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EVALUATION THE ANTIBACTERIAL EFFECT OF ALKALOIDS AND PHENOLS EXTRACTION FROM *HIBISCUS SABDARIFFA* AGAINST UTI INFECTION IN *VITRO*

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ABSTRACT

This study was aimed to evaluate alkaloids and phenols antibacterial activities which were extracted from *Hibiscus sabdariffa* against uti pathogenic organisms which isolated from the patients suffering from urinary tract infection such as *E. coli*, *K. pneumoniae*, *P.aeruginosa* and *S. aureus*, the alkaloids and phenols exhibited strong antibacterial activity against *S.aureus* also gave a noticeable antibacterial activity against *P.aeruginosa* and *E. coli* while the alkaloids and phenols extracts showed no antibacterial activity against *k.pneumonia*e. The results showed best antibacterial activity of alkaloids and phenols of *Hibiscus sabdariffa* in *vitro* against uti pathogenic organisms.

KEYWORDS: *E. coli*, *K. pneumoniae*, *P.aeruginosa* and *S. aureus.*

INTRODUCTION

In recent years antibiotic resistance has become a significant human health issue. Presently, multiple antimicrobial resistant bacteria are considered as a great global threat to public health because they are resistant to many different antibiotics (Levy, 1998). Urinary tract infections (UTIs) are one of the most common infectious diseases (Kolawale et al., 2009; Delanghe et al., 2000; Hryniewicz et al., 2000) Urinary tract infection (UTI) has become a more grievous problem today, due to multidrug resistance of infecting Gram-positive and Gram-negative bacteria . (Mishra etal.,2015). Each year about 150 million people are diagnosed with UTI which classified as uncomplicated or complicated in Worldwide,(Stamm and Norrby.,2001). According to an estimate of World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Hassan etal., 2009) Roselle (*Hibiscus sabdariffa*) have been found to have antioxidant, antihypertensive, anticancer, antidiarrheal, antistress, antispasmodic, anticlastrogenic, hypolipidaemic, hepatoprotective and diuretic activities (Wang etal.,2000)(Joshi and Parle., 2006).

MATERIALS AND METHODS

Hibiscus sabdariffa calyces were purchased from local market in AL-kut city after cleaned and dried, the plant were powdered using a grinder ,then stored in clean conditions until use.

Extraction

1- Crude alkaloids

Extraction of crude Alkaloids from *Hibiscus sabdariffa* were carried out according to Harborne (1984) by using 100 g of plant powder was homogenized with 350 ml of 4:1 ethanol: D.W., in electrical blender for 5 minutes, then filtered with muslin cloth and Buchner funnel under reduced pressure by using Whatman No.1 filter papers. The supernatant was evaporated at 45 °C in a rotary evaporator, drops of 2% sulphuric acid were added until the pH became (1-2), then extracted with chloroform three times in separating funnel . The solution was separated into two layers, the lower layer was chloroform, was neglected. The upper layer was the aqueous layer to be used. Addition of drops of concentrated ammonium hydroxide was added to this layer until pH became (9-10), then extracted was again with chloroform: methanol mixture in ratio of 3:1 twice, and one time with chloroform alone. Two layers appeared, the lower layer was evaporated at 40°C for (1-2) hours. The upper layer, the aqueous layer, was evaporated at 40°C for (1-2) hours, and kept in refrigerator.

2- Crude phenols

Extractions of crude phenols from *Hibiscus sabdariffa* were carried out according to Ribereau-Gayon (1972) and Harborne (1984) by using 200 g of plant powder was divided into two equal parts, 300 ml of 1% hydrochloric acid was added to one part, and 300 ml of D.W. was added to the other, the two mixture parts were homogenized in electrical blender for 5 minutes then were put in boiled water bath for 30-40 minutes. The two mixtures were filtered through muslin cloth and centrifuge 3000 rpm for 10 minutes. The supernatants were mixed with equal quantity of n-propanol and sodium chloride until the solution was separated into two layers. The lower layer was extracted with Ethyl acetate

and then evaporated at 40°C for (1-2) hours .The upper layer was evaporated at 40°C for (1-2) hours .the dry material of both layers were mixed and dissolved in 5ml of 96% ethanol, then transferred to oven and then kept in refrigerator until use.

3- Crude terpenoid

Extractions of crude terpenoid from *Hibiscus sabdariffa* were carried out according to Harborne (1984). A quantity of 10 g of plant powder was mixed with 200 ml of chloroform and placed in soxhlet apparatus for 8 hours for extraction. The solution was evaporated at 40°C for (1-2) hours, and kept in a refrigerator until use.

