

# Isolation and identification of oral candida spp. from leukemic children under chemotherapy and treatment with extraction of different plants in vitro

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## ABSTRACT

Background: Oral candidiasis represents a serious problem for children with cancer during chemotherapy. The aim of this study was isolation and identification of oral candida spp.

Materials and method: Sixty five oral swabs were taken from children suffering from leukemia.

Results and Conclusion: The results of isolation in this research showed prevalence of candida.albicans at 64.62 % and C.glabrata 20%. In vitro antifungal activity of aqueous of (Quercus infectoria , Cinnamon , punica Granatum) at concentration 100-500mg/ml against ( C. albicans, C.glabrata, C.tropicalis and C .famata ) showed the Quercus infectoria was more active against ( C. albicans, C.glabrata, C.tropicalis and C .famata ) compared with others plants extracts which used in this study.

Key word: Oral candidiasis, candida albicans, Quercus infectoria, Cinnamon, punica granatum. (J Bagh Coll Dentistry 2012; 24(4):152-156).

## INTRODUCTION

The term leukemia refers to cancers of the white blood cells. When a child has leukemia, large numbers of abnormal white blood cells are produced in the bone marrow <sup>(1)</sup>. Leukemia is a common malignancy seen in young children and acute lymphoblastic leukemia (ALL) accounts for 75% of all leukemias. Immune suppression caused due to disease and therapy makes these children more prone to bacterial, fungal infections and at times reactivation of viral diseases <sup>(2)</sup>. Candida species are commensal yeast in healthy humans and may cause systemic infections under immunocompromised situations <sup>(3)</sup>.

Candida albicans is the most common species of yeasts isolated from patients with these predisposing factors and non-albicans species have been isolated from the oral cavity of immunocompromised patients, such as C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, C. guilliermondii C. dubliniensis <sup>(4)</sup>.

Leukemia patients undergoing chemotherapy present an optimal environment for the development of oral candidiasis, especially during periods of neutropenia. Candida species are responsible for approximately one-half of all oral infections occurring during antileukemia chemotherapy.

The detection of oral Candida colonization is important in the treatment of leukemia patients, since the oral cavity may serve as a reservoir for systemic infection. While children with leukemia are surviving longer due to advances in the diagnosis and treatment of the disorder, they now have a greater risk of developing opportunistic infections, such as candidiasis, during antineoplastic therapy.

The frequency of an infectious logy patients has been reported as high as 38% <sup>(5)</sup>.

Fungal infections: Bone marrow suppression, oral mucosal lesions and salivary alterations contribute to the development of Candida albicans infection. The most common presentations are pseudomembranous candidiasis, followed by erythematous candidiasis <sup>(6)</sup>.

### Medical plants

Various parts of the medicinal plants used for various treatments include the leaves, barks, tubers, roots, herbs and the plant extracts. These parts secrete various substances contain chemical materials which have great benefits for its physiological effects and healing activities for humans and animals, there are use 3 plants:

**Quercus infectoria:** Olivier (Fagaceae) was studied in order to investigate its antifungal properties. Quercus infectoria is a round-shaped abnormal growth found arising on young branches of the oak tree. The galls are locally known as manjakani in Malaysia, traditional medicine have been used as dental powder and in the treatment of toothache and gingivitis. The galls of Q. infectoria have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal, and anti-inflammatory activities. The main constituents found in the galls of Q. infectoria are tannin (50-70%) and small amount of free gallic acid and ellagic acid . and vitamins A and C, calcium, iron, fiber, protein and carbohydrates and has the ability to be an antibacterial and antifungal agent <sup>(7)</sup>.

**Cinnamon:** The name cinnamon is derived from a Greek word 'Kinnamon', meaning sweet wood.

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The botanical name for the spice *Ci. zeylanicumis* derived from the Sri Lanka's former name Ceylon. It is derived from the inner bark of cinnamon tree that can be used in both sweet and flavoring agent in foods, The *Ci. zeylanicum* is small ever green 10-15meters tall The flowers are arranged in panicles have greenish color, and a distinct odor; it is a purple, one centimeter berry containing a single seed , The bark is pale brown and papery, with thick quills that roll inside one another, and is gathered every 2 years. The antimicrobial properties of essential oils have been known for many years and have been used against a wide variety of bacteria and fungi, including oral pathogens. The principal constituents of *Ci. zeylanicum* bark contains essential oil including 27 components. The main component was cinnamaldehyde (55.79%). The other components in significant percent were limonene (8.31%), eugenol (7.09%) and Cinnamaldehyde propylene (5.55%). It's also contains nonvolatile oils including alkaloids, flavonoid, tannin, Cinnamon is source of minerals, vitamins, fibers and carbohydrates<sup>(8)</sup>.

