In vitro, Study of The Effect of Four Plant Aqueous Extracts on The Growth of Some Candida Species recovered from the stool samples of infants.

Dr. Intesar.M.Juma/ Assistant prof. foundation of technical education /college of medical and health technology.

Mr. Ahmed Ali Mhawesh/ Assist. Lecturer /kufa university ,nursing college.

الخلاصة: شملت هذه الدراسة (32) عينة خروج جمعت من أطفال رضع يعانون من إسهال ، كل العينات شخصت على أنها تحوي إصابات فطرية بواسطة العمليات التشخيصية التأكيدية والروتينية ، بعد العزل الأولي لأنواع المبيضات أظهرت النتائج أن 12 (37.5%) من العزلات كانت Candida albicans ، 11 (34.4%) من العزلات كانت Candida tropicalis و3 (9.4%) لكل من العزلات: Candida globrata, Candida cruzei andCandida parapsilosis. تم اختيار (5) عزلات عشوائياً من كل من Candida albicans و Candida tropicalis لدراسة حساسية تلك العزلات إلى اربع مستخلصات مائية نباتية وهذه المستخلصات كانت: (Pimpinella anisum leaves)اوراق الينسون)، Matrecaria chamomilla) flowers , ازهار البابنج) Camellia sinensis leaves) اوراق الشاي الاخضر)(Citrus aurantifolia fruits peels قشور زهرة النومي بصرة) وتم دراسة التركيز المثبط الأدنى والتركيز القاتل الفطري الأدنى باستخدام طريقة التخفيف في وسط ال (Sabouraud Dextrose Broth) أظهرت النتائج أن التركيز المثبط الأدنى للمستخلص المائي لكل من أوراق الينسون كان (6.25) مايكروغرام / مل والتركيز القاتل الفطري الأدنى لهما كان(12.5) مايكرغرام / مل .، و أن التركيز المثبط الأدنى للمستخلص المائي لكل من زهور البابونج كان (12.5) مايكروغرام / مل والتركيز القاتل الفطري الأدنى لهما كان (25) مايكروغرام / مل، بينما وجد أن المستخلصات المائية لباقي النباتات وهي(أوراق الشاي الأخضر وقشور زهرة الليمون (النومي بصرة)) بأنها ليس لها أو لها القليل جداً من التأثير ضد الفطري على جميع العزلات ، لذا ليس لها تركيز مثبط أدنى ولا تركيز قاتل فطري أدنى. استخدمت طريقة ثانية لاختبار الفعالية ضد الفطرية لنفس المستخلصات المائية النباتية الاربع بواسطة طريقة الانتشار عبر الحفر، وأظهرت النتائج أن أقطار مناطق التثبيط تزداد بزيادة تركيز المستخلص وكانت هذه الطريقة هي الأفضل في التعبير عن النتائج في اختبارات الحساسية.أظهرت النتائج أن المستخلصات المائية لكل من(أوراق الينسون وزهور البابونج) أنتجت منطقة تثبيط للمبيضات البيضاء (Candida albicans) في كل من التراكيز التالية (25، 50 و 100 مايكرو غرام /مل)، بينما باقي المستخلصات المائية النباتية قيد الدراسة أظهرت بأنه ليس لها أو لها منطقة تثبيط قليلة جدا عند التراكيز التالية (25، 50 و 100 مايكرو غرام /مل).عن مقارنة المستخلصات المائية النباتية مع كلا المضادين الفطريين القياسيين وهي الامفوتريسين ب والكيتوكونازول قيد البحث، وجدنا بان المستخلصات المائية لكل من أوراق الينسون، زهور البابونج لها تأثير ضد فطري أكثر من باقي المستخلصات المائية النباتية قيد الدراسة عند التركيز(100) مايكرو غرام / مل لكلاهما ، وكذلك وجد أن المستخلصات المائية لكل من زهور البابونج وتليها أوراق الينسون لها تأثير ضد فطري أكثر من باقي المستخلصات المائية النباتية قيد الدراسة عند التركيز (25) مايكرو غرام / مل لكلاهما .عند المقارنة مع الامفوتريسين ب اكتشف أن المستخلص المائي لزهور البابونج لهما تأثير ضد فطري أكثر من باقي المستخلصات المائية النباتية قيد الدراسة عند التركيز (50) مايكرو غرام / مل .

