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Antimicrobial activity of *Hibiscus sabdariffal* extract against human pathogen

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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* that isolates from the urinary tract infection by microtiterplate method. All 42 strains 30 *E.coli* and 12 *S. aureus* isolated from urine culture of hospitalized patients in Zabol (Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011- 2012 were evaluated and the extract of *Hibiscus sabdariffal* obtained by rotary and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract. The result show that *E. coli* were resistance to 4 of the agent and more resistance to tetracyclin(63.3%), erythromycin(56.6%) and cefixime(40 %), *S.aureus* more sensitive were antibiotic resistance to vancomycin and cefixime. The highest MIC values was found to be 20mg/ml against two *E.coli*. The leas MIC values was found to 1.25 mg/ml against three *S.aureus*.

Keyword: *Hibiscus sabdariffal*, Antibacterial activity, Human pathogen

Introduction

Medicinal plants constitute the base of health care systems in many societies (Almeida et al., 2006; Monteiro et al., 2006). These natural products from plants including saponin, alkaloids, tannins, cardiac glycosides and anthraquinones, are synthesized for defence purpose (Aletor et al., 1993). Sorrel, *H. sabdariffa* L. (Family Malvaceae), a medicinal herb commonly uses to make drink and pickle, is used in folk medicine in the treatment of hypertension, liver diseases, and fever (Dalziel, 1973; Akindahunsi and Olaleye , 2003). *Hibiscus* anthocyanins, a group of phenolic natural pigment present in the dried flower of *H. sabdariffa* and *Hibiscus rosa-sinensis*, have been found to have cardioprotective(Jonadet et al., 1990), hypocholesterolemic(Chen et al., 2003), antioxidative, and hepatoprotective(Wang et al., 2003) effects in animals. Although, native to India and Malaysia, *H. sabdariffa* is also widely available and must have being carried to Africa in early times (Fasoyiro et al., 2005). .Phytochemical screening of the water and

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alcoholic extracts of the calyces showed the presence of such biochemicals as flavonoids, phenols, reducing sugars, combined reducing sugars among others (Builders et al., 2010). *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 (Evans and Brachman, 1991). 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 sabdariffal* against antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus* that isolates from the urinary tract infection by microtiterplate method.

Material and Method

Isolation of bacteria: All 42 strains (30 *E.coli* and 12 *Staphylococcus aureus*)isolated from urine culture of hospitalized patients in Zabol (Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011- 2012 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests (Forbes et al., 2007).

Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (CLSI, 2002). The procedure followed is briefly described here. *S. aureus* and *Ecoli* isolated plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile salin water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ceftazidim(30 µg), tetracyclin (30µg), erythromycin (15µg), ceftazidime (30µg), trimethoprim-sulfamethoxazol (1.25+23.15 µg), penicillin (10µg),oxacillin(30µg) and vancomycin(10 µg).

Plant materials*:*

The flower of *Hibiscus sabdariffal***,** was collection in the region of Iran (Sistan, south-eastern, Iran) and plant in Zabol university herbarium received approval and dried at room temperature .Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts:

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4^0C in air tight screw-cap tube.

Minimum Inhibitory Concentration (MIC) of extract:

The broth microdilution method was used to determine MIC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3mg/ml to 20 mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to

each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

Statistical Analysis:

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

Result

The result show that *E. coli* were resistance to 4 of the agent and more resistance to tetracyclin(63.3%), erythromycin(56.6%) and cefixime(40 %) and ceftazidim(26.6%), *S.aureus* more sensitive were antibiotic resistance to vancomycin and cefixime. The highest MIC values was found to be 20mg/ml against two *E.coli.* The leas MIC values was found to 1.25 mg/ml against three *S.aureus.*

