Antifungal Activity of Diacetyl Compound against *Candida* Isolates in Vitro

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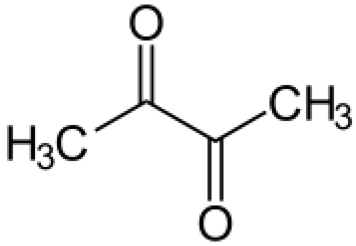
ABSTRACT

To overcome the development of drug resistance, it is necessary to find a new class of antimicrobial compounds possessing different mechanism or chemical properties from those that are used commonly for the treatment of the diseases which are caused by *Candida albicans*. This study was designed to investigate the antifungal activity of Diacetyl against *Candida albicans* isolates with a total of forty eight *Candida albicans* isolates which was isolated from sputum, vagina and urine sample of different patients were tested against several concentrations of Diacetyl compound (30,60,120,180)µg/ml and for different incubation time to examine the inhibitory activity of the compound *in vitro*. All urine isolates showed 100% complete inhibition to(30,60,120,180) µg/ml concentrations after 24,48,72 hrs incubation at 37C°,while only three vaginal isolates 30% and eleven sputum isolates 42.3% showed resistance after 48 hrs incubation to 30 and 60 µg/ml concentrations. It seems that Diacetyl compound has a potent antifungal activity against *Candida albicans in vitro*.

INTROUDACTION

Candida albicans is the major fungal pathogen that affects humans. Although *Candida albicans* is a commensal organism on the cutaneous and mucosal surfaces of oral, gastrointestinal, urinary, and vaginal tracts of healthy individuals [1], [2], it is also an opportunistic pathogen and can cause infections ranging from superficial mucosal infections to hematogenously disseminated candidiasis. In immunocompromised patients, *Candida albicans* is responsible for a number of life-threatening infections [1], [2]. Moreover, with the rapidly expanding use of medical devices (e.g., indwelling catheters) and increases in the number of patients receiving antibiotic and immunosuppressive therapies, there is an increased risk of fungal penetration through mucosal barriers with

subsequent entry into the blood stream, which often leads to multi-organ infections. Consequently, *Candida albicans* is the leading cause of nosocomial bloodstream infections and has a mortality rate of 40% [3], [4]. *Candida albicans* can colonize or infect virtually all body sites because of its high daptability to different host niches by the activation of appropriate sets of genes in response to complex environmental signals [5–6].commensal, which can become a facultative pathogen under altered physiological situations. *Candida albicans* pathogenesis studies have suggested that several steps may lead to mucosal infection, i.e., early colonization, invasion, and late tissue disruption [7], [8]. *C. albicans* first colonizes and proliferates on the mucosal surfaces of host epithelial cells; these events are followed by invasion and tissue damage [9], [10]. Therefore, *C. albicans* adhesion is the first step in infection and allows the pathogen to persist on mucosal surfaces. As the outermost layer of *C. albicans*, the cell wall interacts with the host cells [11]. The cell wall of *C. albicans* contains many different carbohydrates and proteins that come into contact with epithelial cells and facilitate cell-cell interconnections [12] .Diacetyl (IUPAC systematic name: butanedione or 2,3-butanedione) is a natural byproduct of fermentation. It is a vicinal diketone (two C=O groups, side-by-side) with the molecular formula $C_4H_6O_2$. Diacetyl occurs naturally in alcoholic beverages and is added to some foods to impart a buttery flavor.



In food products Diacetyl and acetoin are two compounds that give butter its characteristic taste. Because of this, manufacturers of margarines or similar oil-based products typically add diacetyl and acetoin (along with beta carotene for the yellow color) to make the final product butter-flavored, because it would otherwise be relatively tasteless. [13].In milk products In milk, sour cream, cultured buttermilk or fermented butter are produced by inoculating pasteurized cream or milk with a lactic starter culture, churning (agitating) and holding the milk until a desired pH drop (or increase in acidity) is attained. This cultured sour cream, butter and buttermilk which are made by fermenting pasteurized and homogenized light cream or milk owes its tart flavor to lactic acid bacteria and its buttery aroma and taste to diacetyl[14].http://en.wikipedia.org/wiki/Diacetyl - cite_note-5The aim of the study was the determination of antifungal activity of Diacetyl against *Candida albicans* isolates.