Abstract:

A total of (32) stool samples of infants, complaining from diarrhea were included in this study. All of them diagnosed as fungal infections by making a routine and confirmative diagnostic processes, after primary isolation of Candida species, the results reveal that : 12 (37.5%) of isolates were Candida albicans, 11 (34.4%) of isolates were Candida tropicalis and 3 (9.4%) of each isolate of Candida globrata, Candida cruzei and Candida parapsilosis. Five isolates from each one of C. albicans_ and C. tropicalis were chosen randomly for studying of sensitivity of these isolates to four plant's aqueous extracts, the extracts which were (Pimpinella anisum leaves, Matrecaria chamomilla flowers, Camellia sinensis leaves and Citrus aurantifolia fruits peels), their Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) were studied by using the dilution method by Sabouraud Dextrose Broth .The results show the MIC of Pimpinella anisum (Leaves) aqueous extracts was (6.25) mcg / ml., and the MFC were

(12.5) mcg / ml., the MIC of Matrecaria chamomilla (Flowers) aqueous extracts was (12.5) mcg / ml., and the MFC were (25) mcg/ml , While the rest plants aqueous extracts of (Camellia sinensis (Leaves), Citrus aurantifolia (Fruit Peels)) were results have no or very little antifungal activities in all the isolates, so there were not MIC and MFC for it .A second method was used to test the antifungal activity of the same four plant's aqueous extracts by the agar well diffusion method, and the results showed that the diameters of inhibition zones were increased when the concentrations of extracts were increased .This method was the best in explanation of results of sensitivity tests. Results demonstrated that the aqueous extracts of P. anisum (Leaves) and M. chamomilla (Flowers) Produced inhibition zone against C. albicans at (25, 50 and100 mcg /ml) concentrations, While the other aqueous extracts of plant under study reveal a very little or no inhibition zones at (25, 50 and100 mcg /ml) concentrations. When comparison of the plant aqueous extracts with both standard antifungal drugs of Ketoconazole and Amphotericin B under study, I found that the aqueous extracts of P. anisum (Leaves) and M. chamomilla (Flowers) were more antifungal effective than other aqueous extracts of plants under research at (100 mcg /ml) concentration for both, and detected also that aqueous extracts of M.chamomilla (Flowers) followed by P.anisum (Leaves) were more antifungal effective than other aqueous extracts of plants under research at (25 mcg /ml) concentration for both. In comparison with Amphotericin B, I detected that the aqueous extracts of M. chamomilla (Flowers) was more antifungal effective than other aqueous extracts of plants under research at (50 mcg /ml) concentration for both. And in comparison with ketoconazole, I found that the aqueous extract of M. chamomilla (Flowers) was more antifungal effective than other aqueous extracts of plants under research at (50 mcg /ml) concentration for both.

Key words: Plant Aqueous Extract, Candida Species, Candida albicans

INTRODUCTION:

The last decade witnessed the sustained medical importance of opportunistic infections due to different Candida species mainly because of the worldwide increase in the number of immunocompromised patients including children, who are highly susceptible to opportunistic infections.(1) .Candidiasis is an increasingly common problem in hospitalized patients, with epidemiologic surveys revealing that Candida spp. are now the fourth most common pathogens isolated from the blood of hospitalized patients. Approximately (100) species of fungi pathogenic for humans, and Candida albicans is the most prevalent species in clinical disease. C.albicans has also been shown to be a cause of diarrhea. Candidiasis in neonates is a serious and relatively common cause of late onset sepsis associated with a high mortality. The recent reports that indicate non-albicans infections are on the rise, which often accounting for more than 50 percent of candidiasis found in the infected population (2). Drug resistance may vary by species .Although antifungal resistance in Candida albicans is less frequent than in other species, anincreasing number of resistance strains are emerging..Antifungal drug resistance is quickly becoming a major problem in the expanding population of immunocompromised persons . It has resulted in a drastic increase in the incidence of opportunistic and systemic fungal infections, so a new therapeutic strategies must be considered to reduce antifungal drugs resistance which might be done by using alternative herbal medicine as a natural source(3). The World Health Organization (WHO) estimates that 80 percent of the world's population presently uses herbal medicine for some aspect of primary health care. In fact, according to the World Health Organization, 25% of modern drugs which are approximately used in the United States have been derived from plants.Enormous advances have been made in medical care but many people are still using herbal or alternative remedies (4).