**Peels or scaled of punica Granatum:** *Punica granatum* L. (Punicaceae), commonly called pomegranate, is a plant used in folkloric medicine for the treatment of various diseases, such as ulcer, hepatic damage and snakebite<sup>(9)</sup>. its native to the region from northern India to Iran. Pharmacological effects of pomegranate represent a long history and have been mentioned in the Greek and Egyptian documents, Recently studies have shown that pomegranate has many potential effects including: bacteriocidal, antifungal, antiviral, immune modulation, astringent, stomachic, styptic, laxative, diuretic and anthelmintic. denture stomatitis, vaginitis, and It is widely reported that pomegranate exhibits antiviral, antioxidant, anticancer activities<sup>(10)</sup>, so the current study was established to isolation and identification of oral candida spp. from leukemic children under chemotherapy and treatment with extraction of different plants in vitro.

## MATERIALS AND METHODS

**Collection of the samples:** 65 swabs were taken from hospitalized children undergo acute leukemia during chemotherapy and suffering from (oral thrush, oral ulceration, mucositis , mucosal ulceration and white plaques) , and these swabs were cultured on SDA and differentiated by use (CHROM candida agar), germ tube test and use of API candida .

### Collection of the plants:

**Quercus infectoria and the Cinnamon:** were brought from the local market. The oak galls were washed with distilled water, left for drying at room temperature then the samples were crushed into pieces and grinded into powder using a grinder before being sieved to get only a fine powder the powder was kept in the dry container for using in another time<sup>(11)</sup>.

**Peels or scaled of punica Granatum:** pomegranate was collected from Fruit s shop Peels or scaled separated and then cut into smaller pieces and then first washed with tap water followed by washing with distilled water It was than dried under sunlight until water droplets got completely evaporated. then grinded into powder using a grinder before being sieved to get only a fine powder the powder was kept in the dry container for using in another time<sup>(11)</sup>.

### Preparation of the Aqueous (boil distilled water) of (Quercus infectoria, Cinnamon, punica Granatum) extracts:

18 gm of powder of (*Quercus infectoria* , Cinnamon , punica Granatum) in 3 flask then add 150 ml of boil distilled water for aqueous extracts then left on magnetic stirrer for two days. Suspension was filtrated by Whatman No.2. filter paper and the filtrate was dried in oven at 37 C° for (48-72)hr until fully dried and kept in the refrigerator for usage in another time this powder which used to proper different concentration for different plant extracts<sup>(12)</sup>.

### Proper different concentration of (Quercus infectoria, Cinnamon, punica Granatum) extracts:

Take (1-5g) of powder from each extracts and solvent with 10ml of disitilled water at room temperature 25C° to proper the concentration (100-500 mg/ml).

### Preparation of antifungal susceptibility test:

0.1ml was taken of Candida spp. suspension (10<sup>6</sup> cells / ml) was put on surface of Sabouraud Dextrose Agar (SDA) media and spread by using glass spreader L-shaped , left to dry for (10-20) minutes, then by using sterilized cork borer (5mm) diameters , wells were made in the (SDA) medium then add 0.1ml for each concentrations of the each plants extracts (*Quercus infectoria* , Cinnamon , punica Granatum) in three repeated Petri dishes for each treatment and incubated at 37C° for (24-48) hr . The activity of each concentration of the differents extracts was determined by measuring of diameter of the

inhibition zone around each well using the ruler<sup>(13)</sup>.

## RESULTS AND DISCUSSION

**Results of Isolation:** The presented study included:

- a) 65 hospitalized children swabs which 55 samples had acute lymphoblastic leukemia (ALL) and 10 had acute myelogenous leukemia (AML). The aged ranged from 3 months to 14 years. The ratio of infection with acute leukemia in male 53.9% while in females 46.1% , and the ratio of infection with acute leukemia increase in aged (4-7) year for females and (8-11) year for male (table 1).
- b) The results of culture of the samples showed the ratio of *C. albicans* 64.62% was the main etiological agent of oral infection during chemotherapy compared with other species showed in table 2. this result showed the relationship between the oral candidiasis and treated the children with chemotherapy because the chemotherapy causes changes in oral cavity and increase proliferation of oral candida and buccal infection and the oral candidiasis represents a serious problem for children with cancer the mortality rate of this infection has increased due to fungal septicemia associated with a primary buccal infection<sup>(4)</sup>.
- c) **Inhibitory effects of plants extracts:**

