

**EVALUATION EFFICIENCY OF THYMUS VULGARIS L.PLANT EXTRACT ON
SOME FUNGI ISOLATED FROM MOUTH AND NOSE OF PATIENT CHILDREN
IN MATERNITY AND CHILDREN HOSPITAL IN BABYLON PROVINCE**

NIDAA SHIHAB HAMAD*
ZAMEN SALEM**

*Dept. of Biology, Babylon University, Hilla City, Iraq

**Dept. of Biology, Babylon University, Hilla City, Iraq

ABSTRACT

The aims of this study included isolating & diagnosing species of fungi from the mouth & nose of babies from the first month to the three years of age. Its included (200) babies from stagnant babies in Maternity & Children hospital. (24) species from mould & yeast were isolated, the percentage of fungi species were account, the large ratio for *C. albicans* (%43.61), *Cryptococcus neoformans* (%41.12) of *C. tropicalis* (%8.27) *A. niger* %2.03 and white mycelium (%1.45). Regarding other fungi and yeast of fungi isolated from nose of babies were biggest ratio for *A. niger* (%25.77) of *Cladosporium cladosporoides* (%14.77), *A. flavus* %11.33 and *C. albicans* (%8.21). In addition application of aqueous & alcoholic extract from *Thymus vulgaris* (Thyme) plant on some fungi were tested showed high inhibition activity toward different fungi.

KEYWORDS: Fungal species; Iraq; *Thymus Vulgaris*

1. INTRODUCTION

The mouth is the main passage for most disease-causing germs, therefore, study of oral health contribute in reducing microbial multiple injuries inside the body (Todar, 2002). And show the seriousness of oral disease and tooth decay, especially during the first year of a child's life because the bacteria colonize the mouth during the first 12 months of the child's age and then begins to grow and grow inside the mouth (Grindefjord et al., 1995). The term normal flora on microbiology that could settle the human body healthy without causing injuries disease and characterized by specialization in the colonization of the body where endemic to specific places and under specific conditions and rates almost constant (Brooks et al., 1998 ; Burnett et al., 1976) In the human mouth found a number of fungi, particularly yeast *Candida albicans*, which flora is one of the opportunistic infections that cause gum in people with immunodeficiency, such as people with AIDS (Refai, and Taha, 1990).

Child acquires fungi from its surroundings. Mother is the first source of spores settlement inside the infant's mouth by being the first person who deals with the child at different stages of life. Bacteria are transmitted from mother to child in many ways, and primarily through the mouth, where bacteria are transmitted from the mouth of mother-to-mouth child and in other several ways, including kissing and talking and direct contact and other methods. (Berkowitz and Jordan, 1975 ; Davey and Rogers, 1984 ; Berkowitz and Jones, 1985 ; Caufield et al., 1988 ; Salvador et al., 1998). The mothers can be a reservoir for many fungi transition to the mouth of the child, especially many yeasts including *C.albicans*, *C.tropicalis* and *Cryptococcus*, *Rhodotorulla* and not show the gravity of the reservoir when the mother only after the child's progress in teeth showing a lesion cavities and gum disease different resulting from the settlement germs transmitted from the mouth of the motherland and its effectiveness in various inside the child's mouth (Köhler et al., 1994; Könönen et al., 1992) as the bacteria enter into the child's mouth in other ways it has intervention during childbirth and is usually genital tract bacteria such as *Staphylococcus* bacteria and *Staphylococcus*, *Neisseria* and yeasts and *C. albicans* is the basic germs transmitted to the child's mouth, but it loses over time (Boyd and Hoort, 1981 ; Nolte, 1982).

Respiratory consists of the nose, trachea and lungs that operate accurately and harmony, where the process of inhalation and exhalation, a process that the exchange of oxygen and carbon dioxide , which have importance in the performance of activities that are vital within cells . But could damage as a result of the entry of some microorganisms through a process of breathing through the nose to the human body and cause serious diseases of the lung is the most prominent of aspergillosis caused by fungus *Aspergillus niger* which grows in the bronchi There is also a case of *Aspergillus fumigatus*, which is caused by fungus *A.fumigatus* which invades the alcoves of Foreign Affairs in the lungs or the tissue of the lungs (Imad et al., 2002) and injury with fungi known (Aspergillosis) and some species cause disease in the tissues of animals and humans and is characterized by the presence of inflammatory infrastructure in the skin, and the external ear and sinuses and lungs (Marsh and Martin, 1992) .

