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The study of antibacterial activity of *Hibiscus sabdariffa* flowers extracts

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الخلاصة:

تمت دراسة تأثير المستخلص المائي والمستخلص الكحولي لنبات الكركديه *Hibiscus sabdariffa* بطريقة الانتشار بالحفر وعند التراكيز التالية mg/ml (500, 250, 125, 62.5) ضد البكتريا المرضية التالية:

Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella. pneumoniae, Enterococcus spp., Acinetobacter spp., Pseudomonas aeruginosa, Shigella dysenteriae, Proteus vulgaris

أظهرت النتائج فاعلية المستخلص الكحولي والمائي ضد البكتريا المختبرة باستثناء *E. coli* لم تظهر أي تحسس للمستخلص المائي للكركديه وعند التراكيز المستخدمة.

Abstract:

The aqueous and methanol extracts obtain from flowers *Hibiscus sabdariffa* have been investigated for their antibacterial activity. Antibacterial activity was determined by using agar well diffusion method at concentrations of (500, 250, 125, 62.5) mg/ml, against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Enterococcus spp., Acinetobacter spp., Pseudomonas aeruginosa, Shigella dysenteriae, Proteus vulgaris.*
Hibiscus sabdariffa extracts demonstrated antibacterial effects of methanol and aqueous extracts against bacteria used in this study at different levels. Only *E. coli* was not susceptible to aqueous extract at all concentrations were used.

Introduction:

Multiple drug resistance is world wide growing problem; therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents [1]. Many workers have presented antimicrobial activities of plant extract, and they noticed that plant extracts were inhibited the growth of bacteria, fungi [2, 3] and parasites [4].
Hibiscus sabdariffa (family malvaceae), is a common local drink popularly and is associated with traditional medicine and is reported to be used as a treatment for several diseases such as hypertension [5]. Flowers contain gossypetin, anthocyanin and glucosidehibiscin, which have diuretic, choler tic effects,

decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis. The literature supporting it is using as antibacterial [6], antioxidant [7], hypercholesterolemia [8], hypoglycemic [9], cardio protective [10] and in treatment of cough, cancer and abscesses [11]. The aim of this study is to investigate antibacterial activity of aqueous and methanol extract of *Hibiscus sabdariffa* against pathogenic bacteria.

Materials and Methods:

Hibiscus subdariffa flowers: The flowers were collected from herbal drugs market.

Bacterial strains:

All bacterial strains used in the study are clinical strains, were obtained from medical city hospital laboratories at October–December 2009. They are *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Enterococcus spp, Acinetobacter spp., Pseudomonas aeruginosa, Shigella dysentria* and *Proteus vulgaris.*

Preparation of extracts:

Methanol extract:

To prepare methanol extract 30 gm powder of *Hibiscus sabdariffa* flowers were extracted with 300 ml (80%) methanol by using soxlet apparatus for 10 hrs [12]. The extract was filtered by using whatman no.1 filter paper and the solvent was evaporated using rotary evaporator.

Aqueous extract:

500 mg of *Hibiscus sabdariffa* powder was treated with 8 ml boiling water, the extraction time was 30 min [13]. The extract was filtered and concentrated. Methanol and aqueous extracts were left at 4c until assessments of their antibacterial activity.

Antibacterial Assay:

The agar well diffusion method [14] was used to determine antibacterial activity of *Hibiscus sabdariffa* extracts at different concentrations of (500, 250, 125, 62.5) mg/ml. Inoculums containing 10 bacterial cells/ ml was spread on Mueller-Hinton agar plates. Six millimeters diameter wells punched into the agar and filled with 0.1 ml of different concentrations of each extract. Bacteria were incubated at 37c for 24hrs. At the end of the period, the antibacterial activity was evaluated by measuring the inhibition zone diameter observed. Studies were performed in triplicate. On each plate antibiotic disc of streptomycin 10 mg/disc Oxide was applied for comparing.

Results:

The antibacterial activities of methanol and aqueous extracts of *Hibiscus sabdariffa* flowers at concentration of (500, 250, 125, 62.5) mg/ml against pathogenic bacteria were investigated and results are presented in (Table-1) and (Table -2).