Preparation of different concentrations of plant extracts

Alkaloid and phenol extracts were prepared by dissolving certain weight of each plant extract according the concentration in ethylene glycol. Different concentrations (25, 50 and 75) mg / ml of plant extracts were prepared.

Micro-organisms Used

The micro-organisms (*Escherichia coli, Klebsiella pneumoniae, P.aeruginosa* and *Staphylococcus aureus*) used in the study were collected from the patients suffering from urinary tract infection in Al-kut city ,all bacteria were identified using biochemical tests and confirmed diagnosis by Api test and Api Staph test kits.

Antimicrobial sensitivity test

Antimicrobial sensitivity test was done on Mueller Hinton agar (Baurer etal., 1966) technique to determine susceptibility of gram negative and gram positive UTIs bacteria, the antibiotic disks consisted of ampicilin, Ciprofloxacin, nitofurantoin, gentamicin, ceftriaxone, tetracycline, erythromycin, vancomycin and trimethoprim-sulfamethoxazole.

Antibacterial activity of plant extracts

The antibacterial activity of plant extracts were tested by agar well diffusion method (Perez et al., 1990) nutrient agar plates seeded with 0.1 ml of an overnight broth suspension of bacteria containing 1.5×10^8 CFU/ml of organisms then wells were cut into the pour plates with 6mm sterile cork borer. Using a micropipette100 µl of the plant extracts were added to the wells in the plates then the plates were incubated at 37°c for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) against the bacteria.

Statistical analysis

Data were analyzed by using the general linear model procedure of the Statistical Analysis System Institute, Inc. (SAS Institute, 2001). A $p<0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Different phytochemical compounds were extracted from calyces' of *Hibiscus sabdariffa* such as terpens , alkaloids and phenols and the results showed that phenols are the majority compound with 16 % followed by the alkaloids with 8% and finally terpens with 5 % as shown in (Figure,1)

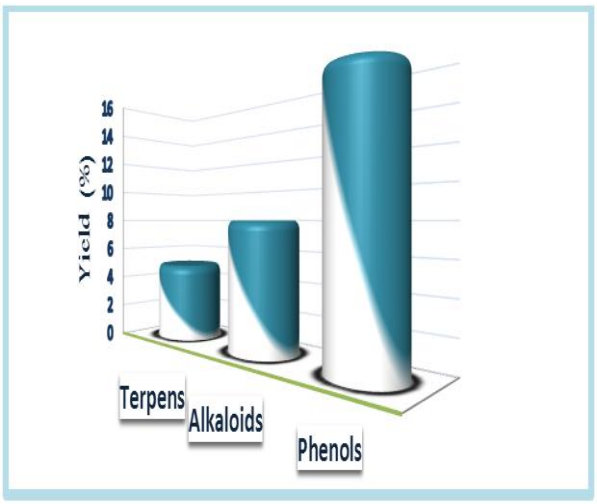

Figure: (1) Phytochemical compounds yielded from calyces extract of *Hibiscus sabdariffa*

Uti gram negative and gram positive bacteria were detected by biochemical tests as shown in (Table, 1). (Amin etal., 2009) revealed that the gram-negative bacilli were responsible for UTI infections and *E. coli* was the most common isolated bacteria from urinary tract infections.Study of (Ilusanyaetal.,2012)(Akortha and Ibadin ., 2008) showed that *Staphylococcus aureus* and *Escherichia coli* are the major pathogen responsible for UTIs.

Table (1): Biochemical tests of gram negative and gram positive bacteria of UTI.

Tests	*E. coli*	*k.pneumoniae*	*P.aeruginosa*	*S. aureus*
Gram stain	-	-	-	+
catalase	+	+	+	+
Oxidase	-	-	+	-
Indole	+	-	-	+
Urease	-	+	+	+
Methyl red	+	-	+	-
Voges- proskauer	-	+	-	-

(+) indicates positive (-) indicates negative

The antibiotic susceptibility testing for gram negative bacteria and gram positive as shown in (Table ,2,3) In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections (Manikandan et al., 2011).

The producer of ß lactamases is the most common mechanism of resistance in gram negative bacteria which inactivate ß lactam antibiotics. Extended Spectrum ß lactamases (ESBL) and Amp C ß-lactamases are most commonly produced (Varsha Gupta, 2007) and the major problem for clinical therapeutics was caused by ESBL-producing organisms (Subitha and Sornajeyanthi., 2015).

Table (2): Antibiotic susceptibility test against gram negative bacteria.