Each of the *A. flavus* , *A.niger* and *A. fumigatus* fungus that spread there spors in the air and when inhaled settle in the lungs and sinuses and lead to the emergence of tuberculosis-like symptoms, especially those with weakened immune, as are the causes of yeast *C.albicans* opportunistic diseases that cause severe diseases in the chest known Mycosis. Thyme returns

to family Labiatae. The oil is extracted from the soft thyme. Thyme oil used as flavoring material, medical and anti-fungal and bacterial.

Leaves and stems contain volatile oils with volatile aromatic odor and sharp taste, and other vehicles such as Linalool, Flavones, Tannins and Caffeic acid and resins. Thyme has many uses, it is used in keeping lotions medical and cosmetic, especially those dealing with skin diseases and cracking (Roberts et al., 1998).

It is also an anti-fungal, anti-bacterial and anti-viruses to contain phenols (Woodruff, 1995) An aqueous extract of thyme leaves uses as property antioxidant drug and attributed its effectiveness to vehicles Flavones and Biphenyl - type that inhibit the effectiveness of lipid high oxidative stress in mitochondria and discourage partly produce ions of high oxidation, aqueous extract also used for the analgesic and intestinal colic treatment of gastritis, intestinal ulcers of the stomach, duodenum, indigestion and repelling gas and worms (Bruneton, 1999).

The objectives of this research are to isolate and diagnose fungal species and yeasts in the mouth and nose from the sick children and healthy aged. Test the effect of some factors on the content and mildew inside the mouth, such as mode of delivery and type of breastfeeding. Test the vulnerability of some fungal isolates on the decomposition of the blood. Study effect of aqueous extract and alcoholic thyme on some types of isolated fungi and yeasts.

2. Materials and Methods

All the culture media are prepared and sterilized by autoclave (121 m) degree and under the pressure of 1.5 lbs / Ang 2, 15 minutes and included the following media.

1. Sabouraud s Dextrose Agar (SDA) attended this media as mentioned in (Kwon-Chung and Bennett, 1992)
2. Potato Dextrose Agar (PDA) attended this media as stated in (Collee et al., 1996)
3. Corn meal Agar with Tween 80 and Trypan Blue. This media was used for the purpose of diagnosis yeasts conformable to the sentiments (Al-Rubiaa, 2001).
4. Sugar Fermentation Medium
5. Urea Agar medium: attended this media as stated in (Collee et al, 1996). Use the middle to diagnose yeast *Cryptococcus* spp as this yeast decomposition of urea and change the color of the center of the red to yellow.

Samples collection

Samples have been collected study from hospital birth and children in the province of Babylon. Total of samples are 600. Four hundred swab oral Swab and two hundred swabs from the nose of children aged from one month to three years and both sexes. Also collected 150 samples from 50 healthy baby of children living in homes in the city of Hilla.

Test of the ability of fungi to haemolysis

The ability of (9) types of molds and yeasts by using (SDA) media (Collee et al., 1996).

Prepare extracts thyme preparation of hot water extract of thyme

The plant has been taking from the local markets in the province of Babylon. The thyme plant from family Labiatae where taking 30 g of powdered leaves and inflorescences of thyme dry, they added (200) ml of distilled water boiling and leave to cool, then nominated the solution through layers of gauze , and then filter paper Whatmann No2, and took the filtrate and dry by electric oven temperature (40-45m) until getting dry powder. The following concentrations (5, 10, 15, 20) mg / ml from the media of Sabouraud Dextrose Agar (SDA) were used (Rios et al., 1987).

Preparation of alcoholic extract of thyme

10 grams of powder dry matter of leaves of Thyme have been taken and extracted with 200 ml of alcohol concentration for 24 hours in a Al-sacsulate, then concentrated by rotary evaporator . Dissolve this product with 5 mL of ethanol, and the alcoholic extract was added to 30 ml of sulfuric acid then used the second evaporator to get rid of ethanol to lag acid solution. This solution is added to a sufficient quantity of ammonium hydroxide concentration of 10 % to become pH (9) then put the solution in the basement separating funnel and add it 10 ml of chloroform. Leave the mixture to settle into two layers separated , I took the lower layer (chloroform layer) dissolved the alkaloids. Then returned to last step three times. Nominated concentrated filtrate rotary evaporator secondly to evaporate chloroform, then dried oven electric temperature (40-45 m) repeated the extraction process several times in order to obtain a sufficient amount of compounds alkaloids and kept in the refrigerator until use (Sousek et al., 1999). Attended concentrations (5,10 , 15, 20) mg / ml of extract with the media (SDA) (Sousek et al., 1999).

