Available online at: www.basra-science-journal.org

ISSN –1817 –2695

Antifungal and antibacterial activity of flavonoid extract from *Terminalia chebula* Retz. fruits

Sameerah Ahmed Zearah
Chemistry department , college science , Basra university
Email: asamera23@yahoo.com
Received 10-11-2013 , Accepted 2-2-2014

Abstract

The present study was designed to determine the antifungal , antibacterial and cytotoxicity activities of the flavonoid extracted from *Terminalia chebula* Retz. fruits . The flavonoid extract showed greater antifungal activity against *Aspergillus funigalus* than *Candida albicans* by using agar well diffusion method for all concentrations , It was found that these activities increased with the extract concentration increases .The Diameter of the inhibition zone of *Aspergillus funigalus* (30 mm) while *Candida albicans* (21 mm) at a concentration of is (150 mg/ml) . The results showed that the antibacterial activity of flavonoid extract against gram positive (*Staphylococcus aureus,* and *Streptococcus spp*.) bacteria greater than gram negative (*Klebsiella pneumonia, Pseudomonas aeruginosa and, E. coli).* The extract showed high activity against *Streptococcus spp.* with inhibition zone (36 mm) than that of (*Klebsiella pneumonia, Pseudomonas aeruginosa and, E. coli)* (30 mm) at a concentration of 500 mg/ml , and minimum inhibitory concentration of flavonoid extract was (25 mg/ml) for *Staphylococcus aureus and* (100 mg/ml) for *E. coli* .The results of cytotoxicity against the human red blood cells proved that the flavonoid extract had no cytotoxic effect at all the concentrations tested (ranging between 0.5-250 ppm) , by using DMSO solution as a control.

Key words :*Terminelia chebula* , flavanoid , antifungal , antibacterial

1. Introduction

Medicinal plants are part and parcel of human society from the dawn of civilization to combat diseases and have been considered valuable and cheap source of unique phytoconstituents which are used extensively in the development of drugs against various diseases [1-3]. Several hundred genera of plants were used medicinally mainly as herbal preparations in the indigenous systems of medicine in different countries which have stood the test of time, and therefore, modern medicines have not been able to replace most of them. The World Health Organization reported that 80% of the world population relies chiefly on traditional medicines involving

the use of plant extracts or their active constituents [4]. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more in countries like India than in the rest of the world. In the last few decades, the field of herbal medicine is getting popularized in both developed and developing countries [5]. This is because the herbal medicines are cheap, and have natural origin with higher safety margins and lesser or no side effects [6]. *Terminalia chebula* Retz. is a plant in the family Combretaceae known as in Thailand "Sa Maw Thai".According to its functions, the fruit of *T. chebula* has been extensively used in Thai traditional medicine for laxative, carminative, astringent, expectorant, and tonic effects [7] . The fruit contains high phenolic content, especially hydrolysable tannins, anthraquinone, flavonol, carbohydrates, glucose and sorbitol [8] . Therefore, the objective of this study was to evaluate the antifungal , antibacterial and cytotoxicity of flavanoid extract from dried fruit of *T.Chebula* .

2. Material and methods

2.1. Scientific Taxonomic of plant

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Combretaceae
Genus: *Terminalia*
Species: ***T. chebula***
Binomial name ***Terminalia chebula* Retz.**

2.2. Plant material

The dried fruit of T. chebula was purchased from a local herbal market in Basra city , after that Milled using electric grinder and kept in a closed glass container while in use .

2.3. Flavonoid extract of *Terminalia chebula* Retz. fruits .

Fifty g of dried fruits powder were extracted with 500 mL of 80% methanol by stirring at 25 °C, for 24 hours. The 80% methanol extract was filtrated , and 25 mL of 1% lead acetate was added to the filtrate. The mixture was filtrated by Buchner funnel, and the precipitate was treated with 25 mL acetone and 30 mL concentrated HCl. The mixture was then filterate and the filtered was evaporated to yield 2.45 g, which was dissolved in 25 mL DDH_2O, and extracted by ethyl acetate (3×50mL). The combined ethyl acetate fractions were concentrated by freeze drier to yield 1.35g [9] .

2.4. Preliminary phytochemicals analysis

A Preliminary phytochemicals study (colour reactions) on flavonoid extract 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), phenolic compound ($FeCl_3$) , flavonoids (Shinoda test) and terpenoids (Salkowsky test) [10-11] .

