogenic bacteria that cause disease epidemic [4].

This has been presence of microorganism's function in water is an indicator of contamination with fecal matter, such as *E. coli*, bacteria coliform and fecal coliform and fecal streptococcus bacteria are used as a function of water pollution [5]. The plants were offered a rich source of antimicrobials, and pesticides, including genus *Melia*, which consists of two types (azedarach and azadirachta).

Melia azedarach (alsabehbeh), which from the family *Meliaceae* is the best known species, whose name is derived from classical Greek [6]. The origin of these species from South Asia (Iran, India and southern China), newly grown in tropical America, from Mexico to Argentina, and spread to other countries such as China, India and Japan to Indonesia and northern Australia, Africa, North America, South America tropical, and southern Europe [7].

Chinese Blackberry trees (chinaberry) *Melia azedarach* L. (Sapindales: Meliaceae) one of the closest species to Al-Neem, which spread culturing in Iraq and called Alsabahbah. The isolated active compounds from Alsabahbah can be a disincentive the growth or inhibitory the feeding or toxic to many insect pests such as Meliartenin and Azadirachtin, who have two negative effect in some aspects of life performance of insect pests [8].

This study came to detect the groundwater contamination through some of microorganism as contamination indicators and tested the activity of methanolic alcohol extract of the Alsabahbah leaves against some bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. This study complements many of the studies carried out in Iraq, which focused on the quality of groundwater. This investigation extent to determine the degree of wells water contamination in different areas of Baghdad and the degree of human use and comparing the results with the permitted levels locally and globally, and to provide a database for other future research.

Material and Methods

Groundwater samples were collected from eight wells situated in Yusufiyah area south of Baghdad. Water samples were placed in plastic bottles of 5 liters from each examined well twice a month from July and January 2014 and each water sample was replicated during each month. The water samples were delivered to the laboratory for bacterial tests. According to previous study [4], Alsabehbeh plant leaves were collected from the Ministry of Science and Technology and diagnosed by the Department of biology / College of Science / University of Baghdad, left to dry under lab condition, crushed and kept in paper bags at 20 C^{o}. Water Most Probable Number (MPN) and the disclosure of evidence of fecal contamination, which includes *E. coli,* were detected following previous study [4].

The bacterial isolates were diagnosed by several tests using culture media to show the form of growth and diagnostics properties which were classified into groups according to Berge's taxonomy manual and subjected to microscopic examination. This examination includes qualities of culture, checking movement [9], Catalase and oxidase tests [10], indole test, Red Methyl test, Vogues Proskauer test, citrate consumption test, urea test, nitrate reductase test, coagulating enzyme test, gelatination test, fermentation of sugars test [11].

The extraction of dried leaves of Alsabehbeh plant was prepared following a method suggested by previous work [12] where 50 g of plant dried leaves were extracted in 500 ml methanol alcohol and placed in Soxhlet for 8 hours at temperature of 78 ^{o}C. The extract was concentrated by using a rotary evaporator. 0.5 ml of the extract was dissolved in 10 ml of solvent to get concentration of 5 % mg/ ml and from this stock extract, other concentrations (3, 1, 0. 5 %) were prepared. Activity tested dishes with metal corking bore in 5 mm diameter to work bore in the culture media were used.

The dishes of Mueller Hinton agar with isolated bacterial after working serial dilution to get the concentration of 1.5× ^{4}10 cells/ ml from the stock solution (bacterial number 1.5 × ^{8}10 cells/ ml) were used and compared with McFarland for calibration at alone. Each culture media was received 0.1 ml from each of these prepared extract concentrations (5, 3, 1, and 0.5 %) in bores and incubated at a temperature of 37 ^{o}C for 24 ± 2 hours. The results of inhibition of bacterial growth [13] were recorded. However, these tests of the three bacterial concentrations were carried out with three replicates of each test .Extract pH was determined following the method of early

study [14] which was reported by previous work [15] where a weight of one gram of powdered dried plant leaves extracted in methanol alcoholic and received 5 ml of distilled water and placed on magnetic mixer for 10 minutes and pH value was measured. Following the method described by a work [16], the glycosides were detected while the method of Fahmy [17] was used to detect alkaloids.

The adopted method [18] as stated in [15] was applied in the detection of both tannins and resins. Also, saponines was detected by strongly shaking of 5 ml of plant. Adopted method described in Geisman study [19] was used to detect coumarins while adopted method described in another study [20] was followed in the detection of flavones. The method of Harborne [21] was applied to detect the phenoles.