Test the influence of alcohol and water extract of thyme on fungi

Culture media (aged 7 days and incubator degree 28) from following fungi *Aspergillus flavus*, *Fusarium longipes*, Black yeast, *Alternaria alternata*, *Candida albicans* and *Cryptococcus* spp isolated from the mouth and nose from the kids where I took tablets diameter of 5 mm from the outer edges of colonies. Then put each disk in the center of the SDA media according to the following concentrations (0.5 , 10, 15.20) mg / ml (Pitt and Hocking, 1997).

Statistical Analysis

The results have been analyzed according to the design of random full Completely Randomized Design (CRD) three-factor and compared by calculates averages less significant difference (LSD) to indicate significant results at the level of probability ($0.05 > p$) (McGinnis, 1980).

3. Results and Discussion

We have isolated 24 species of fungi and yeasts from the mouth and nose and classified depending on the following sources: (Ellis , 1994 ; Hodge and Guarra, 1995 ; Midgley et al., 1997). As shown in the following table : Table (2) Show types of fungi isolated from the cavity of the mouths of babies and prepared as noted from the table that the *C. albicans* highest rate 43.61%. The isolation of yeasts *Cryptococcus* spp and *C. tropicalis* and the genus *Aspergillus* fungi and many other mouth and to varying degrees This may be due to it from opportunistic fungi that are present on host to increase activity and excessive growth and invasion of the host tissue when less host resistance as a result of excessive handling of antibiotics (Domsch et al., 1980; Armstrong, 1989 ; Prescott et al., 1996)

From the table (3) we noted that the fungus *Aspergillus niger* more fungi presence in the nose of the infected children admitted to the hospital and the highest percentage this return to this fungus (25.77%), where the intervention fungal spores into the body through inhalation of air and cause the fungus serious diseases in humans, notably aspergillosis caused by this fungus, which grows in the bronchi . Spores of fungus *A. niger* and *A. fumigatus* occupies sites essential for growth in the open cavities in the lungs of patients with pulmonary tuberculosis disease where the elements necessary for the growth and processing of higher oxygen (Dorko et al., 2002 ; Sharon et al., 2011)

Also we have been isolate two types of fungus Cladosporium they are: Cl. cladosporoides rate (14.77%) and Cl. macrocarpium (0.98%) as this fungus spreads widely in the air and has been isolated at high rates in the winter months and the fungus enters the respiratory tract and lungs through breathing and cause as Cl. cladosporoides symptoms of tuberculosis-like disease in chronic cases (Juan et al., 2007). Through this study, we isolated many types of yeasts *C. albicans* (8.21%), as these yeasts present on the body as Flora natural they possess the ability to grow at 37° its widespread in the air and has a high affinity of a pathogenicity in the event of a weakened immune system.

Alternaria alternata isolate from the mucous membranes of the nose and respiratory tract as opportunistic fungi (2.46%) from the samples of college. The reasons of incidence of these fungi due to its existence and spread widely in the environment and possess some virulence factors that enable them to bring injury and production units reproductive enormous (Juan et al., 2007). From the table (4) we are noted that the fungi isolated from the mouths of children, 60% of the total children, while no fungal growth in 40% of samples taken from children born natural birth either children born by Caesarean section did not show any fungal growth in 64% of samples. Study types of fungi endemic in the mouth require search for their first appearance inside the mouth Table (5). This table showed types of fungi isolated from the cavity of the mouths of children born natural birth while table (6) shows types of fungi isolated from the cavity of the mouths of children born birth Caesarea.

Some sources reported that the child's mouth cavity be free at birth and contain the protein substances and nutrients and other materials only (Stevens, 1996 ; Rick, 2002). Other sources indicate that the child's mouth acquires microbes during childbirth as it passes through the birth canal where the contaminated mouth microbiological endemic in the womb (Brooks, 1998). Also, for a withdrawal of fluids play an important role in the transmission of the species of microbes from child to child (Könonen, 2002). We note from the table (5) that the yeasts *C. albicans* and *Rhodotorulla glutinis* isolation from the cavity mouths of children born only. The interpretation of this result is that these yeasts belong to the group of fungi genital tract for women (Brooks, 1998) and therefore move at the passage of the child in the birth canal of the original home into the baby's mouth during childbirth (Könonen, 2002).

