**RESEARCH ARTICLE**

# Study of the Synergistic Effect of Some Antibiotics and Aqueous Extract of *Hibiscus sabdariffa* Plant against *Salmonella typhi*

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**ABSTRACT**

The rising issue of drug-resistant bacterial species has resulted in the failure of existing treatment systems for *Salmonella typhi*. Lately, a recent treatment strategy was sophisticated to control the problem by using natural products with antibiotics to promote the effectiveness of treatment. The aim of this research was to test the inhibitory efficacy of *Hibiscus sabdariffa* calyxes extract toward *S. typhi* without, and with the following antibiotics, ciprofloxacin, azithromycin, chloramphenicol, ampicillin, amoxicillin, trimethoprim, coftriaxon, and norfloxacin, which were tested by the well diffusion method and disks propagation method, respectively, using Muller Hinton agar. *H. sabdariffa* cups (35 grams) were taken and placed in 250 mL of boiling water for 30 minutes. A series of concentrations of the aqueous extract were then made by dilution method (50, 100, 150, 250, and 500 mg/mL). The results showed that the extract of hibiscus had an inhibitory effect on the studied bacteria at each concentration 150, 250, and 500 mg/mL with diameter of inhibition 25, 27, 18 mm, respectively. The antibiotic susceptibility test was done, where the antibiotics ciprofloxacin, norfloxacin, and ceftriaxon showed areas of inhibition of diameters 25, 31, and 32 mm, respectively.

The synergistic effect was then tested by mixing the aquatic extract of *H. sabdariffa* with the antibiotics; the results showed an increase in the diameters of the inhibition zones compared with the diameters in the use of each of them separately. The highest inhibitory zones were observed with concentration (150 mg/mL) of the extract with ciprofloxacin, and the lowest inhibitory area was recorded at (100 mg/mL) of extract with chloramphenicol. The lowest inhibitory concentration (250 mg/mL) and the lowest killer concentration (500 mg/mL) were also identified.

**Keywords:** Antimicrobial effect, *Hibiscus sabdariffa*, *Salmonella typhi*.

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## INTRODUCTION

There are about 1,500 species of *Salmonella* that have been discovered so far. These bacteria have caused many cases of fear in societies in recent years because of the risk to their lives. *Salmonella* bacteria can be divided into two groups, one causing typhoid fever, and the other causing food poisoning. *Salmonella* is a gram-negative bacilli, do not form spores. *Salmonella* has 1,400 serotypes, some of which cause human diseases and have major medical significance, *S. typhi,* and *S. paratyphi*, which cause enteric fever.[1] *Salmonella* grow and multiply in water, they can live in milk, this is why the fever spreads in the summer and has nothing to do with the sun as it is commonly known, but, it is water pollution with bacteria.[2] Most of the time, bacteria pass through people carrying them in their bodies, especially those working in restaurants and food processing. The main reason for the spread of fever is attributed to low levels of personal and public hygiene, and poor health services.[3]

*Salmonella* bacteria cause approximately 16 million cases of fever each year in the world and the death of 6% of people worldwide annually.[4] The clinical symptoms of typhoid fever go through several stages. In the initial week, the symptoms begin. Signs of the illness include turning, joint pain, headache, and loss of appetite. The temperature begins to rise gradually, arriving 40ºC by the end of the week, as well as, the abdominal pain. In the second week, the patient suffers from increased manifestations of fatigue and may appear rash, as well as, enlargement of the liver and spleen in the third week, and when leaving the patient without any treatment, begins to increase and worsen where the patient suffers from dryness and discoloration, loss of appetite, and loss of consciousness and coma, sometimes the patient suffers from diarrhea and in some cases severe gastric bleeding and ulcers in the intestines.[5]

The development of medical and pharmacological treatment through the prescription of appropriate antibiotics in terms of therapeutic dose and medication duration of 10 to

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14 days gives good results in healing ,and prevent recurrence of the disease again during the delivery of vaccines in endemic areas such as Iraq. The first vaccine was discovered in 1897 by Rieb Edward, and was developed and called the live oral vaccine. The confirmed diagnosis of this infection must be done by isolation of bacteria from blood or urine samples and cultivated them on specific media to ensure the presence of this bacteria,[6] while the Widal test is the oldest method in the diagnosis of *Salmonella* bacteria.[7]

