Comparison of Antimicrobial Activity of Both Seeds and Leaves Extract of Two Type of *Cucurbita pepo L*. (Iraqi&Chinese)

مقارنة الفعالية الضد ميكروبية لمستخلصات بذور و اوراق نوعين من نبات اليقطين (العراقي و الصيني) .

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Abstract:

The seeds and leaves ethanol extracts have been prepared for Iraqi and Chinese *cucurbita pepo* (pumpkin) and the antimicrobial activity of these extracts were evaluated , all alcohol extracts were showed antimicrobial activity against *Staphylococcus aureus* , *Pseudomonas aerugenosa* , *Escherichia coli* and *Candida albicans*. Gram positive bacteria were more sensitive than other microorganisms, all microbial isolates had antibiotic multi resistance. The results were not showed differences between seeds and leaves extracts activity for each pumpkin types while Iraqi pumpkin extracts were more active than Chinese pumpkin extracts. The minimal inhibitory concentrations of Iraqi pumpkin seeds and leaves extracts and Chinese pumpkin seeds extract was 20 mg/ml against *S.aureus* while the highest concentrations were 70 mg /ml and 60mg /ml at Chinese leaves and seeds extracts against *P.aerugenosa* and *C.albicans* respectively .

Key Word: *cucurbita pepo* , ethanol extract, antimicrobial activity.

الخلاصة.

حضرت مستخلصات الكحول الاثيلي لبذور و اوراق اليقطين العراقي و الصيني وقدرت الفعالية البايولوجية لهذه المستخلصات وأظهرت جميع هذه المستخلصات فعالية ضد *S.aureus* و*p. aerugenosa* و*E.coli* و*C.albicans*, البكتريا الموجبة لصبغة كرام(*S.aureus*) كانت هي الأكثر تأثر من بقية الأحياء المجهرية قيد البحث كما وجد أن جميعها متعددة المقاومة للمضادات الحيوية .أظهرت النتائج عدم وجود اختلافات في فعالية المستخلصات الكحولية للبذور والاوراق لكل نوع من اليقطين بينما كانت مستخلصات اليقطين العراقي أكثر فعالية بيولوجية من مستخلصات اليقطين الصيني(البذور و الاوراق) 0 كان التركيز المثبط الادنى لمستخلصات بذور واوراق اليقطين العراقي ومستخلص البذور الصيني هو 20mg\ml ضد بكتريا*S.aureus* في حين كان اعلى تراكيز هي70mg\ml و60mg\ml لمستخلصات الاوراق اليقطين الصيني التي كانت تراكيز مثبطة دنيا لبكتيريا *P.aerugenosa* و*C.albicans* على التوالي .

Introduction

English name: pumpkin
Latin name: *cucurbita pepo*
Family : cucurbitaceae

Cucurbita pepo has broad uses in traditional medicine for many cases, it hasn't side effects. Fruits, seeds and leaves are the most used parts, some of its active compounds are still unknown. The seeds contain material killed tap warm when used as dough evict it out with feces, it is save way without side effects while synthesis drags have serious damage[1].

Fruit's juice of pumpkin has analgesic effect for head ache, also it is considered abdominal laxative. When we make bandage of seeds on the head useful in treatment tumors in brain and its peal use for treatment tumors of ear, eyes and gout[2].

Seeds extract called pepo's oil, active compounds are vitamin A and B ,acids like lucine , tirozine ,pirozine and pepirozine. Seed extract is non toxic used as analgesic, laxative and diuretic. Found in markets as tablets contain 300 mg of seeds oil called pepon (Egyptian product) used to treat prostatic hypertrophy in old men, seed use in treatment insomnia, urinary tract infection and evict tap warm [3].

