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Effect of the Various Culture Media against the Hot Aqueous Extract

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Abstract: *The rising issue of the drug-resistant bacterial species has resulted in the failure of the existing treatment systems for the Salmonella typhi. Lately, a recent treatment strategy was sophisticated to control the problem by using the natural products with the antibiotics to promote the effectiveness of the treatment. The aim of this research was to test the antimimicrobial efficacy of the Hibiscus sabdariffa calyxes extract toward the S.para typhi, E.coli, S.aureus and K.pneumonia by using the well diffusion method using the Muller Hinton agar and Nutrient agar. H.sabdariffa calcyes extract was prepare by crushing them into the electric grinder by adding the ethanol as an additive.*

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

I. INTRODUCTION

There are about 1,500 species of the *Salmonella* that have been discovered so far. These bacteria have caused many cases of the fear in societies in recent years because of the risk to their lives. The Salmonella bacteria can be divided into two groups, one causing the typhoid fever, and the other causing the food poisoning. Salmonella is a gram-negative bacilli, which do not form spores. Salmonella has around 1,400 serotypes, some of which cause the human diseases and have major medical significance, *S. typhi, and S. paratyphi*, which cause the enteric fever. *Salmonella* grow and multiply in the 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 the bacteria. Most of the time, the bacteria pass through the 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. *Hibiscus sabdariffa* (called as the Roselle in English) which belongs to the mallow family (*malvaceae*) is native to the West Africa. *Hibiscus sadariffa* is cultivated in the loamy, well drained soil mainly in the tropical climates and it also requires rainfall averaging about 10 inches (15 cm) each month throughout the growth season. *Hibiscus sabdrriffa* is of several use, it is considered to have anti-hypertensive properties. In some places the plant has been used in the folk medicine as the diuretic, mild laxative and also for the treatment of the cardiac and the nerve diseases and the cancer. The plant (*Hibiscus sabdariffa*) is very rich in the anthocyanin. The dried calyces contained the flavonoids gossypetin, hibiscentine and the sabdaretine. The major flavonoid formerly reported as the hibiscin, has been identified as the aphniphylline. The Small amounts of the myrillin (delphinidin 3- monoglucoside), chrysanthenin (cyanide 3- monoglucoside) and delphinidin are also present in considerable quantity.

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The Bacteria *Salmonella typhi* is a type of enteric bacterium which is responsible for causing the typhoid fever which has affected the mankind since the human population became large enough to contaminate the supply of its water. It is a food borne disease contracted by the ingestion of the bacteria in the contaminated food or the water. The sources of the infection could be through the infected food, poor kitchen hygiene, and the excretions from either the sick people or the infected but apparently clinically healthy people and the animals, polluted surface water standing water and so on. The signs and the symptoms of the disease has 4 phases, first week involves the slow rise in the temperature, headache, cough, malaise and the abdominal pain. In the second week of the infection, high fever in the plateau around 40oC (104oF) and the bradycardiac (Sphygmothermic dissociation) and the delirium is frequent. The patient may be calm but sometimes agitated, thus it gave the typhoid fever the nickname called as the "Nervous fever". In the third week, the intestinal hemorrhage occurs, Encephalitis, Neuropsychiatric symptoms, metastatic abscesses and the endocarditis is seen. The final week (fourth), the patient enters into the typhoid state. The incidence of *Salmonella* infection may not be perfectly known. This is because the majority of the patients are treated as the outpatients and therefore hospital based studies will underestimate the true incidence (W.H.O, 2006). However, the incidence of the typhoid fever in the developing countries is higher compared to the other developed countries.