2.5. Antifungal activity test

The agar well diffusion method [12] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains (*Aspergillus funigalus* and *Candida albicans*) separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with100 µl of flavonoid extracts at various concentration (75 , 100 , 125 and 150 mg/ml) . All the plates were incubated at 27 °C for 24 hours to obtain maximum growth in the culture media. The diameters of inhibition zone were observed measured for each

concentration to estimate the degree of antifungal activity .

2.6. Antibacterial activity test

Antibacterial activity was determined by using Mueller Hinton agar [13]. Petri plates were prepared by pouring 10 ml of Mueller Hinton agar and allowed to solidify. Plates were dried and 100 µl of inoculum's suspension (approximately 10^6 cfu/ml of bacteria) was poured and uniformly. 100 µl of flavonoid extract at various concentration (125, 250, and 500 mg/ml) and used to fill hole bored by cork borer in the inoculated agar. All the plates were incubated at 37 °C for 24 hours to obtain maximum growth in the culture media. For each concentration, the diameter of inhibition zone of growth minus the diameter of the disc was measured to estimate the degree of antibacterial activity.

2.7. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for the flavonoid extract was estimated according to the method of Collee [14] , against different types of clinical isolates of bacteria *Staphylococcus aureus* and *E. coli*, with different concentrations ranging from (25 , 50 , and 100 mg/ml) of the flavonoid.

2.8. Cytotoxicity test

The cytotoxicity activity of flavonoid extract was determined against human red blood cells using a suspension of 1ml of the blood suspended in 20 ml of normal saline . Different concentrations of the extract were prepared separately dissolved in DMSO solution, then 100 µl of each concentration was added to 2ml of blood. Turbidity of the mixture was examined after 10, 30 and 60 minutes before the blood cells were haemolysed completely [15] .

3. Result and discussion

3.1. Qualitative analysis of flavonoid extract

Table (1) Shows that preliminary phytochemicals analysis for flavonoid extract of *Terminalia chebula* Retz. Fruits. The result shows that the flavonoid extract contains only phenolic compounds represented by flavonoid compounds and absence of any other compounds .

Table (1): Qualitative analysis of flavonoid extract *Terminalia chebula* Retz. fruits

Chemical constituent	Remarks of Flavonoid extract
Alkaloids	-
Carbohydrates	-
Glycosides	-
Steroids	-
Phenolic compounds	+
Flavonoids	+
Saponines	-
Terpenoids	-

3.2. Antifungal activity for flavonoid extract

Table (2) show esults of antifungal activity for flavonoid extract of the *Terminalia chebula* Retz. Fruits and figures (1) .The flavonoid extracts showed lowest activity against *Candida albicansfor* for all concentration tested , the diameter of inhibition zone *(8-21 mm)* . More activities of flavonoid extract

were recorded against *Aspergillus funigalus (10-30 mm)* diameter of inhibition zone , These results revealed that antifungal activity of the flavonoid extracts was enhanced by increasing the concentration of the extracts, in effect, the inhibitory activity of the extracts was concentration dependent. They added that the activity was attributed to the presence of phenolic compounds which can held a good promise as a natural fungicidal agent against common pathogens of crops [16] .

Therefore, such results are of a significant value that confirms the therapeutic potency of some plants used in traditional medicine. It should form a good basis for further phytochemical and pharmacological investigation [17]. Useful antimicrobial phytochemicals are: phenolics and polyphenols (such as simple phenols and phenolic acids, quinones, flavones, flavonoids, and flavonols. tannins, coumarins); terpenoids and essential oils; alkaloids; lectins and polypeptides; plus other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisims vary and depend on these compounds [18] . Their mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to the inactivation of the protein and loss of function. They have the ability to form complexes with extracellular and soluble proteins and to complex with bacterial cell walls and disrupt microbial membranes [19] , some have the ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors [20] .

Several studies on pharmacological activities from fruit of *T. chebula* indicated that it has several therapeutic activities in both *in vitro* and *in vivo* tests, for example, antimutagenic [21] , antidiabetic [22] ,antiproliferative [23] , antioxidant [24] , antibacterial [25] , antifungal [26] , and hepatoprotective activities [27] .

