

# ANTIMICROBIAL EVALUATION OF HIBISCUS SABDARIFFAL PLANT EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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## Abstract

The purpose of this study was to examine the evolution of antimicrobial activity of flower extract of Hibiscus sabdariffal against antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus*. The extract of Hibiscus sabdariffal obtained by rotary and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract. Furthermore In the present study, only the susceptibilities of clinical bacterial isolates to the extracts of Hibiscus sabdariffal leaves and flowers have been examined. Therefore, further microbiological studies would be carried out to determine the minimal inhibitory concentrations (MICs) of the extracts prepared from Hibiscus sabdariffal against *Escherichia coli* and *Staphylococcus aureus*. The watery extracts of Hibiscus sabdariffal was evaluated against isolates and the results showed that the crude extracts of them with boiling water were more effective than the cold watery extracts.

**Key words:** Hibiscus, microbial, extract, bacteria, Antimicrobial activity.

## Introduction

Plants constitute the base of health care systems in many . These natural products from plants including saponin, alkaloids, tannins, cardiac glycosides and anthraquinones, are synthesized for defence purpose . Africa in early times. The plants Hibiscus sabdariffal belongs to the family Mallows. Traditionally the flowers can be used as anti asthmatic agent (1).

There are several studies which reveal the presence of such compounds with antimicrobial properties in various plant parts . The genus *Hibiscus* (Malvaceae) comprises about 275 species in the tropics and sub-tropics . Flowers of *Hibiscus tiliaceus* L. are widely used for birth control and for treating skin infections . Leaves and flowers of selected *Hibiscus* species are used in traditional medicine. Information on their antioxidant, antityrosinase and antibacterial activities. Combination of plant extracts with antibiotics help to minimize the minimum inhibitory concentrations (MICs), synergistic activity and this reduces the side effects, the economic cost and reduce sensory impact.

Furthermore, these combinations may also control some bacteria that are known to show consistently high resistance to antimicrobials, alcoholic extracts of the calyces showed the presence of such biochemicals as flavonoids, phenols, reducing sugars, combined reducing sugars among others . *Staphylococcus aureus* is one of the main causes of human infections. It can cause diseases ranging from minor infections such as pimples and boils to serious systemic fatal infections . *Escherichia coli* is a Gram negative rod ( bacillus) in the family Enterobacteriaceae. Most *E.coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. The purpose of this study was to examine the evolution of antimicrobial activity of flower extract of *Hibiscus sabdariffa* against antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* that isolates from humans (2).



**Hibiscus sabdariffa flower**

## Material and Method

Preparation of plant extracts Extraction of aqueous component Cold extraction . A total of 10 g of dried flower was soaked in 50 mL of cold water in a conical flask for 24 h and then filtered off using sterile Whatman No. 1 filter paper into a sterile conical flask and evaporated by using solvent distillation apparatus. The extract was got with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was obtained and stored for further use (3) . Hot extraction Twenty grams of every dried powdered plant material was soaked in 100 ml of distilled boiled water in a sterile conical flask for 48 hours with continuous shaking. Then after filtration through 8 layers of muslin cloth and centrifuged at 5000g for 10 min the supernatant was collected and concentrated (in oven at 45 °C) to make the final volume half of the original volume (stock solutions) (4).

### Determination of the Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for all isolates by a standard agar dilution method as follows: Serial two fold dilution of each antibiotic being tested ranged from 0.05 to 1024µg/ml were prepared. The antibiotic were used (Amoxicillin) against the multi-drug resistant isolates *Escherichia coli* , *Staphylococcus aureus* and *Salmonella*. Mueller-Hinton agar medium was prepared, sterilized by autoclave and cooled to 45°C, then the antibiotics were added in appropriate amount from their stock solution, mixed well and poured into the plates. Few colonies (2-4) from overnight culture were transferred to 2ml of normal saline in order to prepare the bacterial suspension and were adjusted to 0.5 McFarland turbidity equal to  $1.5 \times 10^8$  CFU/ml. Five microliter of each inoculum was spotted on the agar surface by micropipette. The plates were left to dry for 5 minutes and then incubated at 37°C for 18-24 hr. The lowest concentration of antibiotic inhibiting the bacterial growth was recorded as the MIC (5).

Test microorganism for antibacterial assay Agar well diffusion method Muller Hinton agar plates were prepared and wells of 5 mm were cut and swabbed with cultures. The cut wells were then filled with 50. L of aqueous extracts of flowers and the plates were kept for incubation at 37°C for 24 h . Influence of

combination between antibiotics and plant extracts against selected multi drug resistant strains (6). The plant extract were tested in combination with antibiotic Amoxicillin against *Escherichia coli* and *Staphylococcus aureus* by using agar well diffusion method . Each well then filled with 50. L of aqueous extracts and Amoxicillin (7).

## RESULTS

Extracts of Hibiscus was screened for their antibacterial activity against *Escherichia coli* , *Staphylococcus aureus* and *Salmonella* from different bacterial groups using agar well diffusion method. The aim of the study was to investigate whether the folk uses of the herbal preparations derived from Hibiscus sabdariffal in some diseases caused by bacterial infection had any scientific basis. It is clear from the findings of this study, that Hibiscus sabdariffal flowers contain important constituents that confer its antibacterial activity and may be used in treating pathological conditions (8). In the present study, only the susceptibilities of clinical bacterial isolates to the extracts of Hibiscus sabdariffal flowers have been examined.

The watery extracts of Hibiscus sabdariffal was evaluated against isolates and the results showed that the crude extracts of them with boiling water were more effective than the cold watery extracts (crude and diluted ) which recorded inhibition zones ranged from 12 mm to 25 mm. While the hot watery extracts which recorded inhibition zones ranged from 11 mm to 33 mm.



Figure 1. The zone of inhibition of the combination of Amoxicillin (MIC concentration) *Escherichia coli* , *Staphylococcus aureus* and *Salmonella* respectively.

The occurrence and spread of antibiotic resistant bacteria are pressing public health problems worldwide. Many bacteria have become and continue to be resistant nearly against all antimicrobial agents. The resistance rates are higher in

developing countries (9). Emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The study was conducted with a view to investigate the antimicrobial properties of Hibiscus sabdariffa.

## DISCUSSION

Antibacterial activity of medicinal plant extracts on the MDR strains: Medicinal plants continue to play a central role in the healthcare systems of large proportions of the world's population, particularly in developing countries, where herbal medicine has a long and uninterrupted history of use (10). They confirmed that indeed plants can be sources of compounds that can potentiate the activity of antibiotics against resistant bacterial pathogens. These compounds have variably been termed resistance modifying, modulating or reversal agents. This ability of plant extracts to potentiate antibiotics has not been well explained.

## CONCLUSION

Our results are encouraging but precise assessment is utterly necessary before being situated in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation. The synergistic effect of the association of Amoxicillin with Hibiscus sabdariffa extract against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the older and cheaper antibiotic when it is no longer effective by itself as an effective treatment. In addition, the plant extracts can be a potential source of broad spectrum resistance modifying compounds that can potentially improve the performance of antibiotics in the treatment of multi-drug resistant infections.

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