Salmonella bacteria causes approximately 16 million cases of fever each year in the world and the death of 6% of people worldwide annually. The development of the medical and the pharmacological treatment through the prescription of the appropriate antibiotics in terms of the therapeutic dose and the medication duration of 10 to 14 days gives the good results in the healing, and prevent the recurrence of the disease again during the delivery of the vaccines in the endemic areas such as the Iraq. The Treatment of the typhoid is done by the use of the antibiotics such as the ampicillin, chloramphenicol, trimethoprim, sulfomethriazole and the ciprofloxacin. The first vaccine was discovered in the year 1897 by the Rieb Edward, and was developed and called as the live oral vaccine. The confirmed diagnosis of this infection must be done by the isolation of the bacteria from the blood or the urine samples and cultivated them on the specific media to ensure the presence of this bacteria, while the Widal test is the oldest method in the diagnosis of the *Salmonella* bacteria.

Figure 1: *Hibiscus sabdariffa*

The reason for the human use of the medicinal plants in the prevention and the treatment of the diseases since the beginning of the human civilizations and for thousands of the years to the urgent

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need to detect the new antimicrobials with the different chemical structures and the mechanisms because there are cases of the increase in the incidence of the recurrent diseases and the other reason is the other increasing in resistance to the antibiotics. Plants have the capacity to manufacture it's compounds as secondary metabolites that are present in the different parts of the plant. These compounds have a medical role, such as the *H. sabdariffa*, which has therapeutic properties and is used in the communities and the other parts of the world in the foods and the beverages. There are compounds and the organic materials, such as the malic acid, citric acid, alkaloids, vitamin C, glycosides, and the steroid rings.These compounds are considered to be very effective substances to produce the antimicrobial agents of the natural plant extracts, and thus find the alternative, safe, cheap, and very effective treatments at the same time. So, the purpose of this research was to detect the synergistic effectiveness of some of the antibiotics and the aqueous extract of the *H. sabdraffa* calyxes towards the *S. typhi*, *K. pneumonia, E. coli, S. aureus.*

Figure 2: Hibiscus sabdariffa Calyces

The *H. sabdariffa* is also known as the rosella or the rosella fruit in the Australia. Its close relative, *Hibiscus cannabinus* is also known as the meśta/meshta on the Indian subcontinent, Tengamora among the assamese and the "mwitha" among the Bodo tribals in Assam, Gongura in Telugu, Pundi in Kannada, Ambadi in the Marathi, LalChatni or Kutrum in the Mithila Mathipuli in Kerala. Roselle's major enemy is the root-knot nematode, *Heterodera rudicicola*. Mealybugs may be very troublesome. In the Australia, 3 beetles, *Nisotra breweri*, *Lagris cyanea*, and the *Rhyparida discopunctulata*, attack the leaves. The "white" roselle has been found heavily infested with the cocoa beetle, *Steirastoma breve* in the Trinidad, with a lighter infestation of the the red roselle in an intermixed planting. Occasional minor pests are the scales, *Coccus hesperidum* and the *Hemichionaspis aspidistrae*, on the stems and the branches; yellow aphid, *Aphis gossypii*, on the leaves and the flower buds; and the cotton stainer, *Dysdercus suturellus*, on the ripening calyces.

Constituents of *H. Sabdariffa:-*

SR NO.	CONSTITUENTS	PERCENTAGE
1	Moisture	7.6
2	Crude Extract	24
3	Fat	22.3
4	Ash	15.3

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5	Carbon	0.3
6	Phosphorus	0.6
7	Myristic	0.4
8	Palmitic	2.1
9	Palmotheolic	35.2
10	Stearic	2.0
11	Oleic	3.4
12	Lineolic	34
13	Fatty acids	14.4
14	Cholesterol	4.5
15	Malvalic	5.1
16	Sistosterol	61.3

TABLE 1: Constituents of *Hibiscus sabdariffa*

II. REQUIREMENTS

1. Distilled water
2. Normal saline
3. Hydrogen peroxide.
4. Acetone alcohol.
5. Lugol's iodine.
6. Kovac's reagent.
7. Nutrient agar.
8. Muller Hinton Agar.
9. Nutrient Broth.

III. MATERIALS AND METHODS

Collecting the Calyxes of *H. sabdariffa*

Dried calyces of the *Hibiscus sabdariffa* (zobo) flowers were bought from the market,and then 50 grams of the dried leaves were taken and grinded with the electric mixer until very fine powder was obtained.

