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Studying the Synergistic Effect of both Propolis Extract and Green Apple Peel Extract with Fluconazole against *Candida albicans*

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ABSTRACT

Candida albicans were isolated from 36 isolates of the mouth people aged (2.5 – 63) years. A crude sample of bees propolis was collected from several apiaries in Mosul city and green apples were collected from the markets, evaluating antifungal of fluconazole, methanol extract of propolis (MPE) and methanol extract of green apples peels (MEGAP) were different concentration (125,250,375,500,750,1000,1500)ug/ml, each one alone and as combination of (fluconazole 50%+ MPE 50%) and of (fluconazole 50%+ MEGAP50%). Each one alone, the fluconazole only had inhibitory effect against *C.albicans*. As combination, the synergistic effect was observed of both combination (fluconazole 50% + EMP 50%) and (fluconazole 50%+MEGAP 50%) against *C.albicans*. In comparing between two combination observed that it was outdoing in a synergistic effect of (EMP) compared with (EMGAP) specially at concentrations (750 , 1000, 1500) ug/ml.

Keyword: *Candida albicans*, propolis, green apple peel, synergistic.

دراسة التأثير التأزري لكلا من مستخاص العكبر ومستخاص قشور التفاح الأخضر مع مركب الفلوكونازول ضد خميرة *Candida albicans*

الملخص

تم عزل فطر *Candida albicans* من 36 عزلة من فم أشخاص تتراوح أعمارهم بين (2.5 – 63) سنة، كما جمعت عينات العكبر من مناحل مختلفة من مدينة الموصل كذلك تم جمع التفاح الأخضر من الأسواق المحلية لنفس المدينة. اختبرت فعالية كل من المركب الدوائي Fluconazole، ومستخاص العكبر الميثانولي ومستخاص قشور التفاح الأخضر الميثانولي ضد فطر *C.albicans* بتراكيز مختلفة (125, 250, 375, 500, 750, 1000, 1500) مايكروغرام/مل وكل على حده، وكان لمركب Fluconazole فعالية تثبيطية مضادة للفطر دون المستخلصين الأخيرين. وعند دراسة التأثير التعاوني لخليط (Fluconazole 50% + مستخاص العكبر الايثانولي 50%) ولخليط (Fluconazole 50% + مستخاص قشور التفاح الأخضر 50%) وجد أن هناك تأثيراً تعاونياً تثبيطياً ملحوظاً ضد *C.albicans*، وكان هناك تفوق واضح في التأثير التعاوني لمستخاص العكبر مع مركب Fuconazole مقارنة مع التأثير التعاوني لمستخاص قشور التفاح الأخضر ولاسيما عند التركيز (750, 1000, 1500) مايكروغرام/مل.

الكلمات الدالة: *Candida albicans*، العكبر، قشور التفاح الأخضر، التآزر.

INTRODUCTION

In recent times, there has been a rapid increase in fungal infections globally and this could be attributed mainly to increased susceptibility of individuals to infection due to immunosuppression and invasive drug therapy (Schmidt-Westhausen *et al*., 2004; Sims *et al*., 2005; Schwab *et al.*,1997) reported the increased adherence of *Candida albicans* to buccal epithelial cells obtained from AIDS patients compared to non-sufferers. Multiple resistant phenotypes of *C.albicans* have been found to coexist during episodes of oropharyngeal candidiasis in AIDS patients (Lopez- Ribot *et al.,* 1999).*C. albicans* cells are protected by cell walls which mediate interaction with the host for adhesion and modulation of antifungal immune response in their host (Poulain and Jouault, 2004). Hence, the cell membrane may serve as a potential target for antifungal drugs. The mechanism of action of some anti-fungal drugs is by binding to the cell membrane of pathogenic fungi in the presence of certain sterols, which subsequently disturb permeability and transport characteristics of the membrane, resulting in loss of intracellular cations (Katzung, 1984). Apple tree belong to the family Rosaceae, subfamily Maloidea and genus Malus, being a fruit tree with highest importance worldwide, considered one of the main agricultural products (Victoretal, 2015). All sorts of apples have health benefits, but the thing that makes green apples special is that they have a high nutritional density packed with minerals, vitamins, protein and fiber (Barn-don, 2018).

Apple pulp contains catechin, procyanidin, caffeic acid and chlorogenic acid among other components. The skin contains all the afore mentioned substances as well as flavonoids, not present in pulp, such as quercetin glycosides and cyanidin glycosides (Escarpa and Gonzalez, 1998; Van der Sluis *et al.*, 2001). Epidemiological studies associate phenolic consumption with lower mortality, especially caused by coronary diseases. They present multiple biological properties, which are of growing interest for consumers due to the high antioxidant, anti-inflammatory, anti allergic, anti thrombosis and antimicrobial activities (Kanner *et al.*, 1994; Frankel *et al.*, 1995; Koga *et al.*, 1999; Eberhardt *et al*., 2000; Jayaprakasha *et al*., 2003; Baydar *et al*., 2004; Shoji *et al*., 2004).

