

## **Physiochemical Charecters and Antimicrobial Activity of Zamzam Water Against Escherichia coli in vitro.**

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### **ABSTRACT**

Zamzam water provide for billions of muslims and considered as a holy water. In this study, Samples from zamzam water was charecterized chemically and compared with tab and distilled water. In addition to assay the antimicrobial activity. Zamzam water samples were charecterized for physical and chemical constituents and enumerated for total viable count of bacteria and coliform. Antimicrobial activity of zamzam water was studied and MIC, MBC determined. Result showed zamzam water is rich in micro, macroelement and slite saline, with high electric conductivity (EC 70-80 time) higher than tab and distilled water. The concentration of sodium, calicium and magnesium are 42.7, 47.7, and 14.4 µg/ml respectively. The bicarbonate concentration was 74.7 µg/ml and hardness 180.7 µg/ml . Also, high concentration of chlorine (70.7 µg/ml) and sulfate (124.4 µg/ml). Result shows that zamzam water samples were free of microorganisms and coliform, showing antimicrobial activity against E.coli. The bacteria was inhibited for growth after 18 hrs. and killed (no growth) after 24 hrs. The concentration of zamzam water 5:5 V:V inhibit the bacterial growth (MIC) and inhibition zone was started to appear between 5:5 and 6:4 V:V zamzam water and distilled water (MBC), and the inhibition zone increased with the increasing in concentration of zamzam water giving 17 mm at 10:0 V:V zamzam water: distilled water.

**Key words: Zamzam water, Antimicrobial, chemical charecters.**

### **INTRODUCTION**

Water play a pivotal role in carrying out various physiological features in the human body [1]. Our planet has large reservoirs of water, but the potable water is rare. The percentage of freshwater on earth is about 2.8% ; the residue is salt-water that is difficult to use [2]. A source of freshwater, considered as a holy water, called "Zamzam" provide water for billions of muslims. Zamzam source of water located in Mecca city which is located in the western part of Saudi Arabia about 70 km south of Jeddah city on the coast of Red Sea [3].

Many studies have been carried out to invistigate Zamzam water chemical and physical charecterstics. Naeem and Alsanussi used advanced techniques for multi-elemental and hydrochemical study of Zamzam water [4]. Also, Researchers performed neutron activation, inductivity coupled plasma, and different to be had classical techniques to analyze samples of

zamzam water. These techniques determined about thirty-four elements in Zamzam water. The determinations revealed that the concentrations of calcium (Ca), sodium (Na), magnesium (Mg), and chloride (Cl) are higher than natural water. The elements beryllium (Be), antimony (Sb), bromine (Br), bismuth (Bi), iodine (I), cobalt (Co), and molybdenum (Mo) were less than 0.01 ppm. Also, traces of manganese (Mn), chromium (Cr), and titanium (Ti) were detected in Zamzam water. By a hydrochemical study, Zamzam water defined as a sodium chloride water of meteoritic origin. The amount of four toxic elements, arsenic (As), lead (Pb), cadmium (Cd), and selenium (Se), was lower than the danger concentration for human consumption. The pH of zamzam water is more than the pH normal tap water [11]. The higher level of calcium makes Zamzam water healthier [5,6].

Zamzam water is a natural water, has a rich essential minerals profile and antioxidant capacity. It is considered as a potential protective agent against liver toxicity, kidney toxicity and diabetes mellitus, [12].

Researchers do not find any sign of biological growth or contamination in this water [7]. According to quantity of calcium, sodium and magnesium salts in addition to fluoride content, Zamzam water express antimicrobial activity [8]. An experiment was performed by Amalia Tri Utami, 2019, to determine the antimicrobial effect of Zamzam water against *Salmonella typhi* by using MIC and MBC methods. The results show an obvious inhibition in bacterial growth when Zamzam water was included. [9]. The antimicrobial activity of Zamzam water may be done by the ability to inhibit enolase, phosphopyruvate hydratase, is a glycolytic enzyme that catalyze the conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP). This enzyme plays an essential role in metabolism of microbial growth [10].

We have addressed our attention to physical and chemical properties of Zamzam water, because this water might represent a source of potential risk to health. The study also designed to reveal the antimicrobial activity of Zamzam water and showing the differences in testing growth in tap water and Zamam water.