Finally, the method described in Al-Bid [22] was followed to detect steroids & terpenes. The underground water samples were concentrated using a rotary evaporator and one ml of each sample was treated with one ml of the alcoholic leaf extract and the mixture was incubated for three hours at a temperature of 37 ºC. 1 ml was spread on nutrient agar media plates to study the activity of Alsabehbeh leaves extract and incubated at 37 ºC for 24 ± 2 hrs. The results were recorded and compared with water control samples (untreated samples).

Results & Discussion

The total plate count (cfu/ml), MPN, Fecal Coliform, Fecal Streptococci, E. coli and P. aerugiuosa during July and January are given in table 1. Apparently, water samples collected from all examined wells were contaminated with different bacteria species during July and January except well 1 which had MPN values within standards limits and was free from E. coli during the two months. Also, it was free from Fecal Coliform during July and from P. aeruginosa during January.

Water of well 2 was only free from P. aeruginosa during July while water sample of well 3 was only free from fecal streptococci during both months. Water of well 5 was uncontaminated by E. coli during July and P. aeruginosa during July and fecal coliform, E. coli and P. aeruginosa were not detected in well 5 water sample during January and June respectively. Finally, E. coli bacteria was found in water from well 7 during both months while fecal streptococci bacteria was not seen in water sampled from well 8 during January.

Table 1: The total plate count (cfu/ml), MPN, Fecal Coliform, Fecal Streptococci, E. coli and P. aerugiuosa of examined well water samples in July and January

Well No.	Depth M	Total plate count cfu/ml		MPN/1. Ml		Fecal Coliform		Fecal Streptococci		E. coli		P. aerugiuosa	
		July	January	July	January	July	January	July	January	July	January	July	January
1	22	210	1200	3	5	-	+	+	+	-	-	+	-
2	15	480	115	>1600	>1550	+	+	+	+	+	+	-	+
3	11	830	800	920	830	+	+	-	-	+	+	+	+
4	10	1200	835	>1600	>1500	+	+	+	+	+	+	+	+
5	8	850	1300	900	850	+	-	+	+	+	-	-	+
6	12	6070	1015	1000	100	+	+	+	+	+	+	+	+
7	9	555	210	>1600	1500	+	+	+	+	-	-	+	+
8	9	1660	830	>1600	1500	+	+	+	-	+	+	+	+

The total numbers of bacteria were ranged from 210-6070 cfu/ ml in July from 115 - 1300 cfu/ ml in January. These values are well above local and international standards which is 500 cells / ml (Central Organization for Standardization and Quality Control, 2009). Therefore, it indicates that about 75% of examined well water had exceeded standard value in both July and January. Obviously, bacterial well water content was higher by 37% of standard level. However, the count of valuable bacterial numbers in such waters may be considered as indicator bacterial contamination. In case of MPN, water content values were varied from 3 cfu / ml in well 1 to > 1600 cfu / ml in water samples collected from wells 2, 4, 7 and 8 in July while during January, these water

content were varied from 5 cfu / ml in water sample of well 1 to > 1550 cfu / ml in water sample of well 2. Apparently, it seems that 87.5% of well waters in July and January and clearly such MPN water content of most examined water samples except that of well 1 have exceeded local and international standards which is not more than 5,1 cfu / 100 ml.

These results were supported by those of other work [23] which found that the examined well water in Saudi Arabia had exceeded the permitted content by 20% of the wells. Well water contamination by fecal coliform bacteria was found in water samples of all examined wells apart from waters of well 1 and well 5 which were free from these bacteria during July and January respectively.

This may be linked to agricultural and poultry wastes in addition to the soil that contains a lot of human pollutants [24]. The increased temperature in July was probably the reason behind higher water content of total fecal coliform number and may due to the adaption of these bacterial. Regarding fecal streptococci bacteria, it was found that water samples from all examined wells were contaminated during both months except that of well 3 which was free from these bacteria again during both months and water of well 8 showed no bacterial contamination during January.

The current study has found that water samples from all tested wells were contaminated by E. coli during examined months but only those sampled from wells 1 and 7 were free from these bacteria during both months and water of well 5 showed no contamination during July. In case of P. aeruginosa, only water samples of wells 1, 2 and 5 were uncontaminated where water of well 1 was in January, well 2 was in July and well 5 again in July while water samples of all examined wells were found to be contaminated by these bacteria in July and January. Similar study [25] has examined well water samples in Turkey and found that the total numbers of bacteria has exceeded the Turkish standards by 20%.