Table (2): Antifungial activity of flavonoid extract of the *Terminalia chebula* Retz. Fruits

Concentration mg/ml	Diameter of inhibition zone (mm)	
	Candida albicans	*Aspergillus funigalus*
75	8	10
100	13	15
125	17	26
150	21	30

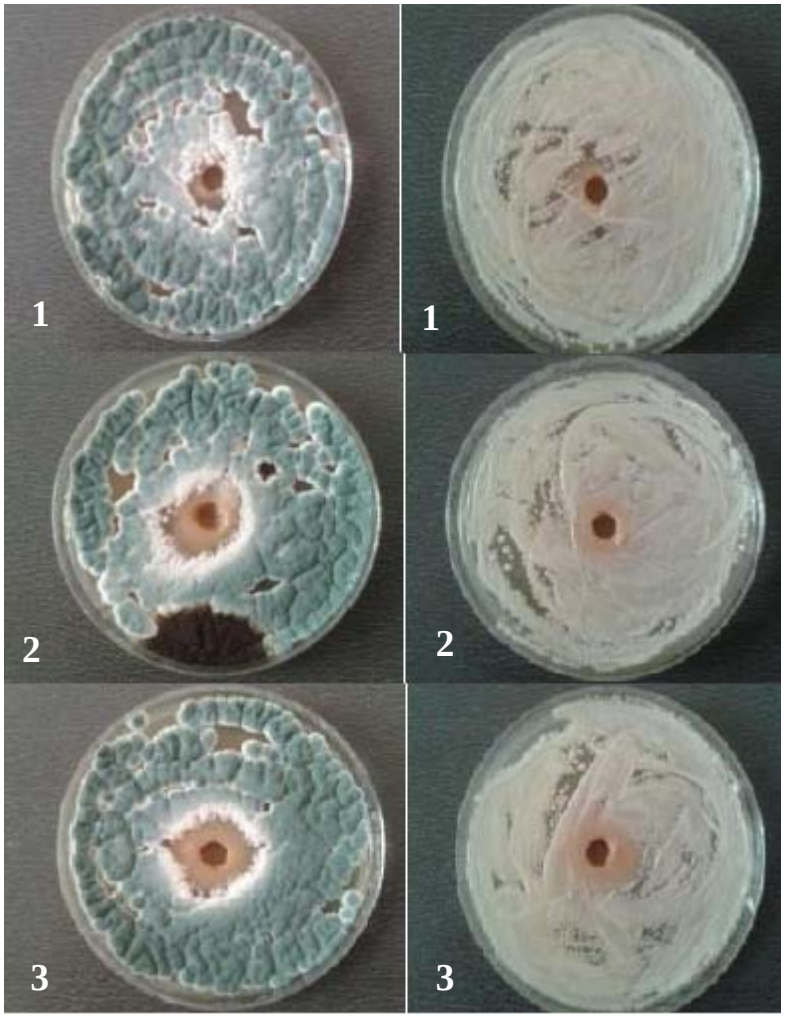

Figure (1): A typical agar plate showing the inhibition zones exhibited by flavonoid extract against *Candida albicans* and *Aspergillus funigalus*
1: (100 mg/ml) ; 2: (125 mg/ml) ; 3: (150 mg/ml)

3.3. Antibacterial Activity for flavonoid extract

The antibacterial activity of the flavonoid compound gave different mean zone of inhibition on the bacterial isolates tested (Table (3) and Figure (2)). The extract gave the mean zone diameter of inhibitions ranging in gram positive isolate from 22- 33 mm for *Staphylococcus aureus* and *Streptococcus spp.* at a range of 20 – 30 mm.) . The mean zone of inhibitions ranging in gram negative isolate from 18-30 mm for *Pseudomonas aeroginosa* , 20-30 mm for *Klebsiella pneumonia* , while for *Escherichia coli* the inhibition ranging from 22-40 mm . It has been postulated that cell membrane of gram negative bacteria contains many be condensed fat layers compared with gram positive bacteria [28] . 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 lead to destroy the cell membrane and then to inhibit the microbial growth and may change the cell protein nature (Denaturation) and increase the permeability of the cell membranes [29] ,as many types of antibacterial compounds [30] .

