ANTIMICROBIAL ACTIVITY OF FLAVONOID COMPOUND ISOLATED FROM *INULA GREAVEOLENS* L. PLANT ON SELECTED PATHOGENIC BACTERIA

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ABSTRACT

The antibacterial activity of the flavonoid compound (B3) isolated from *Inula greaveolens* plant by column – chromatography was determined against several of clinical microbial isolates including Gram positive: (*Streptococcus spp. Staphylococcus aureus*) *and* isolates of Gram negative *:* (*Klebsiella pneumonia, Pseudomonas aeroginosa* and *Esherichia coli*) by using three concentrations of the extract (125 mg /ml , 250mg /ml ,500 mg /ml).The results revealed that the falvonoid compound B3 has varying degrees of inhibition tested microorganisms.

The cytotoxic activity of the falvonoid compound (B3) was determined against the fresh human red blood celles with several concentrations of the extract and the results shown that flavonoid compound did not had toxicity against the human red blood cells.

INTRODUCTION

Medicinal plants have been used for the treatment of various human ailments since long. A revolution came in the medicinal world with the discovery of antibiotics, for treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, giving rise to multiresistant strains, which has become a global concern. Thus, there is a renewed interest in exploring natural resources for such compounds 1.

The need of the hour is to screen a number of new medicinal plants for promising biological activity and there *in vitro* propagation to conserve the biodiversity 2,3,4. Various plants have been documented in the development of novel drugs, by

evaluating their antimicrobial activity, studying active phytochemical constituents and bioactive compounds by various modern analytical techniques. It is believed that crude extract from plants are more effective than isolated components due to their synergistic effect. From the safety point of view, spices and medicinal herbs are mostly targeted to meet the therapeutic demands. Since then efficacy of many medicinal plants in the treatment of many diseases have been put to test in many laboratories 5.

Inula is a large genus of about 90 species of flowering plants in the family Asteraceae, native to Europe, Asia and Africa. The genus is thought by some to be paraphyletic, based on the study of the different phenolic compounds the various species have . *I. viscosa* contains some pharmacologically active compounds 6,7 including sesquiterpenes acids 8, azulenes, lactones, flavonoids, and essential oils 9 which are isolated and identified in its leaves. The aim of this study is to investigate cytotoxic and antibacterial effects of flavonoid compound isolated from aerial part of *I.* graveolens L..

MATERIALS AND METHODS

Plant material:

The *Inula graveolens* L. plant used in this study, was collected in august 2011 from the Abu-Al-Khaseeb region (Southern Basrah), Iraq. The plant was botanically authenticated and 3897 voucher specimens were deposited in the Herbarium of Basrah (Iraq, Basrah, College of Science, University of Basrah).

Figure (1) : *Inula greaveolens* L.

Chemicals:

All of the chemicals were purchased from Sigma- Aldrich Co. (St. Louis, MO, USA), and the solvents were obtained from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

Extract:

A quantity (100 g) of powdered plant was defating by extraction in a Soxhlet apparatus with 500 ml of n-Hexane , for 24 h. the solid residue mixed with 500 ml of 80 % methanol and steering on magnetic sterrier for 24 h , The methanolic extract was filtered and evaporated to half volume under reduced pressure in a rotary evaporator , after that precipitation of flavonoids by 1% lead acetate . the precipitate was collected and dissolved in mixture (30 ml Conc . HCL and 25 ml of Acetone), evaporated to dryness under reduced pressure in a rotary evaporator which afford solid powder , which was dissolved in distil water and mixed with same volume of ethyl acetate and extracted by separating flask 10 . Ethyl acetate extract was collected and evaporated to dryness under reduced pressure in a rotary evaporator to afford 4.47 g. of dry extract.

Preliminary phytochemicals analysis

A Preliminary phytochemicals study (colour reactions) on all extracts was performed using standard procedures in order to determine the presence of alkaloids (Dragendroff test), carbohydrates (Molisch test), glycosides (Benedict test), saponines (Stable foam test), steroids (Liebermann-Burchard test), flavonoids (Shinoda test) and terpenoids (Salkowsky test) 11 ,12 .

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was conducted on extracted flavonoid aliquots which were applied 1cm from the base of the TLC plate. Development of the chromatograms was done in a closed tank in which the atmosphere had been saturated with mixture of [sec- butanol – formic acid – water (7.7 - 1- 1.3)].

For flavonoids identification, plates were sprayed with $FeCl_3$-$K_3Fe(CN)_6$ (1:1) 13. The plates were also stained with Antimony chloride (10% in $CHCl_3$), Folin-Denis, Benedict reagent and lead acetate (basic, 25%) 10 .