Antimicrobial activity of pumpkin was investigated against *S. aureus* , *Bacillus subtillus* , *E. coli* and *P. aerugenosa* by preparing three types of extracts for all parts of plant (water, chloroform and alcohol extracts), an alcohol extracts were more active than other extracts and *S. aureus* was more affects than other bacteria therefore pumpkin has been used in protection and treatment against microorganisms especially bacteria. Pumpkin has expeller effect to insects like house fly and it has been evictor effect to tap warm from intestine [4].

Previous pharmacological tests have shown that it possess antibacterial and antiviral activity. It is treated colds, alleviate aches also have anti- inflammatory and analgesic effects, Pumpkin treat benign prostatic hypertrophy (BPH)[5]. Seeds have been used as supportive treatment in functional disorder of bladder [6].

Aim of this study; was investigate the antimicrobial activity of pumpkin, compare between activity of seeds and leaves extracts for each types of pumpkin and between two types of pumpkin on the other hand.

Materials and methods

plant samples

Two parts of pumpkin seeds and leaves are choice also, two types of plants Iraqi and Chinese.

A. seeds

pumpkin's seeds were brought from market then washed, dried at room temperature and avoided exposure to sunlight to prevent the loss of active components [7] and grinded it to get powder.

B. leaves

Some of seeds were grown in house's garden were taken the leaves from whale plant and washed, dried and grinded to get powder.

seeds and leaves were used as powder to preparing plant extracts.

plant extraction

Ten grams of plants powder were put in the thimbles then put it in soxholate extractor for (24 hr) used (300 ml) of ethanol 95%. The plant's extract evaporated by rotary evaporator under low pressure at temperate (50-60 ^{0}C)[8], the residue was put in Petri dish and dried completely at room temperature. This process have been repeated many times to obtain enough amount of four extracts (Iraqi seeds extract, Iraqi leaves extract, Chinese seeds extract , Chinese leaves extract).

Bacterial samples.

Three isolates of bacteria were used in this research , two of them are gram negative bacteria (G-ve) one was (G+ ve) and one type of yeast. All of these microorganisms were pathogenic isolates table(1)

Table(1) The microorganisms used & source of isolates

Microbial isolation	Source	Gram reaction
Staphylococcus aureus	Burn swab	G+ ve
Esherichia coli	Vaginal swab	G- ve
Pseudomonas aerugenosa	Surgical wound swab	G- ve
Candida albicans	Respiratory tract	Yeast

Antibiotic susceptibility

There are many types of standard antibiotic were tested, by using disk diffusion method [9],

Mueller Hinton agar plates were inoculated with (100 µl) of microbial suspension (containing $10^{\wedge 6}$ CFU/ml) was prepared from suspend many of identical microbial colonies in sterile distilled water the turbidity of this suspension was adjusted to standard density of $10^{\wedge 6}$ McFarland by using spectrophotometer [10], after that antibiotic discs were placed on the surface of solid culture media .These plates were incubated at (37^0C) for (18-24hr) then the diameter of inhibition zones surrounding the discs is measured and these results compared with standard tables to know the susceptibility of tested microbial isolates. the experiment was repeated four times for each microorganisms.

Antimicrobial activity of extractions.

Three concentrations were prepared for each four extracts by dissolving (3gm) of leaves and seeds extracts in (10ml) of di-methyl sulphoxide (DMSO) to preparing stock solutions (300mg\ml) then other concentration were prepared (200 and 100mg\ml).

Antimicrobial activity was carried out by a modification of Agar diffusion method described by [11], Mueller Hinton agar which had been inoculated separately with (100 µl) of same microbial suspensions and allowed to stay at(37^0C) for (3hr), using a sterile cork borer, three well were made (6mm diameter) on the assay plates and these were filed with (100 µl) from three concentrations (300, 200 and 100 mg\ml) of plant extract, these plates were putted in refrigerator overnight to allowed for extracts diffuse throw culture media [12], then incubated at (37^0C) for (24hr) in incubator. the results were recorded by measuring diameter of inhibition zones, these steps repeated for each ethanol extract.