The types *Alternaria alternata* and *A. flavus* they do not belong to the fungi genital tract it isolated from the mouths of children born Natural childbirth and caesarean section and most of the belief that it is transmitted from person helpers in the birth process (nursing staff) through droplets from the mouth either during the process the birth itself or in the course of

cleaning the baby immediately after birth and including that Caesarean sections are usually processes within the hall and in the sterile atmosphere of isolation that the ratio of these two types is less in children born by Caesarean section birth of children born naturally as noted in the table (6)

The inhibitory effect of thyme extracts on *C. albicans* and *Cryptococcus spp*

C. albicans was resistant to aqueous extract and alcoholic extract of thyme but inhibited the yeast at high concentrations 15%, 20% this due to the completion of the contents of the fungal cell and a special cell wall, which is considered a high importance in resistance to fungi (62) and also because the cell wall of the yeast protein contains a substance there are enzymes linked to the wall like Glucanase, Mannase help to softer and analysis of the cell wall of yeast. Figure (1) showed the effect of aqueous extracts of thyme and alcohol on the growth of the yeast *Cryptococcus spp* where the percentage inhibition of 100% in the two extracts and all concentrations. This shows the effect of the thyme extracts events that led to an imbalance in the activity of cells that prevented fungal reproduction and division and growth and influence the permeability of the cell membrane as a result of the inhibition of the manufacturing process of cellular proteins that fall within the composition of the cell wall and cell membrane dysfunction occurs in regulating the permeability of the wall and membrane as well as prevent the synthesis of proteins necessary for cell and involved in activities such as construction and enzymes necessary for the organization (64)

Table (1) fungal species isolated from the mouth and nose of the children in the maternity hospital and children in the province of Babylon

No.	Fungi
1	<i>Aspergillus flavus</i>
2	<i>Aspergillus fumigatus</i>
3	<i>Aspergillus niger</i>
4	<i>Aspergillus ochraceus</i>
5	☉☉ <i>Aspergillus terreus</i>
6	<i>Alternaria alternata</i>
7	<i>Aurobasidium pullulans</i>
8	<i>Bipolaris spicife</i>
9	Black yeast
10	<i>Candida albicans</i>
11	<i>Candida tropicalis</i>
12	<i>Cladosporium cladosporoidies</i>

13	Cladosporium macrocarpium
14	Cladosporium spaerospermum
15	Cryptococcus spp
16	Fusarium longipes
17	Geotrichum candidium
18	Paecilomyces variotii
19	Penicillium chrysogenium
20	Penicillium digitatum
21	Penicillium expansium
22	Rhizopus solani
23	Rhodotorulla glutinis
24	White mycelium

Table (2) types of fungi isolated from the mouths of children

Fungal species	Number of isolated fungal	%
C .albicans	1803	43.61
Cryptococcus spp	1700	41.12
C .tropicalis	342	8.27
Aspergillus niger	84	2.03
White mycelium	60	1.45
Cladosporium cladosporoidies	37	0.89
Flavus A.	19	0.45
Cladosporium sphaerospermum	15	0.36
Penicillium digitatum	12	0.29
Alternaria alternata	10	0.24
Rhodotorulla glutinis	10	0.24
Paecilomyces variotii	8	0.19
Geotrichum candidium	7	0.16
Aurobasidium pullulans	6	0.14
chrysogenum P.	6	0.14
Cladosporium macrocarpium	5	0.12
Black yeast	5	0.12
fumigatus A.	3	0.07
notatum P.	2	0.04

Table (3) types of fungi isolated from the nose of the children

Fungal species	Number of isolated fungal	%
Aspergillus niger	157	43.61
Cladosporium cladosporoidies	90	41.12
A. flavus	64	8.27
C. albicans	50	2.03
Penicillium digitatum	50	1.45
Cryptococcus spp	35	0.89
C. tropicalis	34	0.45

sphaerospermum Cl.	17	0.36
Alternaria alternata	15	0.29
P. chrysogenum	12	0.24
Geotrichum candidum	11	0.24
Rhodotorulla glutinis	10	0.19
Bibolaris spicife	10	0.16
A. terreus	8	0.14
P. notatum	8	0.14
macrocarpum Cl.	6	
A. fumigatus	5	
A. ochraceus	4	
Aurobasidium pullulans	3	
Fusarium longipes	3	
White mycelium	2	

Table (4) types of samples positive and negative obtained from the mouths of children born natural birth and caesarean section.