The reason for the human use of medicinal plants in the prevention and treatment of diseases since the beginning of human civilizations and for thousands of years to the urgent need to detect new antimicrobials with different chemical structures and mechanisms because there are cases of increase in the incidence of recurrent diseases and the other reason is the other increasing in resistance to antibiotics.[8] In addition, scientists have conducted new researches to overcome antimicrobial resistance.[9]

Plants have the capacity to manufacture compounds as secondary metabolites that are present in different parts of the plant. These compounds have a medical role, such as *H. sabdariffa*, which has therapeutic properties and is used in communities and other parts of the world in foods and beverages. There are compounds and organic materials, such as, malic acid, citric acid, alkaloids, vitamin C, glycosides, and steroid rings.[10] These compounds are considered to be very effective substances to produce antimicrobial agents of natural plant extracts, and thus find alternative, safe, cheap, and effective treatments at the same time.[11] So, the purpose of this research was to detect the synergistic effectiveness of some antibiotics and the aqueous extract of *H. sabdraffa* calyxes towards *S. typhi*.

## MATERIALS AND METHODS

### Collection of Bacterial Samples

The bacterial isolates of *S. typhi* were collected from the bacteriological laboratory at Teaching Al-Diwaniyah Hospital from November 2018 to February 2019 after isolation from different diseases and diagnosis by the specialists. The isolates were brought to the laboratory in the Department of Biology, College of Science, University of Al-Qadisiyah, in standard conditions and planted on the appropriate culture media.[12]

### Collecting the Calyxes of *H. sabdariffa*

Dried calyxes of *H. sabdariffa* were purchased from the local market, and then 50 grams of dried leaves were taken and grinded with electric mixer until powder was obtained.

### Preparation of Aqueous Extract of *H. sabdariffa* Calyxes

After obtaining powder from the used plant parts, 50 grams of it was added to 500 mL of hot distilled water in a glass flask and left for a 24-hour period at room temperature. Then, filtered with filter paper and obtained dry extract by subjected to heat of the oven at 45°C until gained heavy liquid, then dry in the incubator at 37°C, after that 35 grams of dried extract were taken and put in 250 mL of boiling water for 30 minutes. A series of concentrations of the aqueous extract were then made by dilution method (50, 100, 150, 250, and 500 mg/mL).

### Antibiotic Sensitivity Test

The Bauer-Kirby standard is used to test the bacterial sensitivity to the following antibiotics, C, CPR, TM, AMP, AMX, AZM, NOR, CIP. A tube containing 5 mL of nutrient broth was taken and inoculated with five colonies isolated from a pure culture of pre-diagnosed bacteria growing on the MacConkey agar medium, and then incubated at 37°C for 24 hours after that check the concentrations with 0.5 McFarland tube. A portion of the cultivated bacteria in nutrient broth that justified to concentrations 0.5 McFarland tube was taken by a sterile cotton swab and distributed evenly in a planner on the Muller Hinton agar medium, and by sterile metal forceps placed the antibiotic tablets leaving spaces between the disks and then, incubated at 37°C for 24 hours. The inhibition area was observed around the disks, and the diameter of the inhibition area was measured using determination of resistance or sensitivity of bacteria to antibiotics according to Vandepitte J *et al*.[13]

### Testing the Inhibitory Effect of the Aquatic Extract of *H. sabdariffa* Calyxes against Bacteria

The inhibitory activity of the *H. sabdariffa* calyxes extract against *S. typhi* was tested using the well diffusion method.[14] Petri dishes containing Muller Hinton agar were attended. Also, the fresh, pure bacterial suspension was prepared and justified to obtain an equivalent concentration of the McFarland tube (108 CFU/mL). The surface of the dish exfoliated from the previous bacterial suspension with a sterile cotton swab and left for 10 minutes, and then, five wells (6 mm) were drilled in the Muller by a cork borer with equal dimensions.

By using a micropipette, 50 to 100 µL were transferred from each concentration of the aqueous extract of the *H. sabdariffa* prepared using sterile, ion-free distilled water (50, 100, 150, 250, and 500 mg/mL) to the wells in the media.