IV. PROCEDURE

1. The calyces of the *H.sabdariffa* were washed under the running water and then it was allowed to dry under the normal/room temperature.
2. After the drying process the calyces of the *H.sabdariffa* were crushed to make the pure sample using the electric grinder.
3. The sample was stored in the air tight container for the further usage.
4. 200ml of Nutrient agar was prepared. {5.6g of Nutient agar was dissolved in 200ml of the Distilled water.}
5. 200ml of Muller Hinton agar was also prepared. {7.6g of Muller Hinton agar was dissolves

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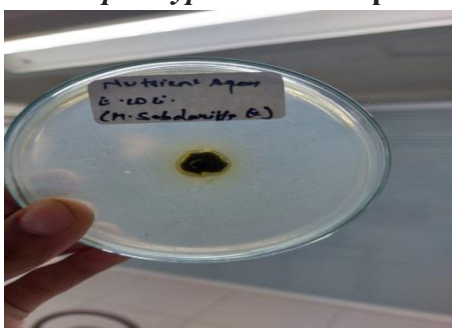
in 200ml of the Distilled water.}

6. Both the agar then sterilized using the Autoclave at 121°C for 15 minutes.
7. After the sterilizing process, both the media's were transferred into sterile petri plates/dishes under the sterile conditions by working between the 2 bunsen burner's.
8. After that, the petri plates/dishes were allowed to cool under the room temperature.
9. On Day 1, petri plates each of both the agar were used for the further process.
10. *Salmonell Para typhi B, Klebsieela pneumonia, E.coli* and *S.aureus* were used as the culture media's.
11. On each 2 plates, each of the cultures were spread using the technique known as the spread plate technique.
12. After the spreading the culture media's , each of the respective media plates were kept in the fridge for 24 hrs.
13. On Day 2, the plates were inoculated with the pure sample of the *H.sabdariffa* using the technique known as the cork borrer method under the sterile condition.
14. After the inoculation process the plates were kept in the incubator at the **37°C**for 24 hrs.
15. On Day 3, the plates were observed.

V. DICUSSIONS OF RESULTS

5.1 Nutrient Agar Plates

Efficiency of the extract against the *S.paratyphi B* on N.A plates



Efficiency of the extract against the *E.coli* on N.A plates

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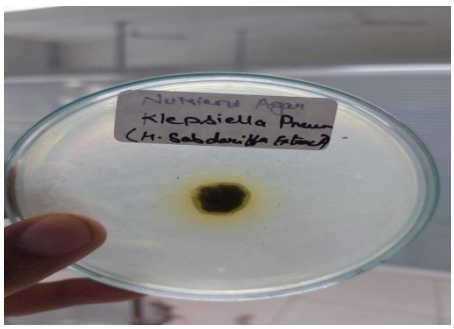
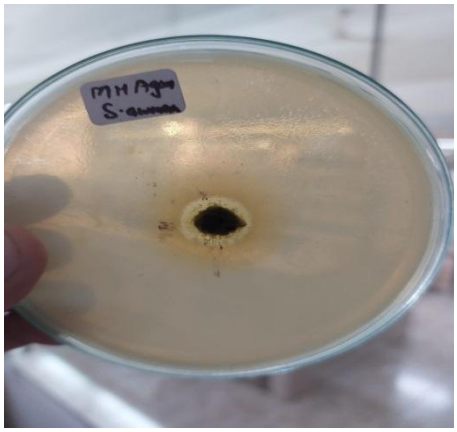


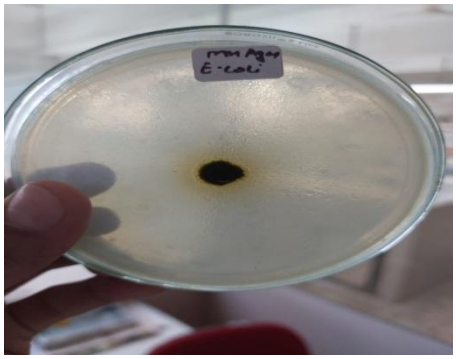
Efficiency of the extract against the *K.pneumonia* on N.A plates