MATERIALS & METHODS

Samples collection:

A total of forty eight isolates of *Candida albicans* were obtained from different specimens urine, vagina and sputum from Medical City Laboratory in Baghdad between March 2009 to January 2010.The samples were cultured on Saburoud agar and incubated for 24 hours at 37C°, then identified by gram staining and germ tube procedure [15] which conducted on each isolate.

Antifungal activity:

By using well assay [16].All isolates were cultured oin Muller-Hinton agar plate (Oxoid) each plate were cultured with overnight growth from nutrient broth by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 5.0 mm was used to bore five wells in each agar plates. Four concentration of the Diacetyl compound (Fluka) were made (30,60,120,180) µg\ml .A 50µL volume of each concentration was applied by micropipette in the wells into Muller-Hinton Agar plate and distilled water used as a control. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37ºC for 24hrs-48hrs-72hrs to examine if Diacetyl compound have cidal or static effect on *Candida albicans* isolates. The zone of inhibition was recorded in mm.

RESULT and DISCUSSION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antimicrobial drug in current clinical use[17]1. Antifungal drug resistance is fast becoming a major problem; particularly with the immuno-compromised population[18].Many works equally reviewed that the emergence of resistance occurred following prolonged therapy, and that switching (the ability of Candida species to generate a variety of phenotypes) is a virulent factor [19].In the present study, we have examined the antifungal properties of Diacetyl compound the result of this study showed that different inhibitory effect of Diacetyl compound concentrations against *Candida albicans* isolates taken from sputum samples were observed. There was eleven isolates(42.3%) showed resistance to 30,60 µg/ml concentration after 48 hrs incubation and the inhibitory effect started at 120 and 180 µg/ml concentration as shown in **table 1**.

Table 1: Antifungal activity of Diacetyl against *Candida albicans* isolated from sputum

isolate	24-48 hrs				72 hrs			
	30µg/ml	60µg/ml	120µg/ml	180µg/ml	30µg/ml	60µg/ml	120µg/ml	180µg/ml
	Inhibition zone (mm)				Inhibition zone (mm)			
*C.albicans*1	(1)R	R	23	31	R	R	23	31
*C albicans*2	(2)C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*3	R	R	14	17	R	R	11	15
*C.albicans*4	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C albicans*5	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*6	C.I	C.I	C.I	C.I	12	17	34	44
*C.albicans*7	C.I	C.I	C.I	C.I	50	C.I	C.I	40
*C.albicans*8	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*9	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*10	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*11	6	16	32	37	6	16	32	37
*C.albicans*12	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*13	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*14	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*15	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*16	R	R	17	19	R	R	17	19
*C.albicans*17	R	R	22	43	R	R	19	40
*C. albicans*18	R	R	25	40	R	R	25	40
*C. albicans*19	R	R	C.I	C.I	R	R	C.I	C.I
*C.albicans*20	R	R	32	40	R	R	32	40
*C.albicans*21	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*22	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*23	R	R	22	46	R	R	22	46
*C.albicans*24	R	R	R	R	R	R	R	R
*C.albicans*25	R	R	37	50	R	R	37	50
*C.albicans*26	R	R	C.I	C.I	R	C.I	C.I	C.I

(1)R: Resistant,(2)C.I.: complete growth inhibition

Preventive antifungal therapies have been adopt-ed to avoid the occurrence of systemic candidosis. Oral *Candida* spp. level control might be an important preventive measure since the occurrence of oral candidosis may be considered a potential risk for the occurrence of systemic diseases among immunocompromised patients. Biocides, better known as antiseptics, disinfectants, or preservatives, are commonly added to mouthwashes, toothpastes, hand soaps, and related consumer products. Pathogenic fungi have received little attention as biocide targets. *Candida albicans* is commonly found at low levels among the normal oral flora, but its overgrowth in immunocompromised individuals or following broad-spectrum antibiotic therapy leads to oropharyngeal candidiasis[20].This is typically treated with fluconazole or related azole antifungal, inhibitors of ergosterol biosynthesis [20-21].However, extended treatment frequently selects for fluconazole-resistant strains that display upregulated expression of multidrug transporters, specifically