Because the Iraqi flora are rich in plants are un submitted to any previous study and the possibility of finding new antimicrobial agents is still widely ahead. And Because the interesting Candida species is an opportunistic pathogen and increases of antifungal drugs resistance in last years varies with different species and increase of researches on medical important plants but only few studies on these researches were done in Iraq, this study aimed at the following:

1- The isolation and identification of Candida species were from stool samples of infected infants.
2- The investigation of the antifungal activity of some aqueous crude extracts were of some plants and some antifungal drugs against Candida species.
3- In vitro, the determination of the ability of using these extracts in determining the lethal dose and inhibitory dose of fungal infections.

Materials And Methods: 1- Chemical Solutions And Their Preparations:

1.1-Normal saline,Dimethyl Sulphoxide And (Methanol 98 %),Gram's Stain Solution,Lacto Phenol Blue cotton Stain Solution,Differential Media And Their Preparations,Sabouraud Dextrose Agar (SDA),Corn Meal Agar (CMA),Sabouraud Dextrose Broth (SDB) or Sabouraud Liquid Medium (SLM) and API 20 C AUX Yeast Identification Medium :

1.2-Preparation Of Stock Solutions Of Antifungal Agents:

I have followed the guidelines of NCCLS for preparing the various concentrations of antifungal drugs. The active components in Amphotericin B obtained was 78% and ketoconazole was 70% .

The stock I solution of Amphotericin B (HiMedia, India) was prepared by using 50 mg of Amphotericin B dissolved in 500 ml of Dimethylsulfoxide (DMSO) , the working solution was done in two fold dilution to obtain concentrations ranging from 3.125 –100 mcg / ml ; DMSO was used to dissolve Amphotericin B following CLSI *Candida parapsilosis* guidelines .The stock II solution of Ketoconazole (Cadila Pharmaceuticals, India) stock solution was prepared by dissolving 20 mg of ketoconazole in 200 ml of 98% methanol according to manufacturer's instructions. Working solution was done in two fold dilution to obtain concentrations ranging from 3.125 –100 mcg / ml, When water was used to dissolve ketoconazole, it resulted in precipitation of the drug. Hence, 98% methanol was used to dissolve ketoconazole , the stock solutions are stable for (3) days in culture at 37 °C. or 2-8 °C for up to 1 month. protected from air and light. Sterilization is done by filtration of stock solutions .

1.3-Preparation Of Plant's Aqueous Extracts:

Four local plant's aqueous extracts were prepared; the basic materials of these extracts were from leaves, flowers, fruits peels and roots of natural plants. They were used as

antifungal agents. The plants were of (*Pimpinella anisum* leaves, , *Matrecaria chamomilla* flowers, , *Camellia sinensis* leaves, and *Citrus aurantifolia* fruits peels) which they were prepared as the following: The extraction method used in this study was a modification of Akinside & Olukoya, and Akinyemi, *i*n line with the traditional methods of preparation, shredded plant materials of the leaves of (*P. anisum* and *C. sinensis*) ,flower peels of *C. aurantifolia* and flowers of *M.chamomile*)) were put in steriled bottles containing distilled water and then plugged, they were oven-dried at temperature of 60°C for 6 days with manual agitation of the flask using a sterile glass rod after every 24 hrs. , they were subsequently grounded into fine powder in 25 ml of steriled distilled water, kept at 60°C for 3 hr. The resulting suspensions were filtered rapidly through four layers of gauge and then by a more delicate filter through Whatman No 1 filters paper., the filtrates were concentrated by evaporation to dryness at 60°C in the hot air oven for 24hrs., then weight 1gm from it and dissolved in 10 ml for getting 100 mcg /ml and the mixture was mixed by hot plate magnetic stirrer for 48 hours at 50-55 °c. .The mixture was put in centrifuge (5000 rpm) for 30 minutes, then made various concentrations from crude extracts (100, 50, 25, 12.5,6.25 and 3.125) mcg/ml (mcg = µg), for each one of plants in respectively in order to study the influence of these concentrations on different Candida spp. under research, the prepared plants extracts sterile by millipore filter, then stored in sterile airtight glass containers protected from sun light and refrigerated at 4°C prior to use for analysis .The antifungal activity of plants aqueous extracts was compared with standard commercial antifungals (as a single pharmacological dose of concentration for each one). Amphotericin B and ketoconazole. The plants then designed for summery as P. anisum (L) , C. sinensis (L) , C. aurantifolia (FP) and M.chamomile (F)) .