Isolation of the components of flavonoids extract :

The component B3 (Spot with higher R_f = 0.835) of flavonoids extract was separated and purified by column chromatography technique. A glass column size (2.8×55 cm)

was plug down to the bottom with small glass wool, then packed with HCl-washed silica-gel (mesh 230-400 μm). The slurry was prepared by dissolving 125 g of silica-gel in (sec- butanol – formic acid – water (7.7 - 1- 1.3)) as eluent. The solid residue was then loaded to the top of the column and fractions of 5mL/min were collected and monitored by TLC. Fractions with the similar R_f were collected and dried at room temperature 14 **.**

Determination of Antibacterial Activity

Antimicrobial activity was evaluated by agar well diffution method of the flavonoid compound (B3) isolated from *Inula greaveolens* against strains of Gram positive *Streptococcus spp. Staphylococcus aureus and* isolates of Gram negative *Klebsiella pneumonia, Pseudomonas aeroginosa E.coli* by using three concentrations of the extract (125 mg /ml , 250 mg /ml and 500 mg /ml), which were tested using plates of Muller- Hinton agar . The antimicrobial activity was defined as the clear zone of growth inhibition 15.

Cytotoxicity test:

Cytotoxicity of flavonoid compound (B3) against human red blood corpuscles RBCs was tested according to Nair *et al*., 1989, in different concentrations ranging from (0.5 - 250) ppm. dissolved with DMSO. DMSO was used as a control sample 16

RESULTS AND DISCUSSION

Qualitative analysis flavonoid extract and flavonoid compound (B3)

The flavonoid extract was isolated in a good yield 4.47 g. from the dried plant . Table (1) indicate the preliminary phytochemicals analysis for flavonoid extract and flavonoid compound B3 of *Inula greaveolens L.* . The results revealed that there was no alkaloids, carbohydrate , glycosides, saponins , tannin ,steroid and terpinoid in the flavonoid extract. The same table indicated that the flavonoid extract contained only flavonoid compounds. TLC procedure was run for the compounds and the results were shown in table (2) and figure (2), the presence of three spots (B1,B2 and B3) of the flavonoid compounds with Rf: 0.126, 0.556 and 0.835 respectively., then subjected to the column to purified of flavonoid compound. Figure (3) shown the presence of one spot (B3) of the purified flavonoid compound by column with Rf equal to 0.835.

Table (1): Qualitative analysis for flavonoid extracts of Inula greaveolens L.

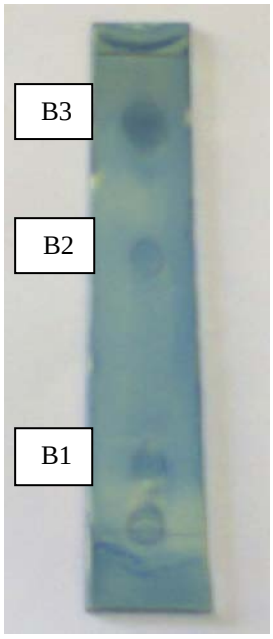
Chemical constituent	Remarks	
	Flavonoid extract	Flavonoid compound B3
Alkaloids	-	-
Carbohydrates	-	-
Glycosides	-	-
Steroids	-	-
Flavonoids	+	+
Tannins	-	-
Saponines	-	-
Terpenoids	-	-

+ Present, - Absence of the chemical constituent

Table (2): Thin layer chromatography for flavonoid extracts of *Inula greaveolens L.*

UV 366 nm		Lead acetate (basic, 25%)		Antimony chloride (10% in $CHCl_3$)		Folin-Denis reagent		Benedict reagent		$FeCl_3$-$K_3Fe(CN)_6$ (1:1)	
FE	FCB3	FE	FCB3	FE	FCB3	FE	FCB3	FE	FCB3	FE	FCB3
0.126 0.556 0.835	0.835	0.126 0.556 0.835	0.835	0.126 0.556 0.835	0.835	0.126 0.556 0.835	0.835	0.126 0.556 0.835	0.835	0.126 0.556 0.835	0.835
Conjugated test		Flavonoid test		Flavonoid test		Flavonoid test		Flavonoid test		Flavonoid test	

FE: flavonoid Extract and **FC B3:** flavonoid compound **B3**



Figure(2): Thin layer chromatography for flavonoid extract

Figure(3): Thin layer chromatography for purified flavonoid compound (B3)

Antibacterial Activity for flavonoid compound (B3)

The antibacterial activity of the flavonoid compound (B3) gave different mean zone diameter of inhibition on the bacterial isolates tested [Table (3) and Figure (4)]. The extract gave the mean zone diameter of inhibitions ranging from 21- 26.3 mm for *Staphylococcus aureus* 23.3- 36.3mm for *Escherichia coli* 19.6 – 24.3 for

