Evaluation of Some Plant Aqueous Extract's Influence on the Candida Species Isolated from the Stool Samples of Infants

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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 and Candida parapsilosis.* تم اختيار (5) عزلات عشوائيــاً مـن كـل من *Candida albicans* و *Candida tropicalis* : لدراسة حساسية تلك العزلات إلى اربع مستخلصات مائية نباتية وهذه المستخلصات كانت اوراق *Thymus vulgaris* leaves وتم دراسة التركيز)جذورعرق السوس *Glyceriza glabra* roots), قشور زهره الرمان *Punica, grantum* fruits peels, الزعتر أظهرت النتائج أن (Sabouraud Dextrose Broth) المثبط الأدنى والتركيز القاتل الفطري الأدنى باستخدام طريقة التخفيف في وسط ال التركيز المثبط الأدنى للمستخلص المائي لكل من أوراق الينسون وقشورزهرة الرمان كان (6.25) مايكروغرام / مل والتركيز القاتل الفطري الأدنى لهما كان(12.5) مايكرغرام / مل .. و أن التركيز المثبط الأدنى للمستخلص المائي لكل من أوراق الزعتر والتركيز القاتـل الفطري الأدنى له كان (25) مايكروغرام / مل, بينما وجد أن المستخلصات المائية لباقي النباتات وهي جذور عرق السوس بأنها ليس لها أو لها القليل جداً من التأثير ضد الفطري على جميع العزلات , لذا ليس لها تركيز مثبط أدنى ولا تركيز قاتل فطري أدنــى. 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 وأوراق الزعتر وقشور زهرة الرمان) أنتجت منطقة تثبيط ل لمبيضات البيضاء 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, أوراق الزعتر, و قشور زهرة الرمان 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 أوراق الينسون وأوراق الزعتر 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 لأوراق الزعتر لهما تأثير ضد فطري أكثر من باقي المستخلصات المائية النباتية قيد الدراسة عند التركيز (50) مايكرو غرام / مل لكليهمـا,وبالمقارنة مع الكيتوكونازول اكتشف أن المستخلص المائي لأوراق الزعتر له تأثير ضد فطري أكثر من باقي المستخلصات المائية النباتية قيد الدراسة عند التركيز(50) مايكرو غرام / مل لكليهمـا

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Abstract- A complete of (32) stool samples of infants, complaining from diarrhoea had been protected in this take a look at. all of them recognized as fungal infections via making a habitual and confirmative diagnostic techniques, after primary isolation of *Candida* species, the outcomes monitor that : 12 (37.5%) of isolates had been *Candida albicans*, 11 (34.4%) of isolates have been *Candida tropicalis* and 3 (nine.4%) of each isolate of *Candida cruzei*, *Candida globrata* and *Candida parapsilosis*. five isolates from every considered one of *C. albicans* and *C. tropicalis* have been selected randomly for reading of sensitivity of these isolates to four plant's aqueous extracts, the extracts which were (*Thymus vulgaris leaves*, *Punica grantum* end result peels and *Glyceriza glabra* roots), their minimal Inhibitory concentration (MIC) and minimal Fungicidal attention (MFC) had been studied by way of the use of the dilution method through Sabouraud Dextrose Broth .The effects display the MIC of *Punica grantum* (Fruit Peels) aqueous extracts was (6.25) mcg / ml., and the MFC was (12.five) mcg / ml., the MIC of *Thymus vulgaris* (Leaves) aqueous extracts turned into (12.five) mcg / ml., and the MFC was(25) mcg/ml , while the rest flora aqueous extracts of (*Glyceriza glabra* Roots)) were outcomes don't have any or very little antifungal activities in all of the isolates, so there were now not MIC and MFC for it .A second approach was used to test the antifungal activity of the same 4 plant's aqueous extracts by using the agar nicely diffusion approach, and the consequences showed that the