2. Methods: 2.1 Samples Collection:

The study included 32 infants complaining from diarrhea in Al –Mansour Hospital for Pediatrics in Baghdad aged from 1 day to 2 years old. They diagnosed as fungal infections by diagnostic processes in the same hospital's laboratory included G.S.E. for showing the pus cell, R.B.Cs and budding of Monillia by direct examination and determination of pH of stool by graduated litmus paper and stool samples were collected from patients by using sterile cotton swabs, which had been immediately suspended in sterile capped test tubes containing 5 ml of sterile normal saline as transport medium . All samples were collected from the beginning of December (2007) to the end of April(

2008) .

2.2 Antifungal Drugs Collection:

They are collected from pharmacies of Baghdad and included : a- Amphotricin B , b-Ketoconazole and c-Chloramphenicol, they were got as tablet 200 mg for (Ketoconazole) , vial 50 mg for (Amphotricin B) and capsule 250 mg for (Chloramphenicol). They are used after special handle techniques for making invitro antifungal susceptibility to certain Candida spp. as shown in the appendix.

2.3 Plants Collection: All plants were collected from local special super markets of Baghdad used by traditional medical practitioners for the treatment of several ailments of microbial and non microbial origins and then washed to remove the wastes and dust, then dried by the air and then blender by electrical blender, kept in dark and dry plastic cases for prevent chemical analytic or spoil by growth of microorganism .

RESULTS:

Table (1): Illustrates the age groups (Year) of *Candida* patients.

Age groups (Year)	No.	%	Comparison of significant	
			P-value	Sig.
<1	14	43.8	0. 458	Non Sig. (P>0.05)
1-1.5	9	28.1		
1.6-2	9	28.1		
Total	32	100		

Table (1) shows that: 14 (43.8%) of the patients were under one year old, 9 (28.1%) of the patients were between the age groups of 1- 1.5 years and 9 (28.1%) of the patients were between the age groups of 1.6-2 years.

Table (2): Illustrates the distribution of patients according to their stool pH.

Stool pH	No.	%	Comparison of significant	
			P-value	Sig.
Acidic	19	59.4	0.007	Highly Sig. (P<0.01)
Neutral	7	21.9		
Alkaline	6	18.8		
Total	32	100		

Table (2) shows that: 19 (59.4%) of stool specimens were acidic pH, (7) (21.9%) of stool specimens were neutral pH and 6 (18.8%) of the stool specimens were alkaline pH.

Table (3): Illustrates the distribution of patients among *Candida* species.

Candida species	No.	%	Comparison of significant	
			P-value	Sig.
albicans	12	37.5	0. 009	Highly Sig. (P<0.01)
tropicalis	11	34.4		
globrata	3	9.4		
cruzei	3	9.4		
parapsilosis	3	9.4		
Total	32	100		

Table (3) illustrate that: 12 (37.5%) of isolates were *C. albicans*, 11 (34.4%) of isolates were *C. tropicalis* and 3 (9.4%) of remaining isolates were *C. Globrata*, *C. cruzei* and *C. parapsilosis* in respectively .

Table (4):- Reveals the sensitivity of isolates of *Candida albicans* to plant's aqueous extracts and antifungal drugs by broth dilution method.

Studied groups	No.	MIC	MFC
Ketoconazole	5	(12.5) mcg / ml	(25) mcg / ml
Amphotericin B	5	(50) mcg / ml	(100) mcg / ml
M. chamomile (F)	5	(12.5) mcg / ml	(25) mcg / ml
P. anisum (L)	5	(6.25) mcg / ml	(12.5) mcg / ml

Table (4) revealed that the MIC of *P. anisum* (L) for all the isolates were (6.25) mcg / ml., and the MFC were (12.5) mcg / ml., the MIC of *M. chamomilla*(F) for all the isolates were (12.5) mcg / ml., and the MFC were (25) mcg / ml., the MIC of Ketoconazole for all the isolates was (12.5) mcg / ml., and the MFC was (25) mcg / ml. The MIC of Amphotericin B for all the isolates was (50) mcg / ml., and the MFC was (100) mcg / ml , while the other plants of (*C. sinensis* (L) and *C. aurantifolia* (FP)) which were detected have no or little antifungal activities in all the isolates, so there were no MIC and MFC.

Table (5): Illustrates the comparison between to plant's aqueous extracts and antifungal drugs in inhibition zone (mm) at (25 mcg / ml) concentration for both .