Minimum inhibitory concentration (MICs)

Agar dilution method was used for determining MICs of four extract of pumpkin on microorganisms [13], nine intermediate concentrations were prepared (900 ,800 ,700 , 600, 500,400,300, 200,100) mg\ml then (1ml) from appropriate concentrations were added to(9ml) of molten media(Muller Hinton agar) in water bath at (50 ^{0}C) after autoclaved it. the agar and extract were mixed thoroughly to get seeded concentrations (90,80,70,60,50,40,30 ,20,10) mg\ml for each one of four alcohol extracts. These media poured into nine Petri dishes and the tenth plate use as control without extract, then inoculated separately with microbial suspensions adjusted to tub No. 0.5 of McFarland on the surface of solid media for each type of microorganisms, the testing plates were left for drying at room temperature then incubated at(37 ^{0}C) after (24 hr) records the results, the minimum inhibitory concentration was regarded as the lowest concentration of extract that completely inhibits growth of the microbial isolates

Results & Discussion

Results showed that all tested microbial isolates have multi resistance because they were resistant for many antibiotics used [14] table (2). The mechanisms of resistance can generally be considered as intrinsic or acquired, Intrinsic resistance is natural property of an organism and usually manifests itself as an impaired uptake of chemically unrelated drugs so that fewer molecules can reach their target site, Acquired resistance is usually demonstrated by mutation at a normally sensitive target site [15] . Also variation in antibiotics activity against microorganism could be due to the bacterial strain, patient being under antibiotic treatment and variation in the nature of bacterial envelope which affects permeability [16].

Although the clinical isolates have multiple resistance against Amoxicillin (AX), Cloxacillin(CX), Rifampin(RA), Cloramphenicol(C), Erythromycin(E) and Clindamycin(DA) but the ethanol extracts for all *Cucuribita pepo* uses parts were active against all of them (*S.aureus* , *E.coil* , *P. aerugenosa* and *C .albicans*) that prove is have antimicrobial activity and these extracts are board spectrum antimicrobial agents .

These activity due to alcohol capacity to extract of active compounds, it is polar solvent and most of Plant active compounds are polar aromatic compounds there for it extract all these compounds (tannins , phenols, flavonoids , terpins and alkaloids)[17,18] There are many studies affirm the activity of an ethanol extract for many medicinal plants [19,20,21 ,22].

Analysis of statistic (ANOVA test) showed significant differences ($p < 0.05$) between types of microorganism , *S .aureus* was more sensitive than other tested microorganism to all four types of alcoholic extracts this results agreement with [4] he founded an alcohol extract pumpkin was active against G+ ve bacteria more than other types of bacteria, also similar observation were made in other studies ,the general expectation that a much greater of extract are active against G+ ve bacteria [18&20] this may scribe to differences in the nature of the cell wall components of G+ ve and G- ve bacteria so these organisms differ in the organization of the structure outside the plasma membrane[23].

Also we noticed significant differences between concentrations ($p < 0.01$) and the relationship was direct between antimicrobial activity and concentration so, the concentration of 100 mg\ml of the ethanol extract had less effect while the more effect concentration was 300mg\ml.

T-test showed there were no significant differences ($p < 0.01$) between activity of seeds or leaves extracts of Iraqi and Chinese pumpkin so, the seed and leaves extracts for two types of pumpkin possess the same antimicrobial activity against tested microorganisms, this results may be due to the nature of active compounds of plants[24].

There were high significant differences ($p< 0.01$) between Iraqi and Chinese pumpkin wherever the Iraqi pumpkin extracts more active than Chinese pumpkin extracts (seeds and leaves),tables (3,4,5,6) & figure(1), major modes of action of plant active compounds include interaction with DNA, inhibition of protein synthesis, alkylation and oxidant stress[25].

In MICs test we noticed that the lowest concentrations have antimicrobial activity was 20 mg\ml of Iraqi seeds and leaves extracts and Chinese seeds extracts against *S .aureus*, while the highest concentrations of Chinese seeds and leaves extracts considered MICs for *P . aerugenosa* and *C . albicans* were 60 mg\ml and 70 mg\ml respectively ,tables (7,8).