diameters of inhibition zones were improved while the concentrations of extracts have been accelerated .This approach was the great in clarification of effects of sensitivity exams. outcomes tested that the aqueous extracts of *T. vulgaris* (Leaves) and *P. grantum* (Fruit Peels) Produced inhibition region towards *C. albicans* at (25, 50 and100 mcg /ml) concentrations, while the alternative aqueous extracts of plant below study monitor a totally little or no inhibition zones at (25, 50 and100 mcg /ml) concentrations.whilst contrast of the plant aqueous extracts with both standard antifungal drugs of Ketoconazole and Amphotericin B under take a look at, i found that the aqueous extracts of *P. T. vulgaris* (Leaves) and *P. grantum* (Fruit Peels) have been extra antifungal powerful than other aqueous extracts of plants under studies at (100 mcg /ml) attention for both, and detected also that aqueous extracts of *T. vulgaris* (Leaves) have been extra antifungal effective than other aqueous extracts of plant life under research at (25 mcg /ml) awareness for both.In comparison with Amphotericin B, I detected that the aqueous extracts of *T. vulgaris* (Leaves) were greater antifungal effective than different aqueous extracts of flowers beneath studies at (50 mcg /ml) awareness for each. And in evaluation with ketoconazole, we found that the aqueous extract of *T. vulgaris* (Leaves) was more antifungal effective than other aqueous extracts of vegetation beneath studies at (50 mcg /ml) concentration for both.

Index Terms- Plant Aqueous Extract, Candida Species, Candida albicans

I. INTRODUCTION

The remaining decade witnessed the sustained clinical significance of opportunistic infections due to extraordinary *Candida* species in particular due to the worldwide increase within the number of immunocompromised sufferers which include children, who are exceedingly prone to opportunistic infections.(1) .Candidiasis is an an increasing number of commonplace problem in hospitalized patients, with epidemiologic surveys revealing that *Candida* spp. are actually the fourth maximum not unusual pathogens remoted from the blood of hospitalized sufferers. approximately (one hundred) species of fungi pathogenic for people, and *Candida albicans* is the maximum standard species in medical disease. *C.albicans* has also been proven to be a cause of diarrhea. Candidiasis in neonates is a extreme and relatively not unusual purpose of overdue onset sepsis associated with a high mortality. The current reviews that imply non-*albicans* infections are on the rise, which frequently accounting for more than 50 percent of candidiasis observed in the inflamed populace (2). Drug resistance may additionally vary by way of species .although antifungal resistance in *Candida albicans* is less frequent than in other species, increasingly more resistance traces are emerging. Antifungal drug resistance is fast turning into a first-rate trouble within the increasing populace of immunocompromised individuals. It has resulted in a drastic increase within the prevalence of opportunistic and systemic fungal infections, so a brand new healing strategies need to be taken into consideration to lessen antifungal pills resistance which is probably accomplished via using opportunity natural medication as a herbal supply (3). The World Health Organization (WHO) estimates that 80 percentage of the arena's populace currently makes use of natural remedy for some component of primary health care. In reality, in step with the sector health corporation, 25% of modern-day drugs that are about used inside the U.S.A. were derived from plant life. Considerable advances had been made in medical care however many people are nevertheless the usage of natural or opportunity treatments (4).

Due to the fact the Iraqi vegetation are wealthy in vegetation are un submitted to any previous examine and the possibility of locating new antimicrobial dealers continues to be broadly ahead. And because the exciting Candida species is an opportunistic pathogen and will increase of antifungal drugs resistance in ultimate years varies with distinct species and increase of researches on clinical crucial plants but most effective few research on those researches were performed in Iraq, this observe **aimed** toward the subsequent:

1 -The isolation and identity of *Candida* species have been from stool samples of infected infants .

2 -The research of the antifungal interest of a few aqueous crude extracts have been of some flora and some antifungal pills in opposition to *Candida* species.