Table (3): Antibacterial activity of flavonoid extract of the *Terminalia chebula* Retz. Fruits

Bacteria	Serial number	Diameter of inhibition zone (mm)		
		125 mg/mL	250 mg/mL	500 mg/mL
Streptococcus spp.	9	20	25	36
Staphylococcus aurens	6	22	38	35
Pseudomonas aeroginosa	2	18	25	30
Klebsiella pneumonia	4	20	25	30
E. coli	8	21	24	30

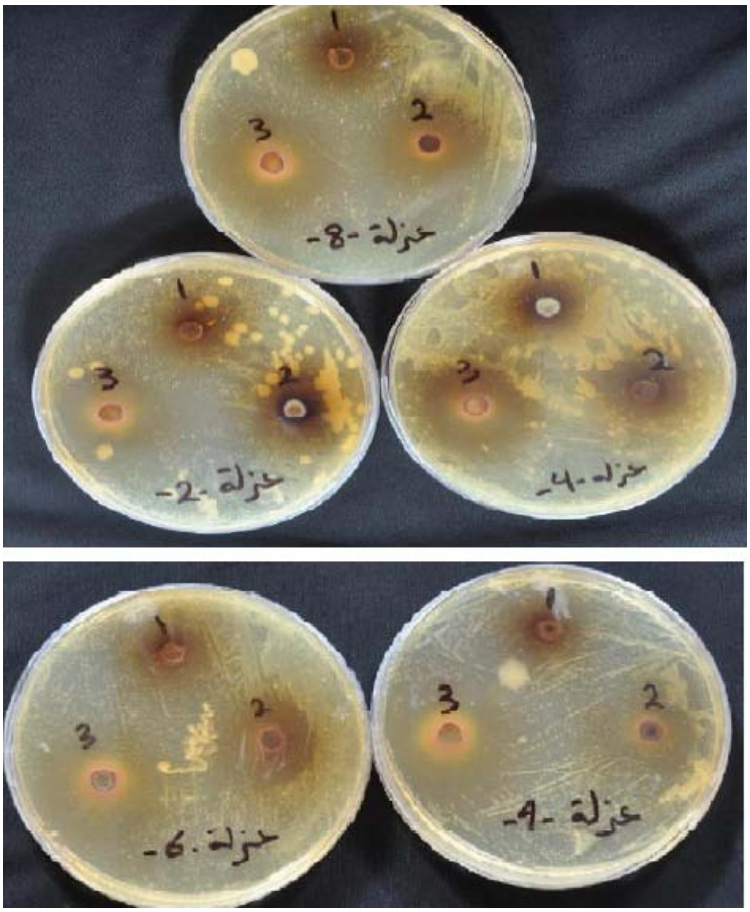

Figure (2): A typical agar plate showing the inhibition zones exhibited by flavonoid extract against all tested bacterial isolates

3.4. The minimum inhibitory concentration for flavonoid extract

Table (4) obtained from the analysis of Figures (3) , shows that the results of the MIC values for flavonoid extract against *Staphylococcus aureus* (25 mg/ml) , while it was (100 mg/ml) against *E. coli*.

Table (4): The minimal inhibition concentration of flavonoid extract

Bacterial strains	MIC (mg/ml)		
	25	50	100
S. aureus	15	15	15
E. coli	-	-	10

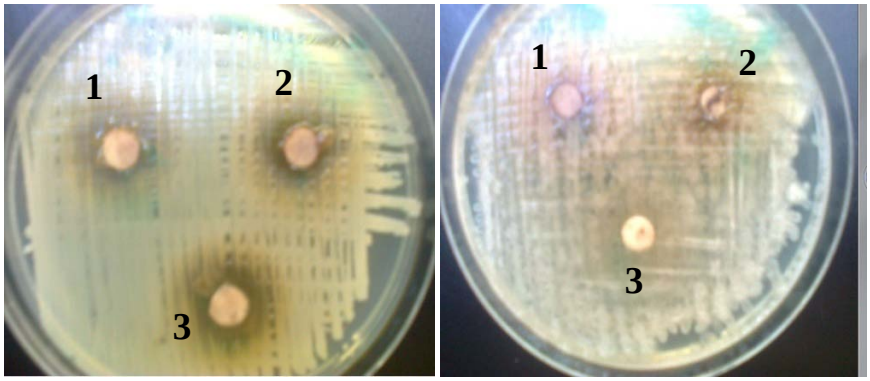

Figure (3): The minimum inhibition concentration of flavonoid extract against *Staphylococcus aureus and E.coli bacteria*
1: (25 mg/ml) ; 2: (50 mg/ml) ; 3: (100 mg/ml)

3.5. Cytotoxicity of flavonoid extract

The result in table (4) and figure(3) , show that the flavonoid extract had no cytotoxicity against the human red blood cells at a concentration ranging from 0.5 - 250 ppm., by using DMSO solution as a control.