## **MATERIALS AND METHODS**

### **1. Physical and chemical properties of zamzam water**

A method described for determining the concentration of Calcium, Copper, Lithium, Magnesium, Manganese, Potassium, Sodium, Strontium, and Zinc in natural water by atomic absorption was used [13]. Samples were filtered through a 0.45 micron micropore membrane. It is necessary to avoid clogging of the burner capillary. Aspirate each sample directly, except for Calcium and magnesium. For Calcium and Magnesium, dilute with 5% (w/v) La solution and HCl to give a final solution concentration of 0.25(w/v) La and 5% (v/v) HCl. Results for Calcium and Magnesium should be corrected by using a reagent blank. Other elements calculated according to the following:

$$\text{Element } (\mu\text{g/ml}) = \frac{(\mu\text{g/ml in diluted solution})(\text{volume of diluted solution in ml})}{(\text{volume of aliquot taken for dilution in ml})}$$

## 2. Assaying Bacteria

The assayed bacteria E.coli which isolated from chicken meat subcultured on nutrient broth media for 24 hours at 37 °C. The cells suspension culture of  $1.5 \times 10^8$  cells/ml is ready for assaying antimicrobial activity of zamzam water.

## 3. Enumeration of total viable bacteria

To enumerate the total viable counts of bacteria in zamzam water samples, pour plate count method was used. One ml. of zamzam water sample was mixed with melted nutrient agar plate tempered at 37°C. Plates were inoculated aerobically at 37°C for 48 hrs. Experiments were conducted in triplicate. All colonies were counted as colony forming unite (CFU) per milliter of water sample.

## 4. Detection and Enumeration of Coliforms

For detection and enumeration of Enterobacteriaceae spp. (coliform), the most propable number (MPN) technique was adopted. One milliter of zamzam water sample was added to each two test tube containing sterile MacConkey broth with Durham's tube. The tubes were inocubated at 44°C for 24-48 hrs. The production of acidic yellow color and gas (appear in Durham's tube) indicate the presence of lactose faecal coliform.

## 5. Bacteriostatic and Bacteriocidal concentration (MIC and MBC):

Difussion method was used for assaying the minimal bactericidal activity of zamzam water against E.coli in vitro. MacConkey agar plates (20 ml. melted media, 5 cm diameter petridish) were sparated with culture broth of E.coli ( $1.5 \times 10^9$  cells/ml). A centre hole of 0.5 centimeter in diameter was made in each plate. A 0.25 ml from each following diluted zamzam water (1,2,3,4,5,6,7,8,9/ 10 ml D.W.) and undiluted once were added to each hole. Distiled water was used as a control. All plates incubated for 24 hrs. at 37°C. Inhibition zones was measured for each dilution considering the minimmum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Experiment was conducted in three replicates for each dilution.

## 6. Antibacterial Activity

Bioassay method is used for detecting the antimicrobial activity of zamzam water. Conical fasks of 250 ml. capacity containing 100 ml of zamzam water or distilled H<sub>2</sub>O were contaminated with  $1 \times 10^7$  cells/ml of E.coli. Viable counts was determined for each flask by

spreading 0.1ml on petridishes containing sterile MacConkey agar. All pates are incubated at 37°C. Colonies were counted at zero time, after 18, 24, 48 and 72 hrs. Three replicates were conducted for each treatment, and the experiment was repeated two times.