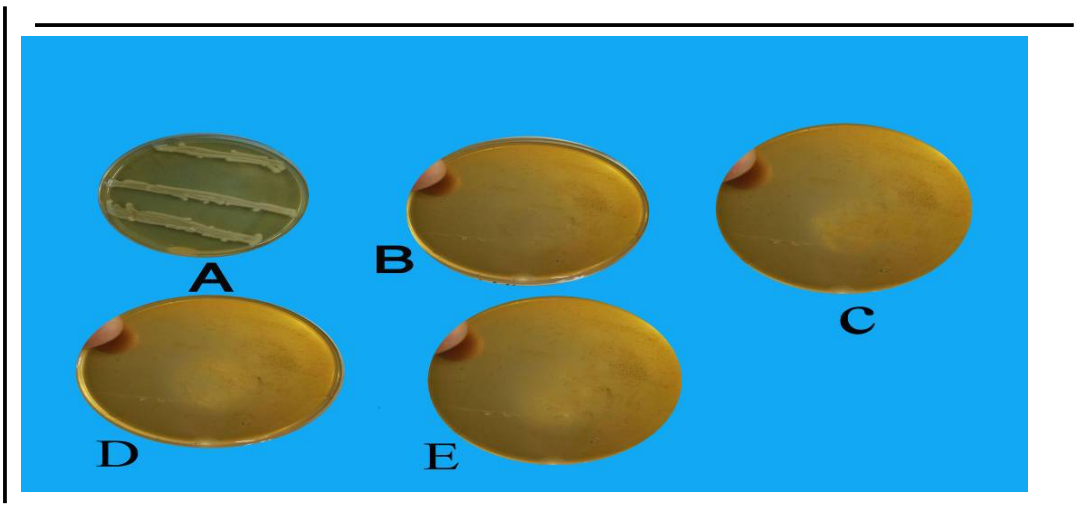
Number of samples	Natural childbirth	%	Caesarean section	%
The number of samples positive	45	60	27	36
The number of samples positive	30	40	48	64
Total	75	100	75	100

Table (5) types of fungi isolated from the mouths of children born cavity newly Natural childbirth.

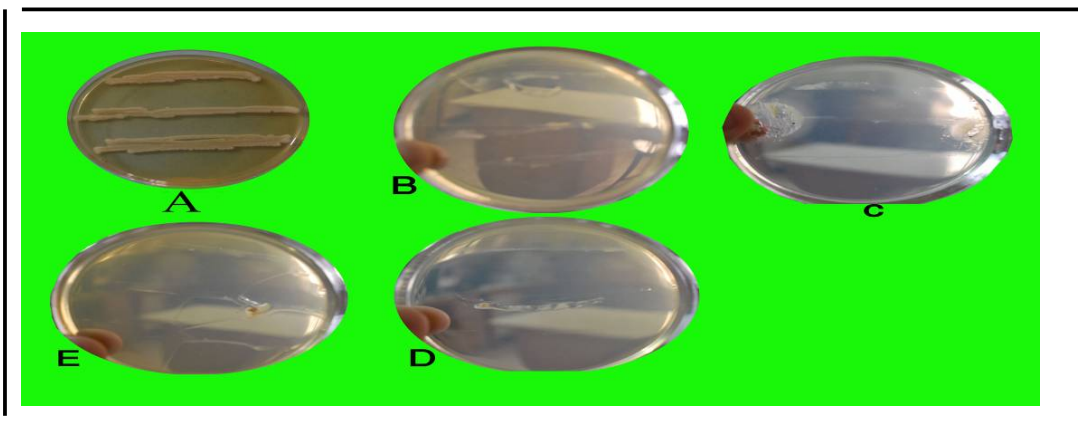
Fungal species	Number of isolated fungal	%
C. albicans	17	37.77
C. tropicalis	10	22.22
White mycelium	7	15.55
Rhodotorulla glutinis	5	11.11
Alternaria alternata	3	1.45
A. flavus	2	6.66
Aureobasidium pullulans	1	4.44
Total	45	100

Table (6) types of fungi isolated from the mouths of newborn babies Caesarean section

Fungal species	Number of isolated fungal	%
<i>C. albicans</i>	9	33.33
<i>C. tropicalis</i>	7	25.92
White mycelium	6	22.22
<i>Rhodotorulla glutinis</i>	5	18.51
Total	27	100



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- 1- The effect of hot water extract of thyme in the rate of diameters colonies of yeast *Cryptococcus* spp incubated under conditions of 7 days 28 degree
- 2- The influence of alcoholic extract of thyme in the rate of yeast colonies diameters *Cryptococcus* spp incubated under conditions of 7 days 28 degree m

A- Concentration of the extract thyme 5 mg / ml (control)

B- Concentration of the extract thyme 10 mg / ml

C- Concentration of the extract thyme 15 mg / ml

D- Concentration of the extract thyme 20 mg / ml

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