Efficiency of the extract against the *S.aureus* on N.A plates

5.2 Muller Hinton Agar Plates



Efficiency of the extract against the S.aureus M.H plates



Efficiency of the extract against the E.coli on M.H plates

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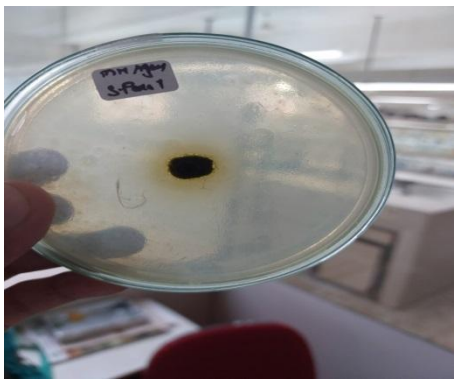

Efficiency of the extract against the S.paratyphi B on M.H plates

FOR N.A PLATES

1. The pure extract of the H.sabdariffa showed the minimum zone of inhibition on the S.aureus plate. The diameter of the zone of inhibition was 38mm.
2. There was no sufficient amount of zone of inhibition was observed for the *E.coli, K.pneumonia, and S.paratyphi B*
3. The above results proved that the pure extract of the *H.sabdariffa* was very effective against the *S.aureus* and it was less effective against the other sample.

FOR M.H PLATES:-

1. The pure extract of the *H.sabdariffa* showed the minimum zone of inhibition against the *S.aureus.* The diameter of zone of inhibition was around 72mm.
2. There was no sufficient amount of zone of inhibition was observed for the *E.coli, K.pneumonia, and S.paratyphi B*
3. The above results proved that the pure extract of the *H.sabdariffa* was very effective against the *S.aureus* and it was less against the other samples.

VI. CONCLUSION

Based on the findings of this great research work, the aqueous extract of the H. sabdariffa possess the antimicrobial activity. The extract exhibited the highest activity on the S. aureus, followed by S. typhi, K. spp. And the E. coli. were all resistant against the extract. From the results of these findings, H. sabdariffa can inhibit as well as promote the growth of the certain microorganisms. This is because it is very rich in the varieties of the constituents which promote the growth of the certain organisms such as the moisture, crude protein, fat, fiber, carbon, phosphorous to mention but a few. H. sabdariffa should be produced in the industrial scale since it has nutritional as well as therapeutic properties. The industrialists should endeavor to maintain the standard operational procedures (SOP) and also pasteurize the finished product in order to reduce the Microbial load in the sample.

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REFERENCES

[1]. Abass AA, Al-Magsoosi MJN, Kadhim WA, Mustafa R, Ibrahim SA, Aljdaimi AI, Al-Nasrawi SJ, Hadi NR, Haider J. Antimicrobial effect of Red Roselle (Hibiscus Sabdariffa) against different types of oral bacteria. J Med Life. 2022 Jan;15(1):89-97. doi: 10.25122/jml-2021-0184. PMID: 35186141; PMCID: PMC8852637.

[2]. https://www.researchgate.net/publication/344286271_Study_of_the_Synergistic_Effect_of_Some_Antibiotics_and_Aqueous_Extract_of_Hibiscus_sabdariffa_Plant_against_Salmonella_typhi

[3]. Apata, L. (1984). Medicinal Plants and Traditional Medicine in Africa. John Wiley and Chichester.

[4]. Calixto, J. B. (2000). Efficiency, Safety, Equality, Control, Marketing and Regulatory Guidelines for Herbal Medicines (phyto therapeutic agent). Brazillain Journal of Medical and Biological Research 33(2), 179-189.

[5]. World Health Organization. (2006). Vaccination Standardization and Effective Health Services.

[6]. World Health Organization. (2008). Weekly Epidemiological Records 83 (6), 49- 60.