The dishes were left for 10 minutes to allow the concentration of the extract to spread in the media and then incubated at 37°C for 24 hours. Growth was then observed if there was an inhibition area around the well where the diameter of the inhibition area was measured in mm. The minimum inhibitory concentration (MIC) and the lowest lethal concentration were determined.

### The Synergistic Effect of the Antibiotic and the Aqueous Extract of *H. sabdariffa* to *S. typhi*

Muller Hinton agar (MHA) was inoculated with *S. typhi* bacteria after adjusting the growth and comparing it with the McFarland tube, the dishes were left at room temperature for 10 minutes. After that, antibiotics were placed and fixed on the medium by slight pressure and then, 25 µL of the five concentrations 50, 100, 150, 250, and 500 mg/mL of the aqueous extract of the *H. sabdariffa* calyxes, and then the plates incubated at 37°C for 24 hours. After the incubation period, the diameters of the inhibition zones were measured around the disks.

## RESULTS AND DISCUSSION

### Results of Antibiotic Sensitivity Test

The results of the antibiotic susceptibility test against *S. typhi* showed resistance of the bacteria to the following antibiotics, AMP, AZM, AMX, TM, where the resistance was 80%. The resistance for (C) antibiotic was 67%, while the antibiotics (NOR, CIP, CRP), the bacteria were sensitive to them by 90 to 95%. These results are further explained in Figure 1.

The diameter of the inhibitory regions for the growth of *S. typhi* was 32 mm for ciprofloxacin, 31 mm for norfloxacin, and 25 mm for ceftriaxon antibiotic, as shown in Figure 2.

The resistance of bacteria to these antibiotics may be due to the erroneous and random use of these antibiotics against fever and cold infections that have been used in the past as first-line antibiotics against various types of bacterial infections. *S. typhi* has acquired multiple resistance to these antibiotics.

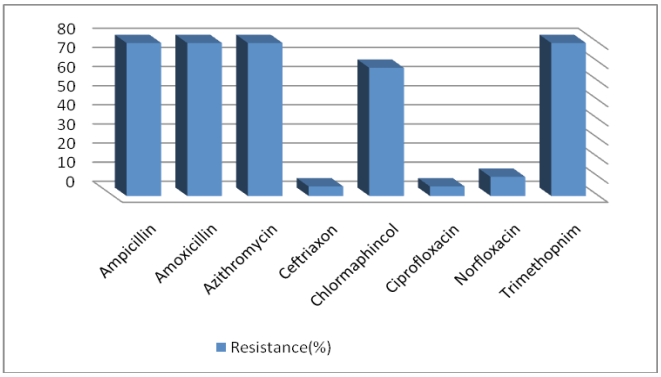


**Figure 1:** Antibiotic sensitivity by *S. typhi*

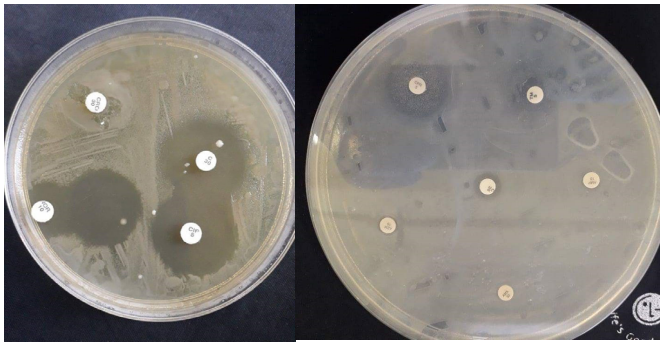

**Figure 2:** Inhibition zones of antibiotics

Also, maybe acquired R-plasmid, which plays an important role in giving resistance to many antibiotics.

### Results of the Test of Inhibitory Efficacy of the Aquatic Extract of *H. sabdariffa* against *S. typhi*

The results of the test of inhibitory efficacy of the aquatic extract of *H. sabdariffa* showed the activity of the extract in inhibiting the growth of *S. typhi* in the concentration of 150, 250, and 500 mg/mL of the extract (Figure 3), with diameter of inhibition 18, 25, and 27 mm, respectively.