Studied groups	N	Mean	SD	SEM	Mini.	Max.	ANOVA	
							P-value	Sig.
Ketoconazole	5	11.6	1.14	0.51	10	13	0.00	Highly Sig. (P<0.01)
Amphotericin B	5	11.6	0.57	0.24	11	12		
P. anisum (L)	5	10.4	0.55	0.25	10	11		
M. chamomile (F)	5	11	0.54	0.21	11	12		
C. sinensis (L)	5	1.4	0.67	0.23	1	2		
C. aurantifollia (FP)	5	1.2	0.45	0.20	1	2		
Total	45							

This Table (5) illustrate that the mean of diameter of inhibition zone of *M. chamomilla* (F) (11 ± 0.54) is the nearest to the mean of diameter of inhibition zone of Ketoconazole (11.6 ± 1.14), followed by *P. anisum* (L) (10.4 ± 0.55) The mean of diameter of inhibition zone of *M. chamomilla* (F) (11 ± 0.54) is the nearest to the mean of diameter of inhibition zone of Amphotericin B (11.6 ± 0.57), followed by *P. anisum* (L) (10.4 ± 0.55)) as a second on the other plants under my research reveal a very little or no antifungal affectivity .

Table (6): Illustrates the multi -comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under study in inhibition zone (mm) at (25 mcg / ml) concentration for both.

Studied groups		LSD (F-test)	
		P-value	Sig.
Ketoconazole	*P. anisum* (L)	0.026	Sig.(P<0.05)
	M. chamomile (F)	0.253	Non Sig.(P>0.05)
	C. sinensis (L)	0.00	Highly Sig.(P<0.01)

	C. aurantifollia **(FP)**	**0.00**	**Highly Sig.(P<0.01)**
Amphotericin B	***P. anisum*** **(L)**	**0.021**	**Sig.(P<0.05)**
	M. chamomile **(F)**	**0.253**	**Non Sig.(P>0.05)**
	C. sinensis **(L)**	**0.00**	**Highly Sig.(P<0.01)**
	C. aurantifollia **(FP)**	**0.00**	**Highly Sig.(P<0.01)**

Table (6) shows depending on statistical analysis (Less Significant Difference (LSD) test (F-test) carried by SPSS computer program), when made a multi comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under study in inhibition zone (mm) at (25 mcg / ml) concentration for both, I detected that the aqueous extracts of plants of *P. anisum* (L) in comparison with Ketoconazole as standard antifungals had a significant difference of (P<0.05). And the aqueous extracts of plants of *M. chamomile* (F) in comparison with Ketoconazole as standard antifungal had a non significant difference of (P>0.05).

The aqueous extracts of plants of *C. sinensis* (L), and *C. aurantifollia* (FP)) in comparison with Ketoconazole as standard antifungal had a highly significant difference of (P<0.01), and the aqueous extracts of plants of *P. anisum* (L) in comparison with Amphotericin B as standard antifungal had a significant difference of (P<0.05). And the aqueous extracts of plants of *M. chamomile* (F) in comparison with Amphotericin B as standard antifungal had a non significant difference of (P>0.05), the aqueous extracts of plants of *C. sinensis* (L), and *C. aurantifollia* (FP)) in comparison with Amphotericin B as standard antifungal had a highly significant difference of (P<0.01) .

Table (7): Illustrates the comparison between to plant's aqueous extracts and antifungal drugs in inhibition zone (mm) at (50 mcg / ml) concentration for both .

Studied groups	N	Mean	SD	SEM	Mini.	Maxi.	ANOVA	
							P-value	Sig.
Ketoconazole	**5**	**14.8**	**1.1**	**0.49**	**14**	**16**		
Amphotericin B	**5**	**14.6**	**0.89**	**0.40**	**14**	**16**		
P. anisum **(L)**	**5**	**12.8**	**1.10**	**0.49**	**12**	**14**		
M. chamomile **(F)**	**5**	**13**	**0.52**	**0.14**	**13**	**14**		**Highly**

C. sinensis **(L)**	**5**	**1.4**	**0.65**	**0.26**	**1**	**2**	**0.00**	**Sig. (P<0.01)**
C. aurantifollia **(FP)**	**5**	**1.4**	**0.63**	**0.29**	**1**	**2**		
Total	**45**							

This table (7) reveals that the mean of diameter of inhibition zone of *M. chamomilla* (F) (13 ± 0.52) is the nearest to the mean of diameter of inhibition zone of Ketoconazole (14.8 ± 1.1), and the mean of diameter of inhibition zone of *M. chamomilla* (F) (13 ± 0.52) is the nearest to the mean of diameter of inhibition zone of Amphotericin B (14.6 ± 0.89), followed by *P. anisum* (L) (12.8 ± 1.10) as a second one and the other plants under my research reveal a very little or no antifungal affectivity .