The differences of MICs values depending on microbial strains [26,27], also the different component diffusing at different rates [19] may have been responsible for the varying of MICs values obtained in our assay against micro-organisms.

Table(2) antibiotic susceptibility for tested antibiotic
R : resistance , S : sensitive , _ : not tested

organisms / Antibiotic, Concentration	*S. aureus*	*E. coli*	*P. aerugerosa*	*C.albicans*
Amoxicillin (AX 25)	8 (R)	5 (R)	0 (R)	_
Cloxacillin (CX 1)	5 (R)	5 (R)	0 (R)	_
Rifampin (RA 5)	20 (R)	14 (R)	0 (R)	_
Erythromycin (E 5)	13 (R)	34 (S)	0 (R)	_
Kanamycin (k30)	22 (S)	18 (S)	14 (R)	_
Tetracycline (TE30)	34 (S)	30 (S)	0 (R)	_
Cloramphenicol (C30)	16 (R)	5 (R)	0 (R)	_
Clindamycin (DA2)	20 (20)	16 (R)	0 (R)	_
Piperacillin (PRL100)	–	_	18 (S)	_
Amphotericim (AMP15)	–	–	–	S
Clotrimazole (C10)	–	–	–	R
Fluconazole (F15)	–	–	–	R
Ketoconazole (K12)	–	–	–	S
Maconazole (M20)	–	–	–	R

Table (3)Diameter of inhibition zones of Iraqi pumpkin seeds extract

Extract / Organism	Iraqi seeds extract concentrations			Average
	100 mg / ml	200 mg / ml	300 mg / ml	
S. anrens	16 mm	20 mm	24 mm	20 mm
E. coli	9 mm	12 mm	15 mm	12 mm
P. aerngerosa	12 mm	14 mm	17 mm	14.3 mm
C.albicans	10 mm	15 mm	18 mm	14.3 mm

Table (4) Diameter of inhibition zones of Iraqi pumpkin leaves extract

Extract / Organism	Iraqi leaves extract concentrations			Average
	100 mg / ml	200 mg / ml	300 mg / ml	
S. anrens	12 mm	18 mm	22 mm	17.3 mm
E. coli	8 mm	12 mm	14 mm	11.3 mm
P. aerngerosa	12 mm	13 mm	16 mm	13.3 mm
C.albicans	14 mm	16 mm	18 mm	16 mm

Table (5) Diameter of inhibition zones of Chinese pumpkin seeds extract

Extract / Organism	Chinese seeds extract concentrations			Average
	100 mg / ml	200 mg / ml	300 mg / ml	
S. anrens	10 mm	16 mm	22 mm	16 mm
E. coli	9 mm	10 mm	12 mm	10.3 mm
P. aerngerosa	10 mm	12 mm	16 mm	12.6 mm
C.albicans	10 mm	14 mm	18 mm	14 mm

Table (6) Diameter of inhibition zones of Chinese pumpkin leaves extract

Extract / Organism	Chinese leaves extract concentrations			Average
	100 mg / ml	200 mg / ml	300 mg / ml	
S. anrens	10 mm	16 mm	20 mm	15.3 mm
E. coli	8 mm	10 mm	13 mm	10.3 mm
P. aerngerosa	10 mm	14 mm	15 mm	13 mm
C.albicans	12 mm	14 mm	16 mm	14 mm

Table (7): Minimum inhibition concentration values of seeds and leaves extract of Iraqi pumpkin

Extracts / Organism	Iraqi Seeds extract concentrations	Iraqi leaves extract concentrations
S.aurens	20 mg/ml	20 mg/ml
E.coli	40 mg/ml	50 mg/ml
P.aerugenosa	50 mg/ml	60 mg/ml
C.albicans	30 mg/ml	40 mg/ml