those encoded by the *CDR1*, *CDR2*, and *MDR1* genes, along with mutations in the *ERG11*-encoded target enzyme [22-23].There have been no reports on the effect of Diacetyl on *Candida albicans*. Furthermore, no studies have examined Diacetyl interaction, i.e., the potential effects of combination treatment on oropharyngeal candidiasis. On the other hand *Candida albicans* isolates taken from vagina samples showed complete inhibition against Diacetyl (30, 60,120,180) µg\ml concentrations except three isolates (30%) was resistant as shown in **table 2**.

Table 2: Antifungal activity of Diacetyl against *Candida albicans* isolated from vagina

isolate	24-48 hrs				72 hrs			
	30µg/ml	60µg/ml	120µg/ml	180µg/ml	30µg/ml	60µg/ml	120µg/ml	180µg/ml
	Inhibition zone (mm)				Inhibition zone (mm)			
*C.albicans*1	R	R	19	39	R	R	19	39
*C albicans*2	R	R	20	24	R	R	20	24
*C. albicans*3	R	R	20	23	R	R	20	23
*C.albicans*4	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C albicans*5	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*6	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*7	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*8	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*9	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*10	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I

(1)R: Resistant,(2)C.I.: complete growth inhibition

While all *Candida albicans* isolates taken from urine samples showed complete inhibition(100%)against Diacetyl (30, 60,120,180) µg\ml concentrations after 24 hrs and the effect lasted up to 72 hrs incubation as shown in **table 3**.

Table 3: Antifungal activity of Diacetyl against *Candida albicans* isolated from urine

isolate	24-48 hrs				72 hrs			
	30µg/ml	60µg/ml	120µg/ml	180µg/ml	30µg/ml	60µg/ml	120µg/ml	180µg/ml
	Inhibition zone (mm)				Inhibition zone (mm)			
*C.albicans*1	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C albicans*2	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*3	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*4	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C albicans*5	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*6	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*7	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*8	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C. albicans*9	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*10	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*11	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I
*C.albicans*12	C.I	C.I	C.I	C.I	C.I	C.I	C.I	C.I

In case of *Candida albicans* infection of the vaginal ecosystem, astudy[24] found that Clotrimazole, although effective against *Candida albicans* infection,

also has a deleterious effect on components of the normal vaginal micro flora. One of the implications for women using clotrimazole for microbiologically undocumented vaginal yeast infections is an increased risk of infection or disease through the disruption of the protective micro flora barrier so it is necessary to find alternative treatment that has no effect on the ecosystem and has no side effect. It seems that Diacetyl compound has a potent antifungal activity against *Candida albicans in vitro.*

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دراسة الفعالية ضد ميكروبية لمركب Diacetyl ضد *Candida albicans*

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الخلاصة

للتغلب على مقاومة المضادات من الضروري ايجاد اصناف جديدة من المركبات الضد ميكروبية ذات ميكانيكية وتركيب كيميائي مختلف عن المواد الضد ميكروبية المستخدمة حاليا لعلاج الاصابات الناتجة عن الاصابة ب *Candida albicans* .اجريت التجربة على ثمان واربعون عزلة من *Candida albicans* المعزولة من القشع،المسحات المهبلية و الادرار من مختلف المرضى وتم استخدام تراكيز مختلفة من Diacetyl 30,60,120,180 مايكروغرام/مل وباوقات حضن زمنية مختلفة 24,48,72 ساعة في درجة حرارة 37° مئوية.كل عينات الادرار لم تظهر اي نمو في كل التراكيز المستخدمة بعد 24,48,72 ساعة بينما اظهرت ثلاث عزلات فقط من*Candida albicans* المعزولة من المهبل 30% واحدى عشر عزلة من العزلات المعزولة من القشع 42.3% مقاومة بعد 48 ساعة من الحضن لتركيز 30 و 60 مايكروغرام/مل من *Candida albicans*.التجربة تشير الى ان مركب Diacetyl له فعالية ضد ميكروبية ضد بعض عزلات *Candida albicans*.