Table (5) : The cytotoxicity of flavonoid extract

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

NT: NOT TOXIC DMSO: DiMethyl Sulfa Oxide

Figure (4): The cytotoxicity of flavonoid extract

4. Conclusion

Based on the results of the present study, it can be concluded that the flavonoid extract of the *Terminalia chebula* Retz. fruits possesses strong (antifungal and antibacterial) , and had broad spectrum antimicrobial effect , and the flavonoid extract had no cytotoxic effect against the human red blood cells .

References

[1] Sarasa D, Sridhar S, Prabakaran E." Effect of an antidiabetic extract of *Trigonella foenum-graecum* on normal and alloxan induced diabetic mice". Int J Pharmacy Pharmaceutical Sci 2012;4(1):63-65.

[2] Agarwal M, Sharma P, Kushwaha S." Antifertility efficacy of 50% ethanolic extract of *Calendula officinalis* in male rats" Int J Pharmacy Pharmaceutical Sci 2011;3(5):192-196.

[3] Gupta PC. *Withania coagulans* Dunal-An Overview. Int J Pharmaceutical Sci Review Research 2012;12(2):68-71.

[4] World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram 1993;28:13-14.

[5] Naik GH, Priyadarsini KI, Naik DB, Gangabhagirathi R, Mohan H." Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radioprotector" Phytomedicine 2004;11:530-38.

[6] Ayyanara M, Ignacimuthu S." Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats in India" J Ethnopharmacol 2011;134:851-64.

[7] Department of Medical Sciences, Ministry of Public Health. 2000. Thai Herbal Pharmacopoeia Volume II.Nonthaburi, Thailand. p 71-89.

[8] Juang LJ, Sheu SJ, Lin TC." Determination of hydrolyzable tannins in the fruit of *Terminalia chebula* by high-performance liquid chromatography and capillary electrophoresis" J Sep Sci , 2004. 27(9):718-724.

[9] Harborne JB " Phytochemical methods " *Champman and Hill*, New York, USA second edition. 1984.

[10] Harbone JB and Baxter HH "Phytochemical Dictionary: A hand book of bioactive compound from plants" *Taylor and Francis; Washington*. 1993 :237-240.

[11] Khandelwal KR "Practical Pharmacognosy" *Nirali Prakashan : Pun*. 16th edition, 2005 : 149-153.

[12] C. Perez, C. Anesini, *J. Ethnopharmacol*., **1993**, 44, 41-46.

[13] Nahayan SS "Antibacterial potential of crude methanolic extract of *Leonotis nepetifolia (L.) R. Br*" *International Research Journal ofPhamacy*. 2012, 3(2) : 277-278.

[14] J. Collee, A. Fraser, B. Marmion, and A. Bimon. Practical Medical microbiology, 14th, p.978,1996.

[15] Nair GM, Putnam RA, Mishra KS, Mulks HM, Taft HW, Keller EJ and Miller RJ "faeriefungin a new broad spectrum antibiotic from *Streptomyces griseus var. autotrophicus* " *J. Natur. Prod*. 1989, 52: 797-809.

[16] Fardos M. Bokhari ″ Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia″ *Mycopath* ,2009 , 7(1): 51-57 **.**

[17] Prasad CS, Ravindra Shukla, Ashok Kumar NK, Dubey Prasad CS, Shukla R, Kumar S, Dubey NK,. "In vitro and in vivo antifungal activity of essential oils of Cymbopogon martini and Chenopodium ambrosioides and their synergism against dermatophytes" *Mycoses*, 2009, 3: DOI: 10.1111/j.1439-0507.2008.01676.

[18] Aly MM, Bafiel S ."Screening for antimicrobial activity of some medicinal plants in Saudi Arabia" *World conference on medical and aromatic*, 2008.

[19] Ali AA,. "Studies on some medicinal plants as a source of antifungal substances in North Africa" 1999 , M.Sc. Thesis, Inst. of African Res. and Studies, Cairo Univ.

[20] Cowan MM. "Plant products as antimicrobial agents" *Clin. Microbiol. Rev.* 1999, **12**: 564-582.