### 1. Inhibitory effect of aqueous of *Quercus infectoria* :

The results of inhibitory effect of aqueous of *Quercus infectoria* on growth of ( *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. famata* ) showed inhibition on growth these pathogens and showed more inhibition on growth of *C. glabrata* compared with inhibition on growth of others pathogens ( table 3 and figure 1-4 ) .that indicate the isolation of *C. glabrata* was more sensitive to aqueous of *Quercus infectoria* because the used of boiled distilled water was need to dissolve out all these these extracts may have high total tannin contents as tannin is a major compound in *Q. infectoria* which is soluble in water , Tannin concentration is high in gall oak; a typical analysis is: gallotannin 53.1%, gallic acid 9.5%, ellagitannin 6.9%. Tannins have structural diversity and are divided into two basic groups: hydrolysable type and condensed type. Hydrolyzable tannins include gallic acid and ellagic acid. Both types of tannins have been used in disease treatment, but hydrolysable tannins have been used more medicinally used to be antifungal and antibacterial activity<sup>(7)</sup>.

### 2. Inhibitory effect of aqueous of the Cinnamon:

The results of inhibitory effect of aqueous of the Cinnamon on growth of ( *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. famata* ) showed no inhibition zone on growth ( *C. glabrata*, *C. tropicalis* and *C. famata* ) , and showed more inhibition on growth of *C. albicans* (table 3 and figure 5) ,because the main component of cinnamon was cinnamaldehyde and also contains nonvolatile oils including alkaloids, flavonoid, tannin. Cinnamaldehyde has been also shown to possess the strongest antifungal activity in comparison with other constituents of cinnamon oil It has been proposed that cinnamaldehyde and eugenol inhibit production of an essential enzyme and/or cause damage to the cell wall of the bacteria and fungi<sup>(8)</sup>

### 3. Inhibitory effect of aqueous of punica Granatum:

The results of inhibitory effect of aqueous of punica Granatum on growth of ( *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. famata* ) showed no inhibition zone on growth ( *C. albicans*, *C. tropicalis* and *C. famata* ) , and showed more inhibition on growth of *C. glabrata* ( table 3 and figure 6) , because the punica Granatum contain Pomegranate polyphenols include flavonoids (flavonols, flavanols and anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins). It means that the antimicrobial effect of tannins is related to its toxicity and molecular structure. Tannins may act on the cell wall and across the cell membrane because they can precipitate proteins, so these composition prevent growth of fungus<sup>(10)</sup>.

In conclusion the showed *C. albicans* was high ratio to causes oral candidal in leukemic children during chemotherapy , and the *Quercus infectoria* was more active against ( *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. famata* ) compared with others plants extracts which used in this study.

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**Table 1: age and gender distribution of children with acute leukemia**

Age	Male	Femal	Total
3m-3year	8	9	17
4-7 year	7	14	21
8-11 year	12	5	17
12-14 year	8	2	10
total	35	30	65

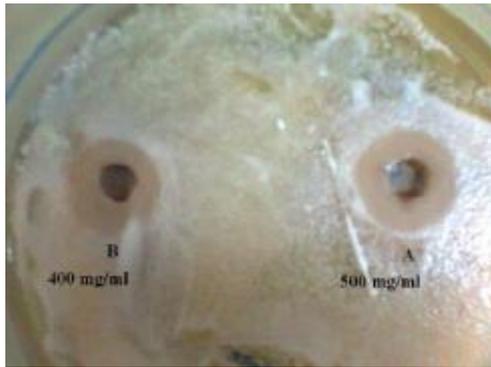
**Table 2: Number of candida isolated and percentage from children with acute leukemia during chemotherapy**

Type of candidal isolates	Number	Percentage %
C. albicans	42	64.62
C.glabrata	13	20
C.tropicalis	7	10.77
C .famata	2	3.07
C .parasilosis	1	1.54
Total	65	100