3- In vitro, the dedication of the capability of the use of these extracts in determining the deadly dose and inhibitory dose of fungal infections.

Materials and Techniques: 1- Chemical Solutions and Their preparations:

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

1.2- Preparation of stock Solutions Of Antifungal marketers:

We have followed the tips of NCCLS for making ready the numerous concentrations of antifungal tablets. The active additives in Amphotericin B received became seventy eight% and ketoconazole changed into 70% .

The stock I solution of Amphotericin B (HiMedia, India) changed into organized by way of the use of 50 mg of Amphotericin B dissolved in 500 ml of Dimethylsulfoxide (DMSO) , the running solution changed into performed in fold dilution to obtain concentrations starting from 3.125 –100 mcg / ml ; DMSO were changed into used to dissolve Amphotericin B following CLSI *Candida parapsilosis* guidelines.The stock II solution of Ketoconazole (Cadila pharmaceuticals, India) stock solution become prepared by using dissolving 20 mg of ketoconazole in 200 ml of ninety eight% methanol in line with manufacturer's instructions. operating solution turned into done in fold dilution to achieve concentrations ranging from 3.125 – 100 mcg / ml, whilst water used to dissolve ketoconazole, it led to precipitation of the drug. subsequently, 98% methanol became used to dissolve ketoconazole , the stock solutions are stable for (three) days in culture at 37 °C. or 2-8 °C for up to one month. covered from air and light. Sterilization is done through filtration of stock solutions.

1.3-Preparation of Plant's Aqueous Extracts:

Four local plant's aqueous extracts have been prepared; the primary materials of those extracts were from leaves, flowers, peels and roots of herbal plants. They were used as antifungal agents. The plants were of (*Thymus vulgaris* leaves, *Punica grantum* fruits peels and *Glyceriza glabra* roots) which they have been prepared as the subsequent:

The extraction technique used in this study was a modification of Akinside & Olukoya, and Akinyemi, in line with the conventional techniques of instruction, shredded plant materials of the (*Thymus vulgaris* leaves, *Punica grantum* fruits peels and *Glyceriza glabra* roots) had been 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 the usage of a sterile glass rod after each 24 hrs. , they have been finally grounded into fine powder in 25 ml of steriled distilled water, stored at 60°C for 3 hr.

The resulting suspensions were filtered rapidly through four layers of gauge after which by using a extra delicate filter via Whatman No.1 filters paper., the filtrates were concentrated by way of evaporation to dryness at 60°C in the warm air oven for 24hrs., then weight 1gm from it and dissolved in 10 ml for getting a 100 mcg /ml and the mixture became mixed via warm plate magnetic stirrer for forty eight hours at 50-55 °c. .The mixture was put in centrifuge (5000 rpm) for 30 minutes, then made various concentrations from crude extracts (a 100, 50, 25, 12.5,6.25 and 3.125) mcg/ml (mcg = µg), for every one among plants in respectively on the way to look at if they have an impact on of these concentrations on exceptional *Candida* spp. underneath research, the organized herbal extracts sterile by millipore filter out, then stored in sterile airtight glass boxes protected from sun light and refrigerated at 4°C prior to use for evaluation .The antifungal potency of herbal aqueous extracts achieved into in comparison with standard commercial antifungals (as a single pharmacological dose of concentration for each one). Amphotericin B and ketoconazole. The plant then designed for summery *Thymus vulgaris* (L) , *Punica grantum* (FP) and *Glyceriza glabra* (R).

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 identified as fungal infections via diagnostic processes within the same hospital's laboratory included G.S.E. for displaying 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 have been collected from patients by using sterile cotton swabs, which have been immediately suspended in sterile capped test tubes containing 5 ml of sterile normal saline as transport medium. All samples were collected from the start 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 had been got as pill 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 have been collected from nearby unique super markets of Baghdad utilized by traditional medical practitioners for the treatment of numerous illnesses of microbial and non-microbial origins and then washed to dispose the wastes and dust, then dried by the air and then blender by electrical blender, saved in darkish and dry plastic cases for prevent chemical analytic or spoil by means of growth of microorganism .