[21] Kaur S, Grover IS, Singh M, Kaur S." Antimutagenesity of hydrolyzable tannins from *Terminalia chebula* in *Salmonella typhimerium*" Mutat Res , 1998,419(1-3):169-179.

[22] Sabu MC, Kuttan R." Antidiabetic activity of medicinal plants and its relationship with their antioxidant properties" J Ethnopharmacol , 2002, 81:155-160.

[23] Saleem A, Husheem M, Harkonen P, Pihlaja K. "Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz. Fruit" J Ethnopharmacol , 2002, 81(3):327-336.

[24] Lee HS, Won NH, Kim KH, Lee H, Jun W, Lee KW."Antioxidant effects of aqueous extract of *Terminalia chebula in vivo* and *in vitro*" Biol Pharm Bull , 2005,28(9):1639-1644.

[25] Kim HG, Cho JH, Jeong EY, Lim JH, Lee SH, Lee HS." Growth inhibitory activity of active component from *Terminalia chebula* fruits against intestinal bacteria" J Food Prot. , 2006, 69(9):2205-2209.

[26] Bonjar GH." Inhibition of Clotrimazole – resistant Candida albicans by plants used in Iranian folkloric medicine" Fitoterapia , 2004, 75:74-76.

[27] Tasduq SS, Singh AK, Salti NK, Gupta DK, Suri K." *Terminalia chebula* fruits prevents liver toxicity caused by sub-chronic administration of refampicin, isoniazid and pyrazinamide in combination" Hum Exp Toxicology , 2006, 25(3):111-118.

[28] Joreme , J. J.Berry and T.Staley, Microbiology Dynamic and diversity, 1997 p.880-881.

[29] Feeny J .phytochemistry,8, p.2116-2129,1998.

[30]Jeffrey Buyten,B.Francis and W.Matthew Ryan, Antibiotics,.viscosa. Phytochemistry. 2005, 30(7):2445–2446.

الفعالية المضادة للفطريات والبكتريا للمستخلص الفلافونيدي لثمار نبات الهليلج الاسود *Terminalia chebula* Retz.

سميره احمد زياره

قسم الكيمياء – كلية العلوم – جامعة البصرة

الخـــلاصة

صممت الدراسة الحالية لقياس الفعالية المضادة للفطريات , البكتريا والسمية الخلوية للمستخلص الفلافونيدي لثمار نبات الهليلج الاسود *Terminalia chebula* Retz. . اظهر المستخلص الفلافونيدي فعالية عالية ضد الفطريات *Aspergillus funigalus* , *Candida albicans* , وان فعاليته ضد الفطر *Aspergillus funigalus* اكثر من الفطر *Candida albicans* ولكل التراكيز , وان هذه الفعالية تزداد بازدياد تركيز المستخلص , واظهرت النتائج ان قطر منطقة التثبيط للفطر *Aspergillus funigalus* (30 ملم) بينما قطر منطقة التثبيط للفطر *Candida albicans* (21 ملم) عند التركيز (150 ملغم / مل) . اظهرتنتائج الفعالية المضادة للبكتريا ان المستخلص الفلافونيدي ذو فعالية عالية ضد البكتريا الموجبة لصبغة كرام and *(Staphylococcus aureus* *Streptococcus aureus)* اكثر من فعاليته ضد البكيريا السالبة لصبغة كرام ,*Klebsiella pneumonia)* , اعلى فعالية سجلت للمستخلص الفلافونيدي ضد البكتريا *Pseudomonas aeruginosa and, E. coli)* وبقطر تثبيط (36 ملم) , اما العزلات البكترييية السالبة فسجلت قطر تثبيط (30 ملم) عند التركيز (500 ملغم / مل) . قدر التركيز المثبط الادنى للمستخلص الفلافونيدي وسجل التركيز (25 ملغم/ مل) للبكتريا *Streptococcus aureus* والتركيز (100 ملغم/ مل) للبكتريا السالبة *E. coli* . نتائج السمية الخلوية ضد كريات الدم الحمراء للمستخلص الفلافونيدي اثبتت ان هذا المستخلص لايملك اي سمية تجاه كريات الدم الحمراء ولكافة التراكيز المستخدمة (0,5 – 250 ملغم/مل) , وباستخدام محلول DMSO كمحلول سيطرة .

الكلمات المفتاحية : هليلج الاسود , الفلافونيد , مضادات الفطريات , مضادات البكتريا