Pseudomonas aeroginosa, while for *Klebsiella pneumonia* diameter of inhibition zone ranging from 17- 25.6 mm and for *Streptococcus spp.* at a range of 23.6 – 40 mm.).It has been postulated that cell membrane of gram negative bacteria contains many condensed fat layers compared with gram positive bacteria 17. The Chemicals and antibiotics or antiseptics face difficulty in penetrating these membranes and, therefore, their effectiveness is diminished, this may be justified due to the combination between hydroxyl group of the flavonoid extract and the phospholipids of the bacterial cell wall, which led to destracion of the cell membrane and then led to inhibition of the microbial growth and may change the cell protein nature (Denaturation) and increase the permeability of the cell membranes 18 , as many types of antibacterial compounds 19.

Table (3) : The biological activity of the flavonoid compound(B3)

Bacterial Isolates	Serial number	Inhibition zone (mm) for flavonoid(B3)		
		125 mg\ml.	250 mg\ml.	500 mg\ml.
Pseudomonas aeroginosa	2	19.6	21.6	24.3
Klebsiella pneumoniae	4	17	22.6	25.6
Staphylococcus aureus	6	21	24.6	26.3
E. coli	8	23.3	36	36.3
Streptococcus spp	9	23.6	37	40

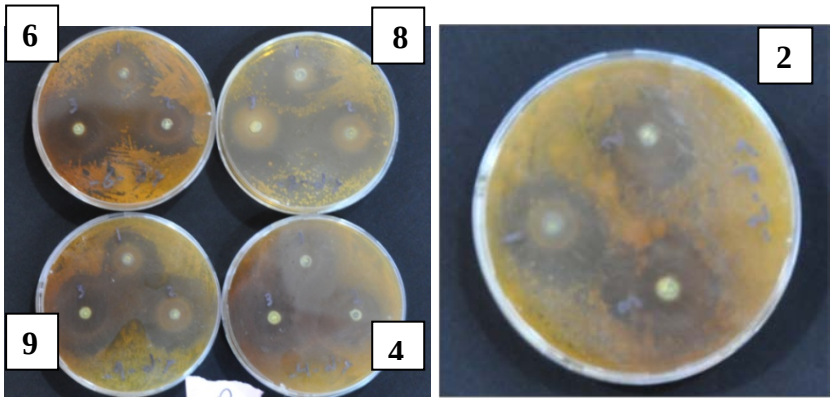


Figure (4) :The inhibition zone of flavonoid compound (B3) antibacterial activity against studied bacteria.

Cytotoxicity of flavonoid compound (B3)

The result in table (4) and figure (5) , showed the flavonoid compound (B3) which had no cytotoxicity against the human red blood cells within a concentration ranging from 0.5 - 250 ppm., by using DMSO solution as a control.

Table (4) : The cytotoxicity of flavonoid compound (B3)

Compound	*Concentration (ppm)*	*Toxicity against RBC*
DMSO	-	NT
Flavonoid B3	0.5	NT
	10	NT
	50	NT
	100	NT
	200	NT
	250	NT

NT: NOT TOXIC DMSO: DiMethyl Sulfa Oxide

Figure (5): The cytotoxicity test of flavonoid compound B3

Conclusion

Based on the results of the present study, it can be concluded that the flavonoid compound B3 isolated from *Inula greaveolens* L. possesses strong antibacterial , and had broad spectrum antimicrobial effect , and the flavonoid compound B3 had no cytotoxic effect against the human red blood cells .

الفعالية المضادة للميكروبات للمركب الفلافونيدي B3 المعزول من نبات الشواصر *nula graveolens* L. ضد انواع مختارة من البكتيريا المرضية

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الخلاصة

تضمنت الدراسة الحالية دراسة الفعالية المضادة للبكتريا للمركب الفلافونيدي B3 المعزول من نبات الشواصر *Inula graveolens* L. بتقنية كروموتوغرافيا العمود ضد انواع مختارة من العزلات السريرية المرضية ، وشملت العزلات الموجبة لصبغة كرام *Staphylococcus aureus* ، *Streptococcus spp*. والعزلات السالبة لصبغة كرام التي تشمل *Klebsiella pneumonia* , *Pseudomonas aeroginosa* , *E. coli* وباستخدام تراكيز مختلفة لهذا المركب (125 ملغرام /مل ، 250 ملغرام / مل ، 500 ملغرام / مل) . اظهرت نتائج الدراسة ان المركب الفلافونيدي B3 يملك درجات مختلفة من التثبيط لهذه العزلات .

درست السمية الخلوية للمركب الفلافونيدي B3 ضد دم الانسان وبتراكيز مختلفة ، واظهرت النتائج ان هذا المركب لا يملك اي سمية تجاه كريات الدم الحمراء .

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