II. RESULTS

Table (1): Clarifies 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): Clarifies the distribution of patients among *Candida* species.

Candida species	No.	%	Comparison of significant	
			P-value	Sig.
Albicans	12	37.5		
Tropicalis	11	34.4		

Globrata	**3**	**9.4**	**0. 009**	**Highly Sig. (P<0.01)**
Cruzei	**3**	**9.4**		
Parapsilosis	**3**	**9.4**		
Total	**32**	**100**		

Table (2) Clarifies 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 (3):- 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**
T. vulgaris **(L)**	**5**	**(12.5) mcg / ml**	**(25) mcg / ml**
P. grantum **(FP)**	**5**	**(6.25) mcg / ml**	**(12.5) mcg / ml**
G. glabra (R)	**5**	**(6.25) mcg / ml**	**(12.5) mcg / ml**

Table (3) revealed that the MIC of G. *glabra (R)* and *P. grantum* (FP) for all the isolates were (6.25) mcg / ml., and the MFC were (12.5) mcg / ml., the MIC of *T. vulgaris (*L) 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 (*G. glabra* (R)) which were detected have no or little antifungal activities in all the isolates, so there were no MIC and MFC for it.

Table (4): 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**		
Amphotericin B	**5**	**11.6**	**0.57**	**0.24**	**11**	**12**		
T.vulgaris **(L)**	**5**	**10.4**	**0.59**	**0.26**	**10**	**11**	**0.00**	**Highly Sig. (P<0.01)**
P. grantum **(FP)**	**5**	**8.8**	**1.10**	**0.49**	**8**	**10**		
G. glabra (R)	**5**	**1.6**	**0.65**	**0.24**	**1**	**2**		
Total	**30**							

This Table (4) illustrate that the mean of diameter of inhibition zone of *T. vulgaris* (L) (10.4 ± 0.59) is the nearest to the mean of diameter of inhibition zone of Ketoconazole (11.6 ± 1.14), and *P. grantum* (FP) (8.8. ± 1.10) as a second one. The mean of diameter of inhibition zone of *T. vulgaris* (L) (10.4 ± 0.59) is the nearest to the mean of diameter of inhibition zone of Amphotericin B (11.6 ± 0.57), followed by *P. grantum* (FP) (8.8. ± 1.10) as a second one, the other plants under my research reveal a very little or no antifungal affectivity.

Table (5): 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.
T. vulgaris (L)	0.028	Sig.(P<0.05)
P. grantum (FP)	0.00	Highly Sig.(P<0.01)
G. glabra (R)	0.00	Highly Sig.(P<0.01)
T. vulgaris (L)	0.025	Sig.(P<0.05)
P. grantum (FP)	0.00	Highly Sig.(P<0.01)
G. glabra (R)	0.00	Highly Sig.(P<0.01)

Table (5) 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 *T. vulgaris* (L) in comparison with Ketoconazole as standard antifungals had a significant difference of (P<0.05). The aqueous extracts of plants of *P. grantum* (FP), *G. glabra* (R) in comparison with Ketoconazole as standard antifungal had a highly significant difference of (P<0.01), and the aqueous extracts of plants of *T. vulgaris* (L) in comparison with Amphotericin B as standard antifungal had a significant difference of (P<0.05). the aqueous extracts of plants of *P. grantum* (FP), *G. glabra* (R) in comparison with Amphotericin B as standard antifungal had a highly significant difference of (P<0.01)**.**

Table (6): 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	0.00	Highly Sig. (P<0.01)
Amphotericin B	5	14.6	0.89	0.40	14	16		
T. vulgaris (L)	5	13.4	0.55	0.24	13	14		
P. grantum (FP)	5	11.2	1.64	0.73	10	13		
G. glabra (R)	5	1.6	0.78	0.33	1	2		
Total	30							

