



# Evaluation Activity of Natural Products as Antifungal Against Diaper Candidiasis

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## Abstract

Many types of fungi have the ability to cause candidiasis, and these fungi live naturally on the skin and mucous membranes as well as on the digestive system, where they colonize the mentioned places for all humans immediately after birth, and one of the common causes is *Candida albicans* that causes diaper rash in newborns. 100 samples were collected from different clinical cases, (100 skin swabs from children with diaper rash who were lying in the halls of the Karbala Teaching Hospital for Children) To diagnosis of candida causing diaper rash and study of its sensitivity to natural products The result was of phenotypic and biochemical tests of the isolated samples showed that (76%) isolates belong to the yeast *Candida albicans*. As a result of the resistance of fungi against chemical antifungal, it was important to search for more effective natural alternatives. The alcoholic extract of natural products showed a great and high ability to inhibit all isolates in comparison with common chemical antifungals.

**Key Words:** Natural Products, Antifungal, Diaper Candidiasis, *Oidium Albicans*.

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1765

## Introduction

*Oidium albicans* in 1853 naming Charles Philippe Robin was launched Referring to *albicans* using *Candida albicans* terms on yeast caused by this fungus. And I knew (Thrush) one of the symptoms of thrush (Whiten). In general Brownlie either. Zopf in the year 1890 by *Monilia albicans* in the name of Then its name settled to become *Blastomyces albicans* 1920, it was called and discovered the ability of yeast. Berkhout in 1923 from *Candida albicans* Slutsky by Switching morphology on *C.albicans* and his group 1985 (Refai, et al. 2015). Fungal pathogens have a significant global impact on human health. It is estimated that, at any one time, more than a quarter of the world's population suffers from a fungal infection of the skin, that 75% of women will have at least one bout of vaginal candidiasis during their lifetime, and that more than one million people die each year from the fungal infection invasive (d'Enfert et al., 2020). Many forms of fungi have the potential to cause candidiasis, this fungus lives naturally on the skin and mucous membranes as well as the digestive system, where it colonizes the mentioned places for

all humans immediately after birth, the widespread use of antibiotics led to the emergence of strains more resistant and able to cause disease *Candida* types cause both skin and systemic infections, and each infection has different clinical manifestations. (Karen C. Carroll, 2016). One of these forms is diaper infection in children. This infection occurs as a result of the *Candida albicans* invasion of the skin due to the availability of appropriate conditions of moisture and temperature, which causes irritation in the skin and dermatitis that may develop and become severe ulcers in the diaper area that are difficult to deal with and treat due to the repetition of conditions suitable for the growth of the fungus, as well as some Diseases that lower the immunity of the child (Holst R. 2018). Although there are many common types of *Candida*, *Candida albicans* causes more than 80% of *Candida* infections. (Scully et al., 1994) (Talapko et al., 2021). Is a multi-form fungus that lives on individuals naturally without causing any harm, but in certain circumstances it can cause infections ranging from superficial infections of the skin to systemic infections.

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There are many factors and activities that contributed to making this type of fungus pathogenic. Among these factors are its ability to attach to host cells, secretion of hydrolytic enzymes, phenotypic alteration, and formation of biofilms. All these characteristics make this fungus capable of causing diseases (Mayer et al., 2013). The statistical rate of the emergence of human fungal infections is on the rise with the increase in cases of resistance to common antifungals, as the use of chemical antibiotics indiscriminately caused the escalation of resistance cases, as in resistance to azoles, for example, where the unprogrammed and unusual use has caused the In the end, the fungi developed forms of this resistance, and one of these results is the high risk of human infection with these opportunistic fungi, as they gradually acquired this resistance against many fungal chemical antibiotics, and for the purpose of controlling these infections, it became necessary to search for alternatives to avoid the dangerous failure of these chemical antibiotics. scientist Dragendra defined that everything is of plant origin and can be used to treat a specific disease it is a medicinal plant. A plant is called a medicinal plant if one or more of its members contains one or more chemicals in low or high concentrations and has the physiological ability to treat a specific disease or at least reduce the symptoms of this disease if given to the patient in its pure form or in the form of fresh herb or reduced or partially extracted (Al-Abed Ibrahim, 2009). During this study, alcoholic extracts of six medicinal plants were used, which are the following: aloe vera, pomegranate, pine, Nigella sativa, oleander, and eucalyptus. The aim of this study is to Isolation, identification of *Candida albicans* from clinical samples in children with diaper rash and Conducting a sensitivity test for fungal isolates against Alcoholic extract of medicinal plants to determine the level of effect on growth.