Table (8): Illustrates the multi -comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under study in inhibition zone (mm) at (50 mcg / ml) concentration for both.

Studied groups		**LSD (F-test)**	
		P-value	**Sig.**
Ketoconazole	***P. anisum*** **(L)**	**0.002**	**Highly Sig.(P<0.01)**
	M. chamomile **(F)**	**0.005**	**Highly Sig.(P<0.01)**
	C. sinensis **(L)**	**0.00**	**Highly Sig.(P<0.01)**
	C. aurantifollia **(FP)**	**0.00**	**Highly Sig.(P<0.01)**
Amphotericin B	***P. anisum*** **(L)**	**0.005**	**Highly Sig.(P<0.01)**
	M. chamomile **(F)**	**0.013**	**Sig.(P<0.05)**
	C. sinensis **(L)**	**0.00**	**Highly Sig.(P<0.01)**
	C. aurantifollia **(FP)**	**0.00**	**Highly Sig.(P<0.01)**

Table (8) shows, when made a multi comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under the study in inhibition zone (mm) at (50 mcg / ml) concentration for both by statistical analysis (Less Significant difference (LSD) test (F-test)), The aqueous extracts of plants of (*P. anisum* (L), *M. chamomile* (F) , *C. sinensis* (L) and *C. aurantifollia* (FP)) in comparison with Ketoconazole as standard antifungal had a highly significant difference of (P<0.01) .And the aqueous extracts of plants of *M. chamomile* (F) in comparison with Amphotericin B as standard antifungal had a significant difference of (P<0.05), the aqueous extracts of plants of (*P. anisum* (L), *C.*

sinensis (L), and *C. aurantifollia* (FP)) in comparison with Amphotericin B as standard antifungal had a highly significant difference of (P<0.01) .

Table (9): Illustrates the comparison between to plant's aqueous extracts and antifungal drugs in inhibition zone (mm) at (100 mcg / ml) concentration for both

Studied groups	N	Mean	SD	SEM	Min.	Max.	ANOVA	
							P-value	Sig.
Ketoconazole	5	16	1.22	0.55	15	18	0.00	Highly Sig. (P<0.01)
Amphotericin B	5	15.8	1.48	0.66	14	18		
P. anisum (L)	5	14.8	1.1	0.49	14	16		
M. chamomile (F)	5	16.8	1.1	0.49	16	18		
C. sinensis (L)	5	2	0.57	0.26	1	3		
C. aurantifollia (FP)	5	1.6	0.45	0.21	1	2		
Total	45							

Table (9) shows that the mean of diameter of inhibition zone of *M. chamomilla* (F) (16.8 ± 1.1) is greater than the mean of diameter of inhibition zone of Ketoconazole (16 ± 1.22), followed by *P. anisum* (L) (14.8 ± 1.1) as a second one, and the mean of diameter of inhibition zone of *M. chamomilla* (F) (16.8 ± 1.1) is greater than the mean of diameter of inhibition zone of Amphotericin B (15.8 ± 1.48), and the mean of diameter of inhibition zone of *P. anisum* (L) (14.8 ± 1.1) is the nearest one to the mean of diameter of inhibition zone of Amphotericin B (15.8 ± 1.48), the other plants under my research reveal a very little or no antifungal affectivity .

Table (10): Illustrates the multi –comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under study in inhibition zone (mm) at (100 mcg / ml) concentration for both

Studied groups		LSD (F-test)	
		P-value	Sig.
1Ketoconazole	*P. anisum* (L)	0.054	Non Sig.(P>0.05)
	M. chamomile (F)	0.192	Non Sig.(P>0.05)
	C. sinensis (L)	0.00	Highly Sig.(P<0.01)

	***C. aurantifollia* (FP)**	**0.00**	**Highly Sig.(P<0.01)**
Amphotericin B	***P. anisum* (L)**	**0.105**	**Non Sig.(P>0.05)**
	***M. chamomile* (F)**	**0.105**	**Non Sig.(P>0.05)**
	***C. sinensis* (L)**	**0.00**	**Highly Sig.(P<0.01)**
	***C. aurantifollia* (FP)**	**0.00**	**Highly Sig.(P<0.01)**