In general, there are several sources may cause microbial pollution in well water such as organic fertilizers and organic wastes which may spoil water quality in terms of the chemical and biological properties [24]. The occurrence of these bacterial species in examined water sampled from 8 wells may act as indicators of bacterial contamination such as fecal coliform, *E. coli* and fecal Streptococci (Table 1).

In other words, obtained results showed that 87.5% of wells waters were contaminated with fecal coliform bacteria and fecal Streptococci and 75% were contaminated with *E. coli* bacteria. Also, it seems that 75 % of well waters were contaminated by Fecal Streptococci and 62.5 % was contaminated with *E. coli* bacteria. However, these results were well above the local and international standards [26]. There are several factors play an important role in the increasing and decreasing the number of fecal coliform such as various wastes from both human and animal sources, the well depth and the method of designing and using of these wells [27].

The presence of fecal coliform was considered as vital indicator of microbial water pollution. In this case, the water is dangerously polluted by fecal coliform. It seems that all wells under study were contaminated because they are shallow and easily subjected to various organic wastes. So, if their depths were expanded deeply enough then may be safe for human use as it has been reported by other studies [28, 29].

However, these current results are backed by previous work (Yusuf and Salman, 2011) which reported that well waters with no more than 10 m deep were contaminated. In the local study [30], it has been found that the wells which have exceeded 35 m deep were free from bacterial contamination compared with those shallow wells which were [31]. Table 2 shows the detected qualitative of active groups in methanol alcoholic extracts of dried Alsabehbeh plant leaves which are glycosides, tannins, resins, saponins, phenols and alkaloids.

Table 2: Detection of chemical qualitative for some activity groups of powder and Alsabehbeh Leaves extract

Active groups	**Alsabehbeh leaves extract**
Glycosides	+
Alkaloids	+
Tannins	++

Resins	+
Saponins	++
Coumarins	+
Flavones	+
Phenoles	++
Steroids	**++**
Terpenes	+

The bioactivity of the plant extract against bacteria was assessed depending on types of bacteria and the concentration used. It can be showed from these results that methanol alcoholic extract gave good inhibition effective against isolated bacteria, especially gram negative bacteria which gave the highest inhibition activity against *Pseudomonas aeruginosa* in terms of the inhibition diameter. The mean inhibition diameter was depended on the extract concentration used where the highest mean was with 5 % concentration and found to be 21±0.31 mm, 17±0.32 mm and 16±0.16 mm for *P. aeruginosa*, *S. aureus* and *E. coli* bacteria respectively while the lowest mean (7±0.02 mm) was recorded at 0.5 % concentration again in *P. aeruginosa* bacteria but other bacterial species did not show such inhibition diameter with this concentration (Table 3).

Table 3: Inhibition effective of methanol alcoholic extract of dried Alsabehbeh plant leaves on isolated bacteria.

Extract Conc. %	**Mean Inhibition diameter mm**		
	S. aureus	*E. coli*	*P. aeruginosa*
5	17±0.32	16±0.16	21±0.31
3	13±0.11	14±0.14	19±0.11
1	10±0.21	11±0.42	14±0.56
0.5	-	-	7±0.02

$P \leq 0.05$

It has been shown that the methanol alcoholic extract of dried plant leaves had good efficiency and ability of inhibiting bacterial growth and this may be due to the content of steroidal and phenolic and tannins compounds which seem to be effective against gram positive and negative bacteria as suggested by previous work [32].

This bio-activity may be linked to azedarach and azadirachta compounds which are the most important active ingredients in Meliceae family and to terpenes and steroid compounds which known very toxic [33, 34] and possibly due to the lack of membrane bacteria's ability to prevent the extract penetrating the bacteria but nevertheless, it was less affective in positive gram stain bacteria being having thick casing surrounding the bacterial cell which prevents the extract from entering the cell [35].

Table 4 shows mean activity ±SD of methanol alcoholic extract of dried Alsabehbeh plant leaves in well water bacteria at 3% concentration with similar activity but without the plant extraction as a control.

Table 4: Activity of methanol alcoholic extract of dried Alsabehbeh plant leaves in well water bacteria

Extract Conc. 3%	**Mean of Total number ± SD**		
	No. of Well waters		
	1	5	6
Methanol alcoholic	150±3.34	120 ±1.95	80±1.23
Control	1200±14.9	1300±16.8	1015±19.9

$P \leq 0.05$

Means of Results for Three Replicates

Obviously, the methanol alcoholic dried plant extract at concentration of 3% was significantly much effective in inhibiting bacterial growth compared to control activity in water samples of examined wells.

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