## Materials and Methods

### Sample Collection

100 samples were collected from different clinical cases, (100 skin swabs from children with diaper rash who were lying in the halls of the Karbala Teaching Hospital for Children). Swabs were taken by swab transport media from the area of the folds between the thighs in the affected place And then transported samples sick by the transport media to the laboratory for diagnostic tests

### Isolation and Identification

Samples were grown on Sabouraud agar medium As cotton swabs were planned on the surface of the food medium The dishes were incubated at 37°C for 24 hours. (Atlas, 1995). *Candida albicans* was diagnosed based on a set of phenotypic and biochemical characteristics, according to (Murray et al., 1999).

1. Direct microscopic examination of the samples. The examination was carried out according to a method (Sood, 1994; Morello et al., 2003).
2. The composition of the germ tube, the examination was carried out according to the method (Baker, 1967; Forbes et al., 2007).
3. The developing colonies appeared on SD Agar medium in the form of smooth white to cream colored colonies (Ellis et al., 2007)) Singh et al., 2013).
4. The cultured isolates gave a positive reaction with gram stain (Boon et al., 2013). As a result of the retention of this pigment, the peptidoglycan present in the cell wall (Sudbery et al., 2004).
5. And when the diagnosed isolates were grown on chromogenic medium for 24-48 hours at a temperature of 37. Showed green growth (Varaiya and Raut., 2009). This medium was used as a differential medium with accurate results in the diagnosis of *Candida* species.

Preparation of the alcoholic extract of the plants used in the study:-

Collecting plant parts after separating and washing they were dried at room temperature (25°C) and then ground with an electric grinder. (To obtain the alcoholic extract of medicinal plants, 300 gm of medicinal plants powder was placed in a glass container, and ethyl alcohol was used as a solvent, where 500 ml of alcohol was placed in an airtight container, and the process of extraction after 7 days when it was filtered and then filtered with filter papers and then the extract was evaporated to obtain a dry powder - The extract was concentrated in a powder form by drying it using a rotary evaporator at a temperature not exceeding 40° C. The concentrated extract (the powder) was kept in polyethylene bags at 4°C until different concentrations were prepared. (Radulovic et al., 2007).



**Preparation of Medicinal Plant Extract Concentrations**

1. A stock solution was prepared for the alcoholic extract of medicinal plant, with a weight of 1.5 gm (1500 mg) of the previously prepared extract powder.
  2. 10 ml of ethyl alcohol was added to it at a concentration of (70%) to get a Stock solution with a concentration of 150 mg/ml.
  3. The concentration of 100 mg/ml was prepared by taking 2 ml of Stock Solution and adding to it 1 ml of ethyl alcohol 70%.
  4. The concentration of 50 mg/ml was prepared by taking 1 ml of Stock Solution and adding to it 2 ml of ethyl alcohol 70%.
  5. The concentration of 25 mg/ml was prepared by taking 0.5 ml of Stock Solution and adding 2.5 ml of 70% ethyl alcohol to it.
  6. The concentration was 12.5 mg/ml by taking 0.25 ml of Stock Solution and adding to it 2.75 ml of ethyl alcohol 70%.
- (Wang et al., 2006) (Al Daami, 2014).