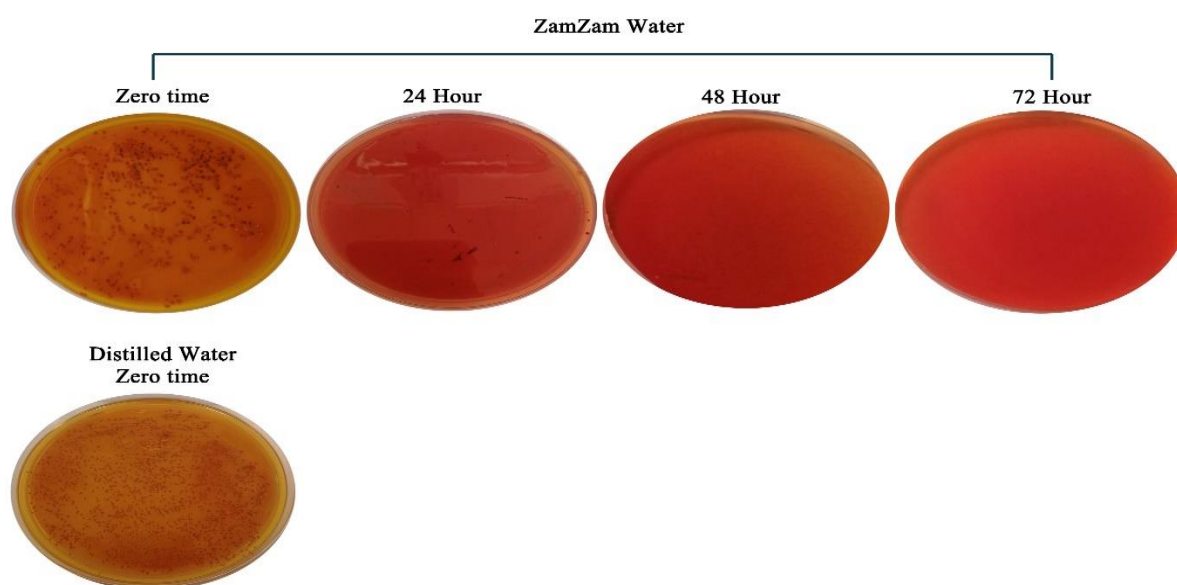
## RESULTS

Chemical analysis of the trace element present in zamzam water in comparison with tab and distilled water is presented in table (1). Results show that only zinc out of trace elements: Fe, Pb and Cd is ten times in concentration higher than the distilled water. Zamzam water showed more salinity than tab and distilled water, (table 1). Also, the electric conductivity (Ec) was eight times higher than that of tab water and 70-80 times than the distilled water, which due to dissolved minirals from rocks and soil of area around Macca that the water seep out through it, also zamzam water more turbid than tab and distilled water as a result of minirals and suspended particles. As a results of salt petre, the concentration of sodium, calicium and magnesium are so high in zamzam water comparing with tab water (42.7 , 47.7, 14.4 respectively). This may be due to passing ground water through rocky places leading to dissolved minirals. Also, bicarbonate concentration is increased significantly as well as the hardness, 74.7and 180.7 respectively (Table 1).

Chlorine content of zamzam water (70.7 µg/ml.) is so high than tab drinking water (13µg/ml). The sulfate constituent of zamzam water (124.4 µg/ml) increased by eight time over the tab water. **Table 1:** Comparison of different parameters between Tab, Distilled and zamzam water.

Parameters	Tab water	Distilled water	Zamzam water µg/ml			Mean value
			1	2	3	
<b>pH</b>	7	5.4	7.9	7.9	7.8	7.9
<b>EC</b>	92	9.5	821	715	780	748
<b>TDS</b>	59	6	391	376	367	378
<b>TH</b>	48	5	187	174	181	180.7
<b>Na</b>	6	1	45	43	40	42.7
<b>Ca</b>	12	0.9	50	45	48	47.7
<b>HNO3</b>	10	0.2	77	75	72	74.7
<b>Mg</b>	4.3	0.6	15.2	15	13	14.4
<b>Cl</b>	13	1.5	70	72	70	70.7
<b>SO4</b>	15	2.5	130	123	120	124.4
<b>K</b>	0.9	-	1.2	1	1	1.7
<b>CO3</b>	0	0	3	2	1	2
<b>DO2</b>	9.5	-	8.5	-	-	8.5
<b>O3</b>	0	0	0	-	-	0
<b>NO3</b>	-	0.07	28.9	-	-	28.9
<b>Turb.</b>	-	1	2.3	2.3	2.1	2.2
<b>Fe</b>	-	0.001	0.001	0.001	0.001	0.001
<b>Pb</b>	-	0.002	0.005	0.004	0.004	0.004
<b>Cd</b>	-	0.001	0.002	0.001	0.001	0.001
<b>Zn</b>	0.002	0.003	0.029	0.03	0.03	0.03

Enumeration of total viable bacterial count result indicate that zamzam water is free of microorganisms. The most probable number (MPN) test tubes showed no reaction after inocubation at 44°C for 24-48 hrs. Which mean zamzam water is free of coliform bacteria. However, the bacteriocidal activity of zamzam water was studied using E.coli as assaying organism. Results showed after 18 hrs. the coliform bacteria was killed and no growth was detected (Table 2), While a heavy growth was found in nutrient agar plates inoculated with suspension of bacteria in distilled water (Fig. 1), (Table 2).