Table (8): Minimum inhibition concentration value of seeds and leaves extract of Chinese pumpkin

Extracts / Organism	Chinese Seeds extract concentrations	Chinese leaves extract concentrations
S.aurens	20mg/ml	30mg/ml
E.coli	40mg/m	60mg/ml
P.aerugenosa	60mg/ml	70mg/ml
C.albicans	60mg/ml	70mg/ml

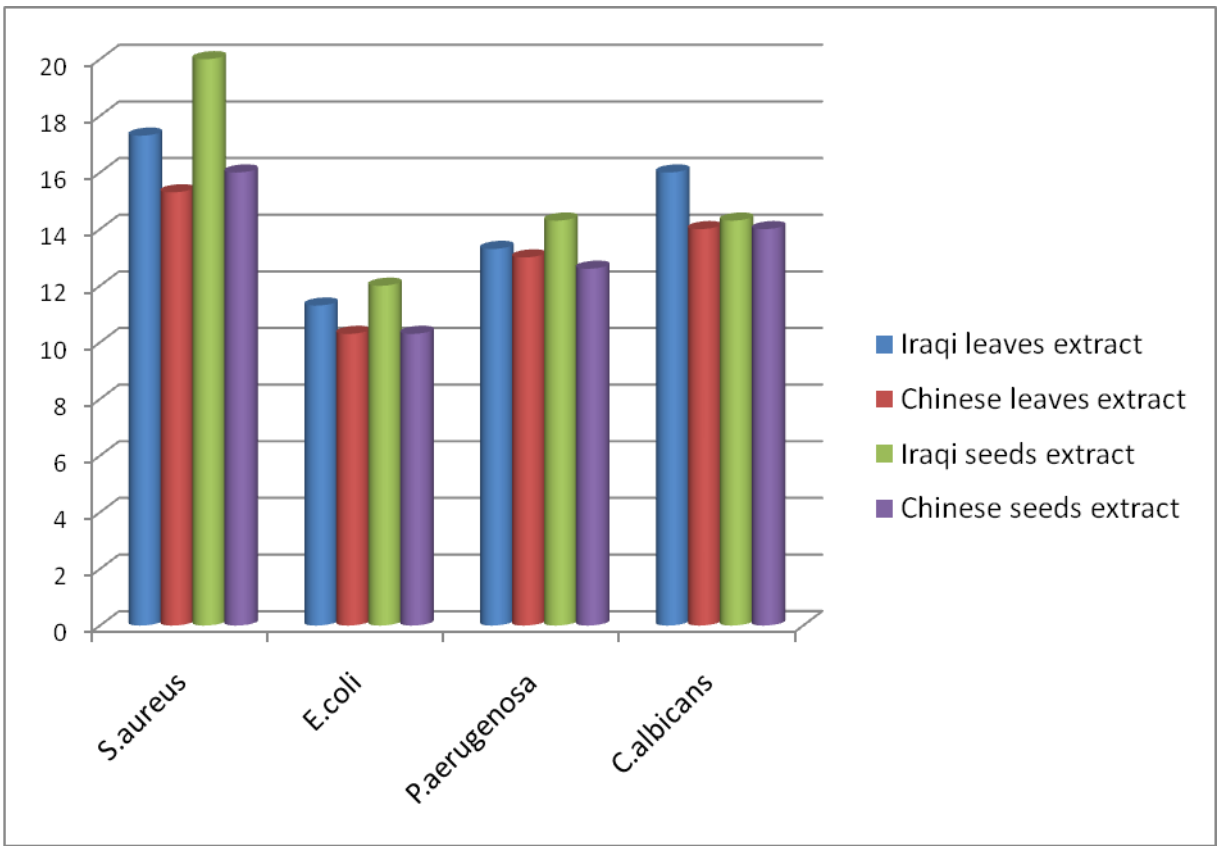

Figure 1: The graphic representation of the average of inhibition zones [mm] of *cucurbita pepo*

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