Original Article

Antibacterial Activity of *Hibiscus rosa-sinensis* Extract and Synergistic Effect with Amoxicillin against some Human Pathogens

Shaymaa Khudhr Al-Alak, Rasha Mohamed Sajet AL-Oqaili*, Basam Basim Mohammed and Nabaa Abd-Alkhalik

Department of biology, Collage of science, University of Al-Mustansiryah, P.O. box 14022, Baghdad, Iraq

ABSTRACT

Objective: Study the Antibacterial activity of *Hibiscus rosa-sinensis* extract and Synergistic Effect with Amoxicillin against some human pathogens.
Methods: Test microorganism for antibacterial assay (Agar well diffusion method) (Influence of combination between antibiotics and plant extracts against selected multi drug resistant strains).
Results: A total of five clinical isolates (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*) belonging to different bacterial species were collected from the Department were obtained from the department of biology, college of science, Al-Mustansiryia university, Baghdad, Iraq. Co-trimoxazol, Tetracycline, Lincomycin, *Enterobacter* isolate was resistant to Amikacin. All bacterial isolates were sensitive to Ciprofloxacin, Norfloxacin. The minimum inhibitory concentrations (MICs) of Amoxicillin most effective antibiotics was tested against the five MDR isolates. Furthermore In the present study, only the susceptibilities of clinical bacterial isolates to the extracts of *H. rosa-sinensis* 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 *H. rosa-sinensis* against (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*). The watey extracts of *H. rosa-sinensis* 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.
Conclusion: Of this work the combination between *H. rosa-sinensis* and Amoxycillin showed synergistic effect against the tested bacteria than each of them alone.

Keywords: *H. rosa-sinensis*, *P. aeruginosa*, *Serratia, Micrococcus, Enterobacter, Salmonella*.

Address for Correspondence

Department of biology, Collage of science, University of Al-Mustansiryah, P.O. box 14022, Baghdad, Iraq.
E-mail: rashaalogali@gmail.com

INTRODUCTION

The situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants[1,2]. Bacterial resistance to chemically unrelated antimicrobial agents is public health concern[3], and may be caused by over-expression of MDR efflux pumps[4]. In Gram negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms[5]. Pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines[6,7].

The plants Hibiscus rosa-sinensis (H. rosa-sinensis) belongs to the family Malvaceae. Traditionally the flowers can be used as anti asthmatic agents[8,9]. There are several studies which reveal the presence of such compounds with antimicrobial properties in various plant parts[10]. The genus Hibiscus (Malvaceae) comprises about 275 species in the tropics and sub-tropics[11]. Flowers of Hibiscus tiliaceus L. are widely used for birth control and for treating skin infections[12]. Leaves and flowers of selected Hibiscus species are used in traditional medicine. Information on their antioxidant, antityrosinase and antibacterial activities is meagre[13]. 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, i.e.: improving the efficacy of antibiotics against resistant bacterial pathogens, (modifying agents)[14,15]. The plants from different geographical locations act synergistically with common antibiotics and exhibited greater antimicrobial activity against MDR pathogens[16,17].

The present study has been designed to determine the role of this flower Hibiscus rosa-sinensis extract in the in-vitro antibacterial activity against human pathogens Gram negative bacteria (*Pseudomonas aeruginosa, Serratia, Micrococcus, Enterobacter and Salmonella*), investigate the effect of some commercial antibiotics against multi-drug resistant human clinical bacterial isolates and combination effect of plant extracts with these antibiotics against the resistant bacteria pathogens.

MATERIALS AND METHODS

Collection of samples

A total of 5 clinical isolates (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*) belonging to different bacterial species were collected from the Department were obtained from the department of biology, college of science, Al-Mustansiryia university, Baghdad, Iraq.

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 at 4. for further use[18].

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 ºC) to make the final volume half of the original volume (stock solutions)[19,20].