As already mentioned that *H. sadariffa* has antibacterial characteristics towards various pathogens, foodborne pathogens, methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* These antibacterial activities can be due to strong chemical properties of secondary phytochemical metabolites of *H. sadariffa* cycles.[9,14] The former study on Sudanese Roselle appears the existence of alkaloids, phenolic compounds, flavonoids, and saponins; these are components of major groups of antimicrobial compounds in plants.[15]

### Results of the Test of Synergistic Effect of Antibiotics and Aqueous Extract of *H. sabdariffa* against *S. typhi* Bacteria

Table 1 and Figure 4 illustrate the results of the synergistic effect test of antibiotics and the used concentrations of aqueous extract of *H. sabdariffa*, where it is clear that the diameters of the inhibition zones of antibiotics have been increased with the different concentrations of the extract, which were resistant to bacteria when used separately.

The method of mixing antibiotics with plant extracts has a significant financial impact from reconstituting existing drugs,

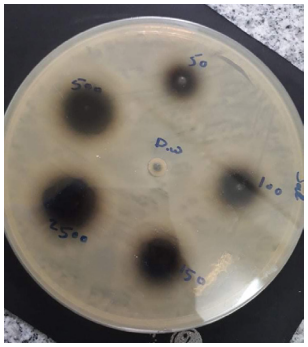

**Figure 3:** Inhibition zone of *S. typhi* by aqueous extract concentrations of *H. sabdariffa*, where D.W represents control

**Table 1:** Inhibition zones of *S. typhi* by antibiotics with different concentrations from the aquatic extract of *H. sabdariffa* plant

| | *Concentrations of Hibiscus sabdariffa calyxes (mg/mL)* | | | | |
|---|---|---|---|---|---|
| | *50* | *100* | *150* | *250* | *500* |
| *Antibiotics* | *Inhibition zone (mm)* | | | | |
| Ciprofloxacin | 31 | 39 | 40 | 35 | 30 |
| Trimethoprim | 0 | 0 | 0 | 13 | 18 |
| Ampicillin | 0 | 25 | 27 | 22 | 30 |
| Amoxicilin | 0 | 0 | 0 | 23 | 30 |
| Azithromycin | 0 | 13 | 16 | 32 | 28 |
| Chloramphenicol | 0 | 12 | 14 | 16 | 20 |

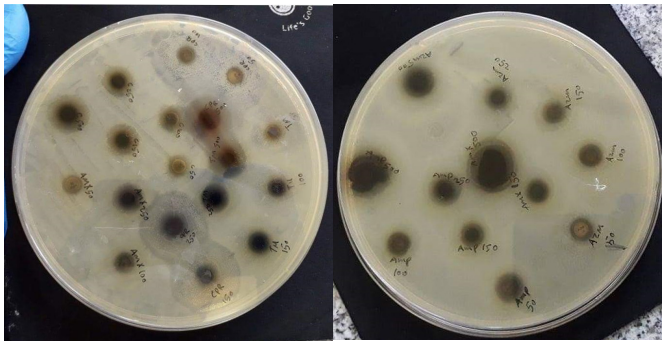

**Figure 4:** The inhibitory areas of *S. typhi* by antibiotics added to them of different concentrations of aquatic extract of *H. sabdariffa*

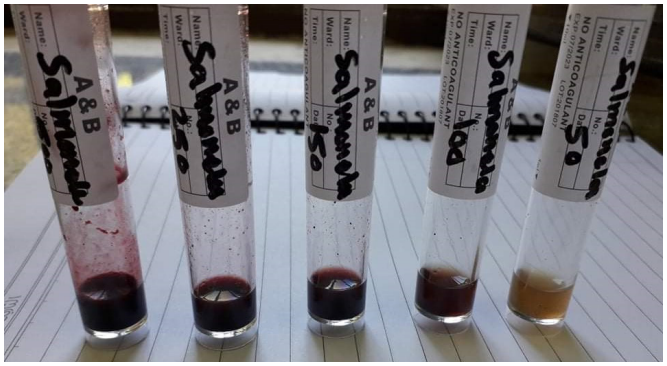

**Figure 5:** Growth of *S. typhi* in nutrient broth containing concentrations 500, 250, 150, 100, and 50 mg/mL of aquatic extract of *H. sabdariffa*

where reconstituting or mixing is the most applicable choice rather than developing a new drug that requires extensive clinical trials to prove its effectiveness.