This table (6) reveals that the mean of diameter of inhibition zone of *T. vulgaris* (L) (13.4 ± 0.55) is the nearest to the mean of diameter of inhibition zone of Ketoconazole (14.8 ± 1.1), followed by *P. grantum* (FP) (11.2 ± 1.64) as a second one, and the mean of diameter of inhibition zone of *T. vulgaris* (L) (13.4 ± 0.55) is the nearest to the mean of diameter of inhibition zone of Amphotericin B (14.6 ± 0.89), followed by *P. grantum* (FP) (11.2 ± 1.64) as a second one, and the other plants under my research reveal a very little or no antifungal affectivity.

Table (7): 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.
T. vulgaris (L)	0.028	Sig.(P<0.05)
P. grantum (FP)	0.00	Highly Sig.(P<0.01)
G. glabra (R)	0.00	Highly Sig.(P<0.01)
T. vulgaris (L)	0.057	Non Sig.(P>0.05)
P. grantum (FP)	0.00	Highly Sig.(P<0.01)

	G. glabra **(R)**	**0.00**	**Highly Sig.(P<0.01)**

Table (7) 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)), I detected that the aqueous extracts of plants of *T. vulgaris* (L) in comparison with Ketoconazole as standard antifungal had a significant difference of (P<0.05). The aqueous extracts of plants of (*P. grantum* (FP), *G. glabra (R)*) in comparison with Ketoconazole as standard antifungal had a highly significant difference of (P<0.01), the aqueous extracts of plants of *T. vulgaris* (L) in comparison with Amphotericin B as standard antifungal had a non significant difference of (P>0.05). the aqueous extracts of plants of (*P. grantum* (FP), *G. glabra (R)*) in comparison with Amphotericin B as standard antifungal had a highly significant difference of (P<0.01)**.**

Table (8): 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**		
T. vulgaris **(L)**	**5**	**15.4**	**0.55**	**0.24**	**15**	**16**		
P. grantum **(FP)**	**5**	**14.8**	**1.1**	**0.49**	**14**	**16**		
G. glabra (R)	**5**	**2**	**0.57**	**0.26**	**1**	**3**		
Total	**30**							

Table (8) shows that the mean of diameter of inhibition zone of *T. vulgaris* (L) (15.4 ± 0.55) is the nearest to the mean of diameter of inhibition zone of Ketoconazole (16 ± 1.22), followed by *P. grantum* (FP) (14.8 ± 1.1) as a second one, and the mean of diameter of inhibition zone of *T. vulgaris* (L) (15.4 + 0.55) is the nearest one to the mean of diameter of inhibition zone of Amphotericin B (15.8 ± 1.48), followed by *P. grantum* (FP) (14.8 ± 1.1) as a second one, the other plants under my research reveal a very little or no antifungal affectivity.

Table (9): 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.
T. vulgaris **(L)**	**0.326**	**Non Sig.(P>0.05)**
P. grantum **(FP)**	**0.054**	**Non Sig.(P>0.05)**
G. glabra **(R)**	**0.00**	**Highly Sig.(P<0.01)**
T. vulgaris **(L)**	**0.511**	**Non Sig.(P>0.05)**
P. grantum **(FP)**	**0.105**	**Non Sig.(P>0.05)**
G. glabra **(R)**	**0.00**	**Highly Sig.(P<0.01)**

Table (9) 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 *T. vulgaris* (L) and *P. grantum* (FP) in comparison with Ketoconazole as antifungal drug had a non significant difference of (P>0.05) and the aqueous extracts of plants of *G. glabra (R*) in comparison with Ketoconazole had a highly significant difference of (P<0.01). The aqueous extracts of plants of *T. vulgaris* (L) and *P. grantum* (FP) in comparison with Amphotericin B as antifungal drug had a non significant difference of (P>0.05). And the aqueous extracts of plants of *G. glabra (R)* in comparison with Amphotericin B had a highly significant difference of (P<0.01).