Antibiotic sensitivity test

Antibiotic sensitivity was tested using disk diffusion with the following antibiotics: the following antibiotics: Ciprofloxacin CIP (5 µg), Norfloxacin NOR (10 µg), Amikacin AK (30µg), Co-trimoxazol STX (30 µg), Lincomycin L (15 µg) and Tetracycline TE (30 µg). Isolates were categorized as susceptible (S), moderately susceptible (I), and resistant (R), based upon the interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI)[21].

Determination of the Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for all isolates according to the CLSI[21], criteria 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; (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter 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 1.5×10^8 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.

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 different cultures. The cut wells were then filled with 50. L of aqueous extracts of flowers and leaves separately and the plates were kept for incubation at 37°C for 24 h[22].

Influence of combination between antibiotics and plant extracts against selected multi drug resistant strains

The plant extract were tested in combination with antibiotic Amoxicillin against five selected MDR strains; (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*) by using agar well diffusion method according to[15,16]. Each were then filled with 50. L of aqueous extracts and Amoxicillin.

RESULTS

Extracts of Hibiscus was screened for their antibacterial activity against five isolates 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 *H. rosa-sinensis* in some diseases caused by bacterial

infection had any scientific basis. It is clear from the findings of this study, that *H. rosa-sinensis* flowers and leaves contain important constituents that confer its antibacterial activity and may be used in treating pathological conditions caused particularly by isolates (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*). In the present study, only the susceptibilities of clinical bacterial isolates to the extracts of *H. rosa-sinensis* 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 *H. rosa-sinensis* against (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*). Biochemical and pharma-cological investigations would also be necessary to establish the exact antibacterial principles for use in complementary alternative medicine. The watery extracts of *H. rosa-sinensis* 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 10 mm to 27 mm. While the hot watery extracts which recorded inhibition zones ranged from 10 mm to 32 mm. figure 1.

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[23]. Emergence of multi-drug 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 some medicinal plant extracts against some human clinical bacterial isolates; (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*). Identification of the bacterial isolates was carried out using morphological and biochemical properties. Morphological studies were carried out by microscopic observation and studies on growth characteristics in Petri dishes. All clinical isolates were identified biochemically by the API 20E systems according to manufacturer's instructions.

The results of antibiotic susceptibility test of the bacterial isolates indicated that high proportion of test organisms were resistant to Co-trimoxazol, STX; Tetracycline TE; Lincomycin, L. *Enterobacter* isolate was resistant to Amikacin, AK. All bacterial isolates were sensitive to Ciprofloxacin, CIP; Norfloxacin, NOR. (Table 1). Results showed[24] that the susceptibility rate of clinical isolates was the highest for amikacin (87.2%), followed by ciprofloxacin (74.8%), ceftazidime (71.5%), gentamicin (70.4%) and nitrofurantoin (53%).While, Yuksel *et al.* (2006) showed that nitrofurantoin was the most active agent (2.2% resistant isolates), followed byamikacin (4.9 %) and ciprofloxacin (12%). Table (1).

Amoxicillin most effective antibiotics was tested against the five MDR isolates (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*). The MICs was determined for all isolates according to the CLSI[21], criteria by a standard agar dilution method. The results showed that the highest rate for some isolates was 128 µg/ml and the break point is (64, 16, 4 µg/ml). The highest MIC against *Micrococcus* and *Salmonella*.

The combination of Amoxicillin (MIC concentration) and crude extract *H. rosa-sinensis* was determined and the results showed that the mixture of Amoxicillin and crude extract of *H. rosa-sinensis* was more

active against (*P. aeruginosa*, *Serratia, Micrococcus, Enterobacter and Salmonella*) isolates comparison with Amoxicillin alone or crude extracts alone. Amoxicillin and crude extract of *H. rosa-sinensis* recorded inhibition zones ranged from 33 mm to 62 mm. figure 2.

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[25].