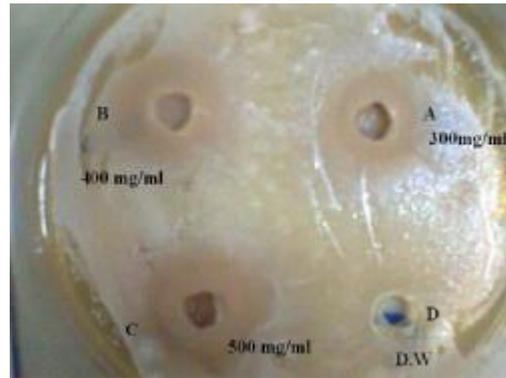
**Table 3: inhibition Zone (mm) of Aqueous of (*Quercus infectoria*, Cinnamon, punica Granatum) extracts on (*C. albicans*, *C. glabrata*, *C. tropicalis* and *C .famata*) in vitro**

plant extract	Concentration	<i>Candida albicans</i>	<i>C.glabrata</i>	<i>C.tropicalis</i>	<i>C .famata</i>
	mg/ml				
<i>Quercus infectoria</i>	100	11±0.5	15.66±0.66	6.66±0.33	—
	200	14.66±0.32	20.33±0.33	9±0.57	6.66±0.33
	300	16.33±0.62	21.66±0.33	13.33±0.88	8.66±0.33
	400	16.66±0.33	22.66±0.66	17.66±0.33	11±0.57
	500	17.66±0.66	23.66±0.33	20.33±0.33	14.66±0.33
Cinnamon	100	11.33±0.33	—	—	—
	200	16.33±0.66	—	—	—
	300	16.33±0.41	—	—	—
	400	23±0.33	—	—	—
	500	24±0.33	—	—	—
punica Granatum	100	—	15.66±0.66	—	—
	200	—	17±1	—	—
	300	—	20.33±0.33	—	—
	400	—	23.33±0.97	—	—
	500	—	24±0.91	—	—

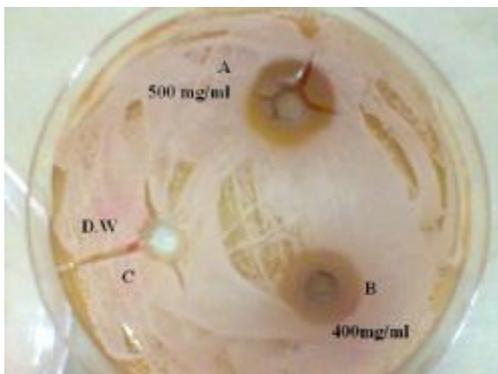
- The inhibition zone represented by mean± SE



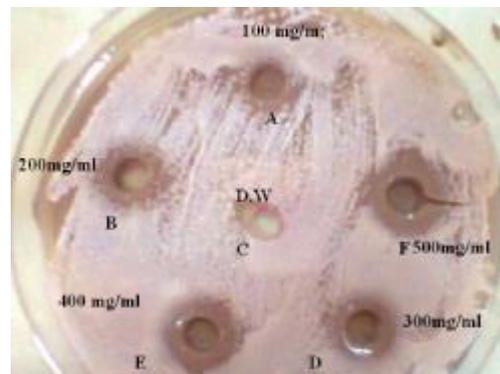
**Fig.1.** The effects of the Aqueous of *infectoria* on *C. albicans* in vitro at different concentration.



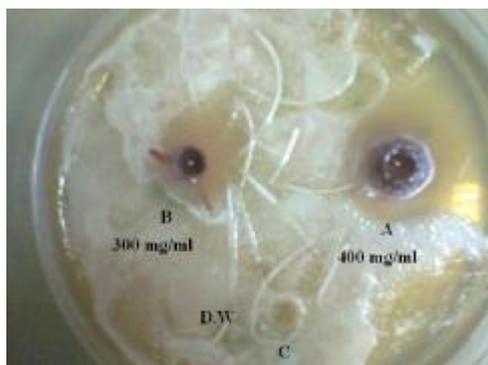
**Fig.2.** The effects of the Aqueous of *Quercus infectoria* on *C.glabrata* in vitro at different concentration.



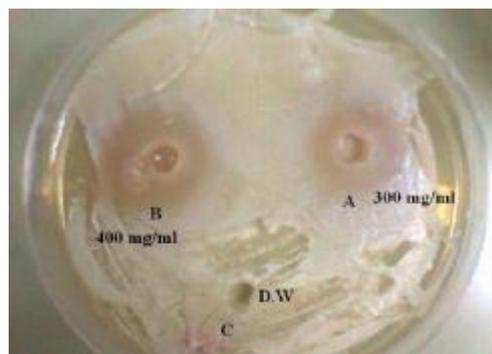
**Fig. 3.** The effects of the Aqueous of *infectoria* on *C.tropicalis* in vitro at different concentration.



**Fig. 4.** The effects of the Aqueous of *Quercus infectoria* on *C. famata* in vitro at different concentration.



**Fig. 5.** The effects of the Aqueous of Cinnamon on *C. albicans* in vitro at different concentration.



**Fig. 6.** The effects of the Aqueous of *punica Granatum* on *C. glabrata* in vitro at different concentration.