Discussion

The result show that *E. coli* were more resistance to tetracyclin(63.3%), erythromycin(56.6%), ceftazidim(26.6%) and cefixime(40 %). The study of Akond, 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested Escherichia coli strains from poultry sources were found resistant respectively to penicillin, ciprofloxacin, riphampicin, kanamycin, streptomycin, cefixine, erythromycin, ampicillin, tetracyclin and chloramphenicol and neomycin(Akond et al., 2009). Tricia reported 43% isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin (Tricia et al., 2006). In the study *S.aureus* more sensitive were antibiotic resistance to vancomycin and cefixime. The study of Soltani, The overall susceptibility of isolated *S. aureus* strains to antimicrobial agents was 100% for vancomycin, 49.4% for amikacin, 43.8% for gentamicin, 36.8% for co-trimoxazole and tetracycline, 36.3% for cefazolin, 30.6% for cephalexin, 24.4% for oxacillin, 23.8% for erythromycin, and 3.1% for penicillin(Soltani et al., 2012). The highest MIC values was found to be 20mg/ml against two E.coli. The leas MIC values was found to 1.25 mg/ml against three S.aureus. The study of Fullerton, the findings indicated that sorrel was effective at all levels in inhibiting *E. coli* O157:H7 (Fullerton et al., 2011). The study of Jung, E. coli was strongly inhibited by the Roselle water extract at concentrations of 25 and 50 mg mL^{-1} as determined by a paper disc method (Jung et al., 2013). The study of Nwachukwn, the extracts showed a significant activity on *S. aureus* with zones of inhibition range of 27.7+ 0.47 – 11.3 + 0.94mm and minimum inhibitory concentration range of < 5-15% (v/v) while *E. coli* had zones of inhibition range of 14.3 + 0.47 – 7.0 + 0.00mm and minimum inhibitor concentration range of 15 – 50% (v/v)(Nwachukwu et al., 2009). The another study, the aqueous extract of *H. sabdariffa* showed equal inhibition ability against *E. coli* and *S. aureus (*40 mm) which was highest than *Str. mutans* (28 mm) and *P. aeruginosa* (27 mm). Alcoholic extract exhibited higher inhibition ability against *E. coli* (47 mm) than against *S. aureus* (20 mm), *Str. mutans* (30 mm) and *P. aeruginosa* (17 mm) (Al-Hashimi, 2006). Aqueous-methanolic extract of *H. sabdariffa* L. calyces have been found to exhibit antibacterial activities against *S. aureus, Bacillus stearothemophilus, Micrococcus luteus, Serratia mascences, Clostridium sporogenes, E. coli, K. pneumonae, B. cereus* and *Pseudomonas fluorescence* (Olaleye, 2007). The another study, the strongest antibacterial activity was shown by the methanol-HCl (99:1) extract 30%

with an average GI of 2.714 cm against both of Staphylococcus aureus and Streptococcus pyogenes(Dien, 2008). *Hibiscus sabdariffa* extract exhibited higher activity against *Salmonella* (10mm) than against *Shigella* and *Enterobacter* (9mm) (Nwaiwu et al., 2012). However, further studies about the isolation of active compounds and the absence of toxicity of plant extracts are necessary to propose these plants as alternative approaches to resistance management.

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Table1: Antimicrobial susceptibility and MIC of extract against Ecoli

Bacterial Cod	MIC for Extract plant(mg/ml)	Antibiotic-resistant
1	5	A_1, A_2, A_3, A_4
2	5	A_1, A_3
3	10	A_2, A_3, A_4
4	10	A_1, A_2, A_3, A_4
5	10	A_1, A_2, A_3, A_4
6	10	-
7	10	-
8	10	A_4
9	10	A_1, A_4
10	20	A_1, A_4
11	10	A_4
12	20	A_1, A_2, A_3, A_4
13	10	A_3, A_4
14	10	A_1, A_4
15	10	A_4
16	10	-
17	5	A_1, A_2, A_4
18	10	A_4
19	5	-
20	5	A_1, A_2
21	5	A_1, A_2, A_4
22	5	A_1, A_2, A_4
23	10	A_1, A_2, A_3, A_4
24	10	-
25	10	A_1
26	10	A_1, A_2, A_4
27	10	A_1, A_2, A_4
28	10	A_1, A_2
29	10	-
30	10	-

A_1=Erythromycin, A_2=Cefixime, A_3=Ceftazidime, A_4=Tetracyclin

Table 2: Antimicrobial susceptibility, MIC extract plant for *S.aureus*.

Bacterial Cod	MIC for Extract plant(mg/ml)	Resistance pattern
1	5	A_1, A_2, A_3, A_4, A_5, A_6,
2	20	A_1, A_2, A_4, A_5, A_6,
3	1.25	A_1, A_2, A_4, A_5, A_6,
4	10	A_4, A_6
5	10	A_4, A_6
6	5	A_6
7	5	-
8	1.25	-
9	1.25	A_6
10	5	A_2, A_4, A_5, A_6,
11	5	A_1, A_2, A_4, A_5, A_6,
12	20	A_4, A_5, A_6,

A_1= cefixime , A_2= trimethoprim-sulfamethoxazol, ,A_3= Vancomycin, A_4= Ceftazidime A_5= penicillin, A_6= Oxacillin .