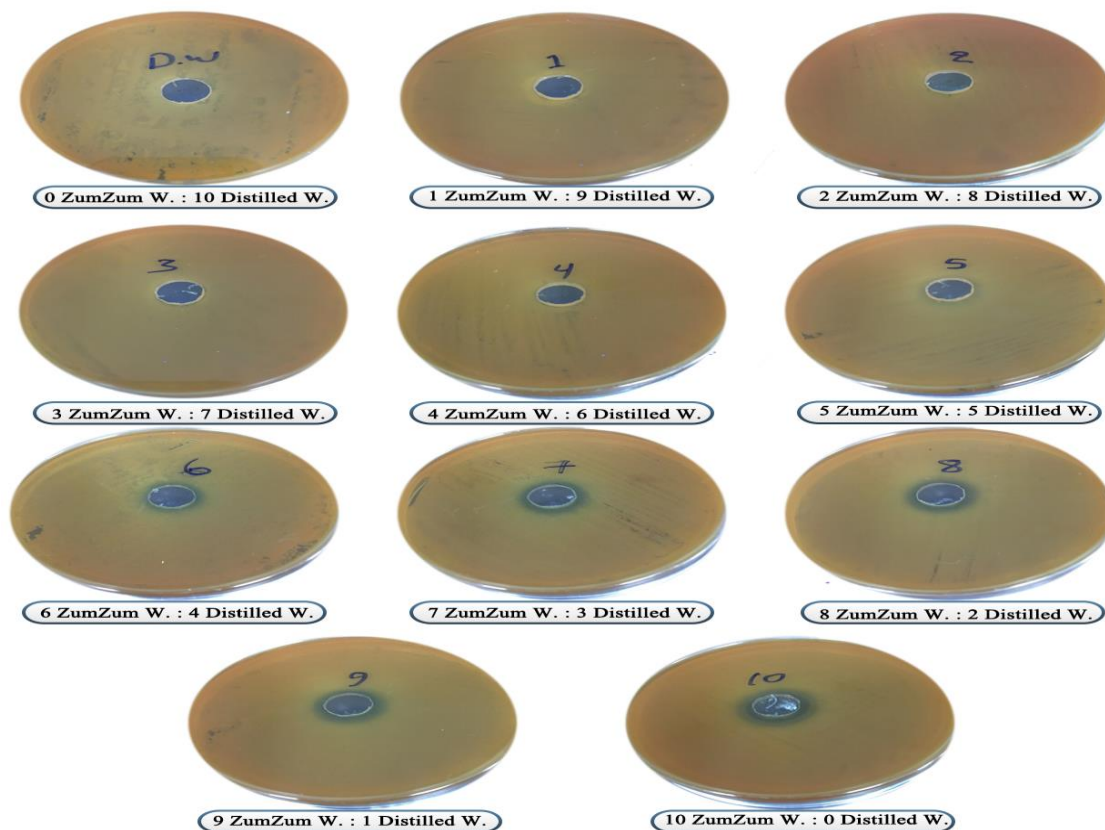
**Figure 1:** Antimicrobial activity of zamzam water against E.coli within interval of incubation period.

**Table 2:** Bacterial growth per time intervals in Zamzam and Distilled water.

Time/hrs.	Zamzam water (cells/ml)	Distilled water (cells/ml)
Zero time	$4 \times 10^7$	$2.6 \times 10^7$
18 hours	No growth	Heavy growth(uncounted)
24 hours	No growth	Heavy growth (uncounted)
48 hours	No growth	Heavy growth (uncounted)
72 hours	No growth	Heavy growth (uncounted)

For determination the minimum inhibitory concentration (MIC) of zamzam water, gradual concentration was prepared, starting from zero (distilled water) to 10 (100 zamzam water only). As shows in figure (2), 5:5 (50%) showed starting the inhibition of bacterial growth, (MIC) and the inhibition zone was started appear between 5:5 and 6:4 ratio of (MBC)

increased as zamzam water increased giving maximum inhibitory zone (17 mm) with 100% zamzam water (table 3) (Fig. 2).