while 39 isolates were resistant to the drug.alcoholic extract of pomegranate peel at a concentration of 50 mcg/ml there are 76 isolates were affected by 20 mm inhibition zones out of 76 isolates. alcoholic extract of pomegranate peel at a concentration of 100 mcg/ml there are 76 isolates were affected by 24 mm inhibition zones out of 76 isolates. alcoholic extract of eucalyptus at a concentration of 50 and 100 mcg/ml It slightly and ineffectively affected the growth of some Candida isolates(there are 5 isolates were affected by <12 mm inhibition zones out of 76 isolates).alcoholic extract of oleander at a concentration of 50 and 100 mcg/ml Little effect (there are 7 isolates were affected by <10 mm inhibition zones out of 76 isolates).alcoholic extract of aloe vera at a concentration of 50 and 100 mcg/ml It was not effective.alcoholic extract of pine at a concentration of 50 and 100 mcg/ml very limited effect (there are 3 isolates were affected by <10 mm inhibition zones out of 76 isolates) alcoholic extract of Nigella sativa at a concentration of 50 and 100 mcg/ml It was not effective.

**Sensitivity Test of Medicinal Plant Extract**

The filter disc is divided into two groups with a diameter of 0.22 cm. The first group was added at a concentration of 50 µg/ml and the second group was added at a concentration of 100 µg/ml to test the sensitivity of the isolates against these extracts. Drug sensitivity was tested using medicinal plant extract compared with common chemical antifungals, Inhibition zones are measured in millimeters (mm).

**Results**

From a total of 100 samples taken from children with diaper rash, 76 isolates of Candida albicans were diagnosed. The results of tests for sensitivity to chemical antifungals and medicinal plant extract it showed a variance in the effect against isolates there are 31 isolates were affected Fluconazole drug 25 microgram/disk by > 20 mm inhibition zones out of 76 isolates, while 45 isolates were resistant and did not affected by the antifungal. While the number of isolates affected by nystatin drug 50 microgram/disk is only 28 by >15 mm inhibition zones out of 76 isolates, and the remaining 48 isolates were resistant to the drug. Also, amphotericin B 100 microgram/disk sensitivity showed that only 37 isolates were affected by >15 Inhibition Zone out of 76 isolates,

**Table 1.** The sensitivity test of *C.albicans* isolates against common antifungals and alcoholic extracts of medicinal plants

Antifungal drug	Concentration By mg	Inhibition Zone by mm	Isolates No.	Sensitivity
Fluconazole	25	>20	31	S
		<15	45	R
Nystatin	50	>15	28	S
		<10	48	R
Amphotericin B	100	>15	37	S
		<10	39	R
Alcoholic extract of pomegranate peels	50	>20	76	S
		0	0	R
	100	>24	76	S
		0	0	R
Alcoholic extract of eucalyptus	50	0	0	-
	100	<12	5	-
Alcoholic extract of oleander	50	0	0	-
	100	<10	7	-
Alcoholic extract of aloe vera	50	0	0	-
	100	0	0	-
Alcoholic extract of pine	50	0	0	-
	100	<10	3	-
Alcoholic extract of Nigella sativa	50	0	0	-
	100	0	0	-



During the study, we have found that there is a large spread of *Candida albicans*, and this is natural, as it is the most widespread species and causes infections (Talapko et al., 2021).

The excessive use of random antifungals led to the occurrence of resistance of *Candida albicans* due to the occurrence of genetic mutations in the genes of the treatment target in the treatment of nystatin and azoles.

(Sultan & Zghair, 2020). The use of a natural alternative instead of fungal chemical compounds produces an outlook in the field of safe and effective drug treatment, It is believed that the alcoholic extract of the pomegranate peel contains many of the active secondary compounds (tannins, terpenoids, flavonoids, alkaloids and others) (Sadeghin et al., 2011). The sources indicate that it is highly effective in controlling the growth of fungi and may lead to the presence of the active secondary compounds because it has the ability to inhibit the growth of *Candida albicans* cells and other microbes (Singla et al. 2013) (Ahmed et al. 2013). There is resistance of *Candida albicans* isolates against common chemical antifungals, and the alcoholic extract of pomegranate peels showed sensitivity and high efficacy against these isolates:

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