Bacteria	Antibiotics					
	AM	CIP	FM	SXT	GM	CRO
E. coli	R	S	R	R	R	R
K. pneumoniae	R	S	R	R	R	R
P.aeruginosa	S	S	R	R	R	S

R=Resistance, IS=Intermediated sensitive, S=Sensitive AM, Ampicillin ; CIP, Ciprofloxacin ; FM, Nitrofurantoin ; SXT, Sulfamethoxazole-trimethoprim ; GM, Gentamicin and CRO, Ceftriaxone.

Table (3): Antibiotic susceptibility test against gram positive bacterium.

Bacteria	Antibiotics						
	AM	GM	FM	E	V	TE	SXT
S. aureus	R	S	S	R	I	R	S

R=Resistance, I=Intermediated sensitive, S=Sensitive AM, Ampicillin; GM, Gentamicin; FM, Nitrofurantoin ;E, Erythromycin; V, Vancomycin; TE, Tetracycline and SXT, Sulfamethoxazole-trimethoprim. Alkaloids and phenols were used to determine antibacterial effect against gram negative and gram positive pathogenic organisms such as *E. coli*, *K. pneumoniae* , *P.aeruginosa* and *S. aureus* ,we don't use terpens extract because lower extraction yield.

Table (4): The antibacterial activity of the alkaloid and phenol extracts of *Hibiscus sabdariffa* on *E. coli*

Plant extract	Concentrations (mg/ml)		
	25	50	75
Alkaloid	C 13.33±0.33a	B 16.00±0.57a	A 19.00±0.57a
Phenol	B 13.00±0.57a	B 14.66±0.33a	A 17.66±0.33a

Table (5): The antibacterial activity of the alkaloid and phenol extracts of *Hibiscus sabdariffa* on *K. pneumoniae*

Plant extract	Concentrations (mg/ml)		
	25	50	75
Alkaloid	B 0	B 0	A 9.00±0.57a
Phenol	0	0	0b

Table (6): The antibacterial activity of the alkaloid and phenol extract of *Hibiscus sabdariffa* on *P.aeruginosa*

Plant extract	Concentrations (mg/ml)		
	25	50	75
Alkaloid	C 14.66±0.33a	B 18.33±0.33a	A 20.33±0.33a
Phenol	C 15.00±0.57a	B 16.66±0.33b	A 20.00±0.57a

Table (7): The antibacterial activity of the alkaloid and phenol extracts of *Hibiscus sabdariffa* on *S. aureus*

Plant extract	Concentrations (mg/ml)		
	25	50	75
Alkaloid	C 14.00±0.57b	B 19.33±0.66b	A 24.33±0.66b
Phenol	C 23.33±0.66a	B 28.66±0.66a	A 34.00±0.57a

*Values with different small letters are significant differences vertically at ($p < 0.05$).

*Values with different capital letters are significant differences horizontally at ($p < 0.05$).

The in *vitro* antibacterial activity of alkaloids and phenols extracts of *Hibiscus sabdariffa* against *E. coli* as shown in (Table,4) the maximum effect of alkaloids extracts was observed with (19.00±0.57mm) and phenols extracts with (17.66±0.33mm) at higher concentration 75 mg / ml.

Table 5 illustrate that the alkaloids and phenols extracts of *Hibiscus sabdariffa* showed no antibacterial activity against *K. pneumoniae*. *K. pneumoniae* was more resistance to alkaloids and phenols extract of *Hibiscus sabdariffa* because it was expressed capsule polysaccharides (Domenico etal.,1994) which act to decrease the uptake of antimicrobials (Campos etal.,2004). The effect of alkaloids and phenols extracts of *Hibiscus sabdariffa* against *P.aeruginosa* were equally inhibited with value (20.33±0.33mm) and (20.00±0.57mm) respectively at higher concentration 75 mg / ml (Table, 6)

Results showed that the phenols extract of *Hibiscus sabdariffa* was exhibited strong antibacterial activity against *S. aureus* which gave the maximum zone of inhibition (34.00±0.57mm) at higher concentration 75 mg / ml compared with alkaloids extract was recorded (24.33±0.66 mm) at same concentration 75 mg / ml, (Table,7).

Our results were found the alkaloids and phenols were more effective in inhibiting gram positive bacterium than gram negative bacteria. (Osei-Djarbeng etal.,2014) founded that calyx of *H. sabdariffa* has exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as the yeast-like fungi.

(Mutalib etal.,2015) revealed that the *H. sabdariffa* have antibacterial activity against various standard bacterial species. (Ewansiha., 2014)showed that the extract of *Hibiscus sabdariffa* exhibited antimicrobial activity with potential applications in pharmaceutical industry for controlling infections caused by the gram negative organisms. In conclusion, best antibacterial activity of alkaloids and phenols of *Hibiscus sabdariffa* in *vitro* against uti pathogenic organisms so further study should be needed to evaluate its activity in *vivo*.

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