All bacteria strains demonstrated degree of sensitivity to extracts tested, (Table -1) showed results obtained in our study that methanol extract posses a potential antibacterial activity against all tested bacteria, the most susceptible bacteria was *S. Aureus, K. Pneumonia, Ps. aeruginosa, Enterococcus spp., S. typhi* for all concentrations were used and at different levels, they showed a zone of clearance ranging 7-25 mm.

Acinetobacter spp. and *E. coli* were sensitive to (250–500) mg/ml, they showed inhibition zone ranging 13–18 mm. While the least susceptible bacteria were *Shigella dysentriae*. And *Proteus vulgaris* at concentration of 500 mg/ml, they showed inhibition zone 15 mm, 10 mm respectively.

(Table -2) showed the antibacterial activity of aqueous extract. *S. aureus, Acinetobacter spp*, and *Ps. aeruginosa* were sensitive to all concentrations; they showed a zone of clearance ranging 8 - 17 mm.

Enterococcus spp., S. typhi, K. pneumonia were sensitive to (500, 250, 125) mg/ml, their inhibition zones were 7 -22 mm.

Shigella dysenteriae, Proteus vulgaris were the least sensitive at concentration of 500 mg/ml. There was inhibition zone for *E. coli* at all concentrations of aqueous extract.

Streptomycin (10 mg/disc) showed inhibition zone ranging 10–25 mm against tested bacteria.

Our results suggest that *Hibiscus sabdariffa* extracts have potential use as antibacterial.

Tested bacteria	**Inhibition zone (mm) concentrations (mg/ml)**			
	500	**250**	**125**	**62.5**
Staphylococcus aureus	20	18	10	7
Klebsiella pneumonia	18	17	15	8
Acinetobacter spp.	18	17	-	-
Escherichia coli	15	13	-	-
Shigella dysenteriae	15	-	-	-
Pseudomonas aeruginosa	20	15	12	10
Enterococcus spp.	25	23	15	13
Salmonella typhi	15	14	12	10
Proteus vulgaris	10	-	-	-

Table-1: Antibacterial activity of methanol extracts of *Hibiscus sabdariffa* flowers.

Tested bacteria	Inhibition zone (mm) concentrations (mg/ml)			
	500	250	125	62.5
Staphylococcus aureus	17	12	10	8
Klebsiella pneumonia	13	8	7	-
Acinetobacter spp.	14	13	12	8
Escherichia coli	-	-	-	-
Shigella dysenteriae	14	-	-	-
Pseudomonas aeruginosa	15	14	11	10
Enterococcus spp.	22	10	10	-
Salmonella typhi	13	7	7	-
Proteus vulgaris	9	-	-	-

Table-2: Antibacterial activity of aqueous extract of *Hibiscus sabdariffa* flowers.

Discussion:

The intensive use of antibiotics has of ten resulted in the development of resistant strains [15].

Because of this drug resistance, is should continuous investigation new, safe and effective antimicrobials alternative against [16].

The antimicrobial activities of various plants have been reported by many researches [17, 18, 19], Some researches reported there is a relationship between chemical structures of the most compound in the tested extracts and antimicrobial activity. As the plant produce secondary metabolites in order to protect them selves from microorganisms, herbivores and insects, therefore antimicrobial effects is some how expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide ranging of microorganisms. Antibacterial effects of *Hibiscus sabdariffa* extracts against tested bacteria suggest that they have remarkable therapeutic action in the treatment of skin disease, gastrointestinal infection and diarrhea. The antibacterial activity of methanol and aqueous extracts of *Hibiscus sabdariffa* is due to the presence of the secondary metabolites presents in the extracts, such as Flavonoid, glycosides [20], tannins, saponins, alkaloids, and anta quinines [21]. Glycosides can get hydrolyzed to release phenol which is toxic to microbial

pathogens [22], or the activity may due to impairment of variety of enzyme systems including those involved in energy production and structural components synthesis [23]. According to the antibacterial assay done for screening purpose, all tested bacteria were susceptible to the extracts, except *E. coli* was resist to aqueous extract.

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