Our results agree with the findings of[14,16]. 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. It is speculated that inhibition of drug efflux, increasing permeability, inhibition of β- lactamase and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics[26].

CONCLUSION

Our results are encouraging but precise assessment is utterly necessary before being situate 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 *H. rosa-sinensis* 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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Table 1. Antibiotic susceptibility test of the bacterial isolates

Isolates	STX	L	TE	CIP	AK	NOR
Salmonella	R	R	R	S	S	S
Enterobacter	R	R	R	S	R	S
P. aeruginosa	R	R	R	S	S	S
Serratia	R	R	R	S	S	S
Micrococcus	R	R	R	S	S	S

Ciprofloxacin, CIP; Norfloxacin, NOR; Amikacin, AK; Co-trimoxazol, STX; Tetracycline TE; Lincomycin, L. sensitive S; resistance R.

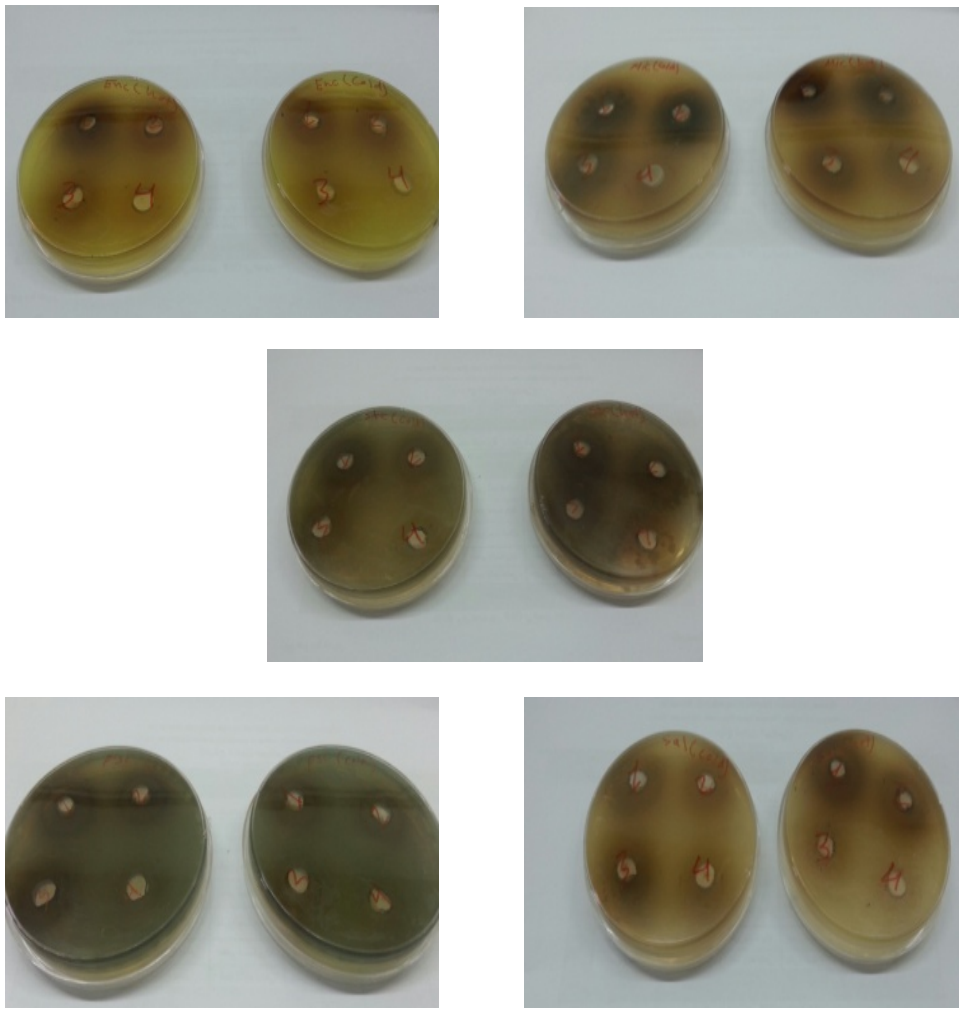

Figure 1. The zone of inhibition of concentrations the watey extracts (cold and hot watey extracts) of Hibiscus on *Enterobacter, Micrococcus, Serratia, P. aeruginosa*, and *Salmonella* respectively