III. 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 (Sayyada and *et al*, 2013).[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 (Sara and *et al*, 2012) [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 (Jan and *et al*, 2010) [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 .(Vishnu Chaturvedi and *et al*. (2015) [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 a very little studies on *T. vulgaris* (L), *M.* and *P. grantum* (FP) had been done, however, a recent research of (Pina,2004)[14] . found that the antifungal activity of *Thymus* extracts and their major compounds showed inhibitory activity on *C. albicans*. In the study of (Duke,2002) [15]. He found that there are a lot of complex compound in *P. granatum* Peel Extracts of Gallagic acid, punicalins, punicalagins and these compounds showed inhibitory activity on *C. albicans*. Results of **(Table 5,6,7,8 and 9)** were agreed with (Kosalec,2005) [16]. Which were studied *in vitro* on clinical isolates of *Candida* species. Other studies reveal that the seeds of it are antiseptic, antispasmodic, antifungal, aromatic, carminative, digestive, expectorant, pectoral, stimulant, stomachic and tonic. The results of thesis supported the results of the advanced studies that used *Thymus* spp. extracts as antimicrobial agents depend on the presence of thymol, these studies are also suggested the use of thyme as an antibiotic, thymol is 25 times as effective as phenol, but less toxic [17]. Other studies reveal that the thymol is considered to be antihelmintic (antiworm) with particular effectiveness against hookworm, and together with carvacrol is both antibacterial and antifungal . Recent studies show that thyme may be effective against a variety of microbs . *T. vulgaris* extracts has also been shown to be effective against the fungus that commonly infects toenails . And externally it has been used to treat fungal infections, which help treat minor arthritis, gum disease and tonsillitis [18] . Further studies confirm the use of *T. vulgaris* in folk medicine as: antifungal, sedative, antiseptic, antipyretic to control menstruation and cramps and in the treatment of dermatitis and Laboratory studies demonstrate that thymol has antifungal activity against a number of species, including *Cryptococcus neoformans*, *Aspergillus*, *Saprolegnia*, and *Zygorhynchus* species [19]. The results were agreed with the Iraqi researchers who showed that the pomegranate peel extract have sub inhibitory effect on fungal growth [20]. And on the other hand, the *P. granatum* peel extracts have antioxidant and antibacterial activities , In another research the (Laurylene, 2003)[21] .revealed that the use of *P. granatum* as an antifungal agent against candidiasis is associated with denture stomatitis , in further studies in Iraq researchers revealed that pomegranate peels extract have fungicidal activity for some types of fungi, nematodes and other infectious agents [21]. In Iraq a very little studies on *G. glabra* had been done, however, the results of my thesis disagreed with (Mahboubeh Irani, and *et al* .2010)[22] . who revealed in his study that the *G. glabra* showed effectiveness against *Candida albicans* , it was also disagreed with (Natália Martins, and *et al* .2016)[23]. Who illustrated in their studies that the Glycyrrhizin was shown to protect mice from *Candida albicans* infection when administered for 15 days, the mortality rate of infected mice was decreased from 100% to 65% by the Glycyrrhizin treatment .

IV. Conclusions

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. 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. The aqueous extracts of *T. vulgaris* (L), 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. Other aqueous extracts of *G. glabra* (R) under my research reveal a very little or no antifungal affectivity at (25, 50 and100 mcg /ml) concentrations. The aqueous extracts of *T. vulgaris* (L) was 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 .The aqueous extract of *T. vulgaris* (L) were 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) 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 .The aqueous extracts of *T. vulgaris* (L) and *P. grantum* (FP) were more effective than *G. glabra* (R) in comparison with the standard antifungals of Ketoconazole and Amphotericin B under study at (100 mcg /ml) concentration for both.

V. 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 *T. vulgaris* (L), 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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