In this study, the sensitivity of *S. typhi* bacteria to the extract of *H. sabdariffa* was demonstrated in combination with antibiotics AMP, AZM, AMX, C, CIP, TM. This may be due to the chemical properties of flavonoids and anthocyanin, which were identified in the aqueous extract of *H. sabdariffa* plant. The results showed that the extract could enhance the inhibitory activity of antibiotics against the tested bacteria. Furthermore, no antagonistic interactions were observed, when mixing the antibiotic with the extract.

Although the synergistic activity of aquatic extract of *H. sabdariffa* with antibiotics against *S. typhi* was confirmed, further studies should be undertaken to prove these activities, as well as, *in vivo* and in clinical trials.

### Results of the Minimum Inhibitory Concentration Test (MIC) and the Minimum Bactericidal Concentration (MBC) of Aqueous Extract against *S. typhi*

For testing the minimum inhibitory concentration, the bacteria were grown in tubes of the nutrient broth with different concentrations of aquatic extract of *H. sabdariffa* flowers (50, 100, 150, 250, and 500 mg/mL). The growth was observed after incubation period for the five concentrations, where there was a growth in the tube containing the concentration 50, 100, and 150 mg/mL of the extract, and this indicates the resistance of bacteria to these concentrations and confirmed by re-cultivation from these tubes on the nutrient agar, and

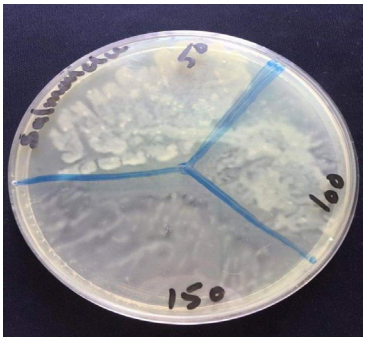


**Figure 6:** Growth of *S. typhi* on nutrient agar medium after taking an inoculation from growth at a concentration of 50, 100, and 150 mg/mL of the aquatic extract of *H. sabdariffa*

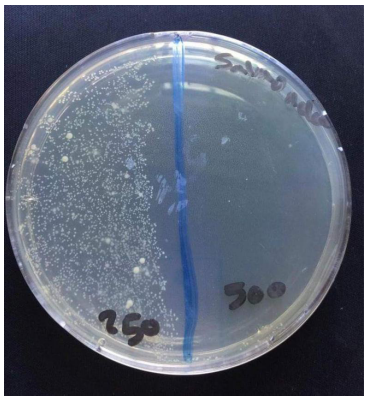


**Figure 7:** Growth of *S. typhi* on nutrient agar after taking an inoculation of growth at a concentration of 250 and 500 mg/mL of aqueous extract of *H. sabdariffa*

the results are shown in the Figures 5 and 6, respectively. Whereas, in the concentration of 250 mg/mL, there was very little growth compared to previous concentrations (Figures 5 and 7). This indicates that the MIC inhibitor concentration was 250 mg/mL, while the tube containing a concentration of 500 mg/mL showed no growth (Figure 5). When cultivating from this concentration on the nutrient agar and after 24 incubation period, no bacterial growth was observed, which indicates that it represents the MBC (Figure 7).

*H. sabdariffa*, which is consumed by people around the world in the form of tea extract, has a wide range of antimicrobial events.[16] In addition, a study by Hassan *et al*.[17] indicates that the aqueous extract of *H. sabdariffa* plant can be a useful factor when mixed with antibiotics to enhance the efficacy of treatment against *Helicobacter pylori* infection.

Several studies have reported that compounds in the aqueous extract of *H. sabdariffa*, such as, flavonoids and anthocyanins are responsible for possessing antimicrobial properties.[18]

The inhibitory effect of *H. sabdariffa* aqueous extract against *S. typhi* bacteria may be due to its capability to inhibit protein synthesis in bacteria.[19]

## CONCLUSIONS

According to the present study, the calyxes of *H. sabdariffa* can be used as a substitute source of the current ineffective synthetic antibiotics used against multidrug bacteria as

*S. typhi*, and can be used with antibiotics or separately to treat bacterial infections.

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