Table (10) shows, when made a multi comparison between the aqueous extracts of plants & standard antifungals of Ketoconazole and Amphotericin B under study in inhibition zone (mm) at (100 mcg / ml) concentration for both by statistical analysis (Less Significant Difference (LSD) test (F-test)), I detected that the aqueous extracts of plants of *P. anisum* (L) and *M. chamomile* (F) in comparison with Ketoconazole as antifungal drug had a non significant difference of (P>0.05) and the aqueous extracts of plants of (*C. sinensis* (L) and *C. aurantifollia* (FP)) in comparison with Ketoconazole had a highly significant difference of (P<0.01). The aqueous extracts of plants of *P. anisum* (L) and *M. chamomile* (F) in comparison with Amphotericin B as antifungal drug had a non significant difference of (P>0.05). And the aqueous extracts of plants of (*C. sinensis* (L) and *C. aurantifollia* (FP)) in comparison with Amphotericin B had a highly significant difference of (P<0.01).

DISCUSSION:

These results in **(Table 1)** may be attributed to the immunocompromised infants especially a Very Low Birth Weight (VLBW) (<1500 gm) infants who usually require invasive therapies, such as central vascular catheters and endotracheal tubes, and are exposed to broad-spectrum antibiotics and parenteral nutrition. In addition, they occasionally receive postnatal steroids. All of these factors place them at high risk for fungal infection.

The results in **(Table 1)** were agreed with (Feja, 2005) [(5).] Who detects that the complicated GI disease in both preterm and term infants increases risk of fungal infections. Complicated GI disease in which infants receive nothing by mouth (not enterally feed) and/or antibiotics for more than 7 days increases the risk for fungal sepsis. Examples include intestinal atresia, tracheoesophageal fistula disease**.**

The results in **(Table 2)** were supported by (Hornby, 2003). [(6)] .who explained that the effect of environmental conditions as pH medium on morphological forms of Candida spp., where growth below 30^{o}C , pH 4.0 were promoted yeast growth, and growth at pH 6.0, 35^{o}C were promoted pseudohyphae growth and growth at pH 7.0, 37^{o}C were promoted hyphae growth .The results also were supported by (Soll, 1983).[(7)] who described that the switch in pH suffices had effect on the production of either the yeast form (pH 4.5) or the hyphal form (pH

6.5), and both being cultured at 37°C . The species of C. albicans and C. tropicalis had a greatest percentage, so I focused on them in this study. the results in **(Table 3)** were agreed with (Pfaller, 2006) [8] who explained that his study in Asia included (518) of Candida isolates and that the C. albicans remained the most frequently isolated yeast species in infected neonates which compromise (60.2% of isolates) , followed by C. parapsilosis infections (16.2% of isolates), which were increased during the past decade. C. glabrata (7.3 % of isolates) and C. tropicalis (12.5 % of isolates) were also increased in frequently. In another study the results were also agreed with (Talwar, 1990) [9] who explained in three-years study, included (854) patients (640) children and (214) adults) with acute or chronic diarrhea were screened, fungal proliferation was noted in 54.8% of these patients (53.6% in children, 58.4% in adults).And C. albicans has been shown to be a cause of diarrhea and the predominant fungal species isolated were Candida albicans (64.5%), followed by C. tropicalis (23.3%), C. krusei (6.9%), and C. glabrata (1.6%).

These results in **(Table 4)** were agreed with (Ingroff, 1999) [10] who explained that Ketoconazole has an effect on C. albicans and the MIC which was range from (12.0- 50.0) mcg / ml, and also the results were also agreed with (Shadomy, 1984) [11] who explained that Ketoconazole has a wide range of MIC values and has been reported for Candida. in vitro studies, the MIC of ketoconazole for C. albicans, C. parapsilosis, and C. tropicalis was 1–16 mcg/ml, However, these organisms required ketoconazole concentrations of 25 mcg/ml or greater for in vitro inhibition.