**Figure2:** Minimum inhibitory concentration and Minimum bactericidal concentration of zamzam water against E.coli

**Table 3:** Minimum Inhibitory concentration of zamzam water against E.coli bacteria.

Dilution of zamzam water in 10 ml distilled water	Percentage (%) of zamzam water	Inhibition zone (mm)
0:10	0	Negative
1:9	10	Negative
2:8	20	Negative
3:7	30	Negative
4:6	40	Negative
5:5	50	5 (hole diameter)
6:4	60	12
7:3	70	13
8:2	80	14
9:1	90	15
10:0	100	17

The results

lead to a

conclusion that zamzam water is rich in essential Macro and Microelememts, having antimicrobial activity and TDS within acceptable range (WHO standard 1000 mg/l).

## DISCUSSION

All biochemical and enzymes activity inside living cells happened in electrolyte solution, and water is a liquid phase for reactions. Fresh drinking water over world is limited, representing 2.8% of the total water on earth [1].

Chemical analysis of materials in three samples of zamzam water as compared with constituents of tap water and distilled water indicate a high concentration of essential minerals and water hardness. Also, zamzam water has higher electrical conductivity in comparison with bottled water [14]. Shomar (2011) studied the concentration of trace elements in zamzam water, he found the quality of water did not change for two years, and the water is alkaline (average pH is 8), the average of Ca and K were (95 and 50 mg/ml) respectively. The concentration of As and  $\text{NO}_3$  three times higher than WHO standard. The healing power of zamzam water may due to the presence of trace element of As, Li and alkalinity [18]. Wangde (2016) explain the partial melting of the minerals which comprise the rock cause any existing pores to close up, and its flows through long stretches of rock from which it gains its high mineral content, the main source of water is the crack in the rock seeping water from the mountains that surround Makkah [19]. Badar *et al.* (2019) concluded the prolonged use of zamzam water did not induce any significant differences in Met Hb (methemoglobin) concentration in rat pups, which might indicate that it is safe for infants [20]. Antimicrobial activity and microbial contents of zamzam water was studied by many researchers. Rauthah (2015), mention in a thesis of titled: "Microbial diversity and antimicrobial resistance of bacterial isolates in zamzam water" that zamzam water shows high bacterial contamination with existence of *E. coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* up to  $4.5 \times 10^2$  CFU/ ml and there was risk in waterborn disease from zamzam water and association between source of zamzam water and antimicrobial profile [21].

El-Desoky Rehab *et al.* (2020) found zamzam water, and zamzam water with ruqyah possessed antimicrobial activity against all examined pathogens used in their study. However, Saulawa and Hamisu (2018) found zamzam water was inactive against *C. albicans* [22].

*Streptococcus mutans* is considered one of the most important cariogenic species of the human oral microbial flora and there is a strong association with dental caries. Therefore, zamzam water found to have an inhibitory effect on *S. mutans* and higher statistically significant inhibitory values with probiotic strain group than that of zamzam water group [23]. Also, zamzam water can be used as solvent for extraction of antimicrobial substances from medical plants [24], thus the least MIC and MBC was found using zamzam water /acetone as solvent for extraction of flavonoid and phenolic content of *Moringa oleifera* leaf. Fares *et al.* (2013) founds that all zamzam extracts of five medical plants inhibit the growth of multidrug resistant clinical isolates of *Streptococcus pneumoniae* as a result of synergetic effect between active substances and zamzam water [25]. The antimicrobial activity of zamzam water on different microbes has been studied by Alwan *et al.* (2020). She

demonstrated that zamzam water inhibit the growth of Enterobacter, Bacillus and E.coli O157:H7. Also, Utami concluded that zamzam water has an antimicrobial activity against *Salmonella typhii* using MIC and MBC methods is 1.5% and 1.75% respectively [9]. Zamzam water has healing properties, that has the ability to stimulate AQPS, which play a critical roles in controlling the water content of cells [15], and stimulating the reproductive system through endometrial prolectin,  $\alpha$  and  $\beta$  defensin, luteinizing hormone (L.H). Zamzam water significantly decreased tumor size of colon cancers in mice as a result of biochemical nature of zamzam water and indirect influence on endocrine immunology and population of lymphocytes [16]. Also, zamzam water might have potential therapeutic efficacy for lung cancer (Omer U. M. et al. 2017). Alsokari (2011) found a positive effect on growth and protein content of lentil seedling irrigated with zamzam water and increased RNA, DNA and antioxidant content of lentil seedlings [17].

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