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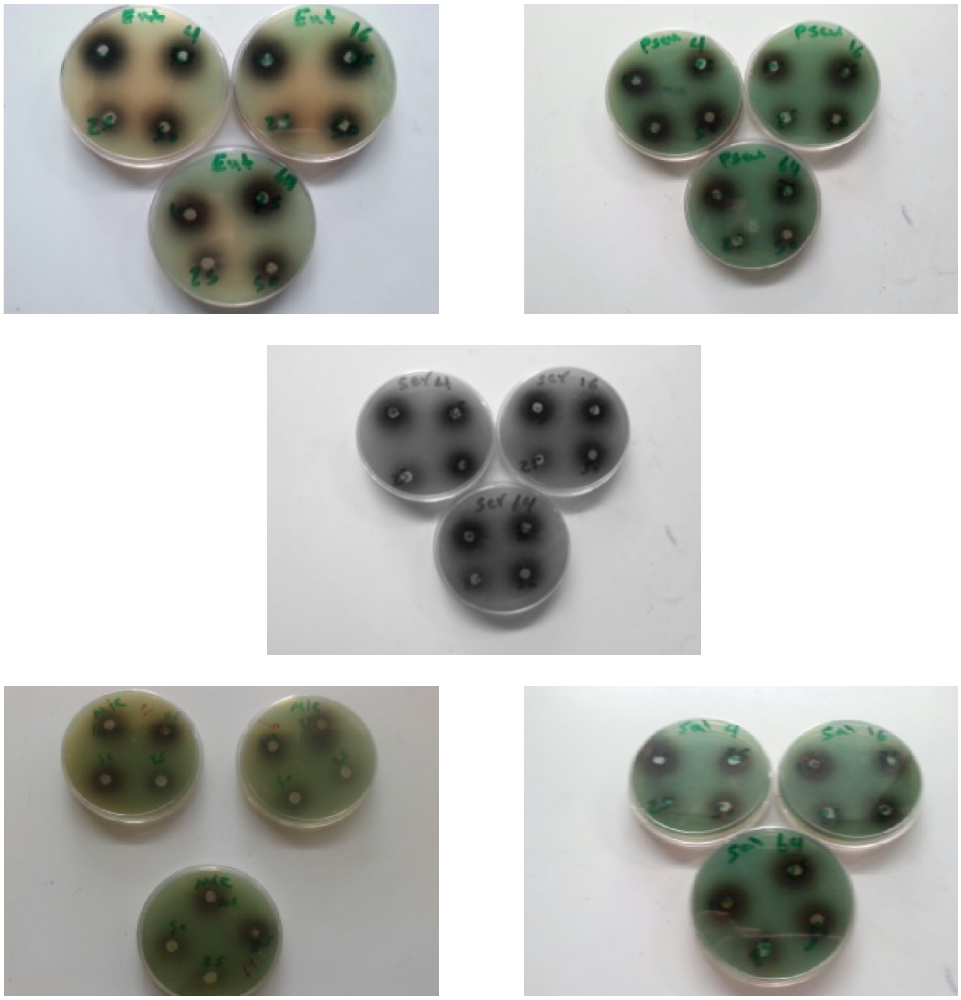

Figure 2. The zone of inhibition of the combination of Amoxicillin (MIC concentration) and concentrations the watey extracts (cold and hot watery extracts) of Hibiscus on *Enterobacter, P. aeruginosa, Serratia, Micrococcus* and *Salmonella* respectively