Other results were also agreed with my thesis results where, (NCCLS, 2003) [12] explained for the most part Amphotericin B MICs for Candida species cluster between 0.25 and 1.0 mcg /ml .(NCCLS, 1997) [13] revealed that MICs were determined by the NCCLS microbroth dilution method M27-A MICs of ketoconazole for the reference ATCC strains were within the range from $\leq$0.03-$\geq$16 μg/ml and the MICs of Amphotericin B for the reference ATCC strains were within the range from 0.06-2.0 μg/ml. In Iraq no studies on M. chamomile (F), and P. anisum (L) had been done, The results were agreed with (Mann et al., 1986) who explained that aqueous extracts of M. chamomilla had a lot of inhibitor compounds for several organism included Bacteria and fungi, this was attributed to contain a lot of compounds like aznlene (Mowrey .V. , 1990) .Bisabobls and Matricine (Ahmed . 1994)[14]. Apigenin and Flavonids (Avallone, 2000) [15]. which had inhibitor activities for fungi especially C. albicans.

Results of **(Table 5,6,7,8,9,10)** were agreed with (Kosalec,2005) [16]. which were studied in vitro on clinical isolates of Candida species]. In Iraq alittle studies on leave extract of P. anisum had been done, however, other studies reveal that the seeds of it are antiseptic, antispasmodic, antifungal, aromatic, carminative, digestive, expectorant, pectoral, stimulant, stomachic and tonic . These results were agreed with (Al-A'ni, 2005)[17] who revealed in his study In Iraq that the M. chamomilla aqueous extract have inhibitory effect on C. albicans growth in vitro

diagnosis, further studies confirm that the M. chamomilla has fungicidal properties. The aqueous extracts of M. chamomilla had also Vitamin C which had the antioxidant action (Wesibuger et al . , 1998) [18] and contains Authamidin, Chamazulene, Matriearin and Bisabolols and all of these compounds had inhibitor action for several mutagens (Mann et al . 1986) [19] . The results of thesis disagreed with (Hashim, 1988) who revealed that the green tea aqueous extract have inhibitory effect on C. albicans growth in vitro diagnosis, and also disagreed with number of studies (Al-Khayat , 2002). [20] and (Hirasawam , 2004) [21], who explained in their studies that the aqueous extracts of Camellia sinensis had a lot of inhibitor compounds for several organism included Bacteria and fungi.

CONCLUSIONS:

1- The prevalence of infections with C. albicans and C. tropicalis were of the most percentile value in infant than the other species of Candida patients. 2- The agar well dilution method can be adopted for in-vitro antifungal testing sensitivity, as it is a simple, reproducible, cost effective and easy to perform technique in a routine clinical microbiology laboratory.3- The aqueous extracts of P. anisum (L), T. vulgaris (L), M. chamomile (F) and P. grantum (FP) produced inhibition zone against C. albicans at (25, 50 and100 mcg /ml) concentrations and the activity of inhibition increased with the increase of concentrations. This also indicates the presence of potent antifungal activity, which confirms its use as anti-infective drug as treatment. 4- The other aqueous extracts of C. sinensis (L), G. glabra (R) and C. aurantifollia (FP) under my research reveal a very little or no antifungal affectivity at (25, 50 and100 mcg /ml) concentrations. 5- The aqueous extracts of M. chamomile (F) followed by P. anisum (L) and T. vulgaris (L) were more effective than other aqueous extracts of plants under research in comparison with standard antifungals of Ketoconazole and Amphotericin B under study at (25 mcg /ml) concentration for both .6- The aqueous extract of T. vulgaris (L) was more effective than other aqueous extracts of plants under research in comparison with the standardantifungals of Ketoconazole under study at (50 mcg /ml) concentration for both, and the aqueous extracts of T. vulgaris (L) followed by M. chamomile (F) were more effective than other aqueous extracts of plants under research in comparison with the standard antifungals of Amphotericin B under study at (50 mcg /ml) concentration for both .7- The aqueous extracts of P. anisum (L), T. vulgaris (L), M. chamomile (F) and P. grantum (FP) were more effective than C. sinensis (L), G. glabra (R) and C. aurantifollia (FP) in comparison with the standard antifungals of Ketoconazole and Amphotericin B under study at (100 mcg /ml) concentration for both.

RECOMMENDATIONS:

1- Further researches and studies are needed to elucidate the importance of the plants extracts as a source of new antifungal agents

2- Identifying the active chemical compositions of P. anisum (L), T. vulgaris (L), M. chamomile (F) and P. grantum (FP), and in vivo testing of their antifungal activities.

3- Encouraging the use of natural herbs extracts which were detected as active antifungal agents with suitable non toxic doses.

4- Studying of the relationship between the infant diarrhea and Candida spp. infections.

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