The word propolis originates from Greek: «pro» = in front, «polis» = city. The meaning in front of the city, suits well the protecting role of propolis for the bee colony. The Greek word propolis means also to glue and describes also the role of propolis to cement openings of the bee hive. Another name of propolis is bee glue. Propolis was already known in ancient Egypt, where it was probably used as an adhesive. Propolis was mentioned by the Greek philosopher Aristoteles. In his Historiaanimalium it was referred to a substance which the bees smeared at the hive entrance and used as cure for bruises and sores (Crane, 1999). As the major constituents of propolis, flavonoids contribute greatly to the pharmacological activities of propolis. The quantity of flavonoids is used as a criterion to evaluate the quality of temperate propolis (Zhang *et al*., 2014) Flavonoids have a broad spectrum of biological properties, such as antibacterial, antiviral and anti-inflammatory effects (Bueno-Silva *et al*., 2013; Nijveldt *et al*., 2001). According to the chemical structure, flavonoids in propolis are classified into flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans, isoflavans and neoflavonoids. From 2000 to 2012, 112 flavonoids were identified in different type of propolis (Righi *et al*., 2011).

MATERIALS AND METHODS

Strains

Candida albicans was isolated from 36 isolates from the mouth of people aged (2.5 – 63) years, using sabroud dextrose agar (SDA), and tryptone soya agar (TSA) as growth media, and using serum blood (SB) as diagnostic media, incubate in 37 C˚ for 24 day (Sagar, 2016; Bougnoux *et al.*,1999).

Disc Diffusion Test

Disc diffusion test was used as an alternative measure of susceptibility and a counterpart method of minimum inhibitory concentration (MIC) and maximum inhibitory concentration

(MXIC), (NCCLS, 1996). Aseptically TSA plates were swabbed by *C.albicans*. Sterile paper discs (6 mm) were dipped in different dilution of (flucoconazole, green apple peels and propolis) each one alone and prepared combination of (fluconazole 50%+propolis 50%) and (fluconazole 50% + green apple peels 50%) in different dilution and placed on swab plates for *C.albicans* in specific dilutions and placed in media plate. In each dilution of each one was replicated thrice under factorial completely randomized design. Observed media plates and measured inhibition's zone from each paper disc in mm. If the test organism grows on the disc it may safely be assumed that the test organism is resistant (R).

The Extraction

A crude sample of bees propolis was collected from several apiaries in Mosul city and green apples were isolated from market. Methanol of propolis extract (MPE) and methanol of green apple peels extract (MGAPE) were performed according to the method described by Ran *et al*.,with minor modification (Junjian *et al*., 2013). (MPE) and (MGAPE) were extracted 125 gm with 500 ml of methanol that mean (1 : 4), in an ultrasonic bath at 37 c^o for 40 min. Two produced extracts were dried under negative pressure in rotary evaporation at 40 c^o and then re-dissolved 4g in 40 ml of edible alcohol.

The extracts was filtered through a 0.45-um membrane (Millipore) and stored in refrigerator at 4 c^o until analysis. The evaluating anti fungal effects were different concentrations (50,125, 250, 375, 500, 750, 1000, 1500) ug – ml.

RESULT AND DISCCUSION

After evaluating different concentrations of disc dilution of (fluconazole, green apples peels and propolis) each one alone, It was observed that the fluconazole only had inhibitory effect against *C.albicans* (Table 1). The (MIC) of fluconazole drug was 2 mm at concentration (125) ug/ml, and (MXIC) was 3.6 mm at concentration (1500) ug/ml. While, the synergistic effect was observed of combination (fluconazole 50% + MPE 50%) against *C.albicans*, the highest synergistic effect was 19.8% at concentration (1500)ug/ml , (Table 2).

Also there was a synergistic effect of combination (fluconazole 50% + MGAPE 50%), the highest synergistic effect was 12.9 % at concentration (1500)ug/ml, (Table 3).

At comparing between (Table 2) and (Table 3) observed that it was outdoing in a synergistic effect of (MPE) on (MGAPE) specially at the last three concentration (750, 1000, 1500) ug/ml.

Table 1: The inhibitory effect of fluconazole, (MPE) and (MGAPE) each one alone against *C.albicans*.

Fluconazole Concentration ug/ml	Average diameter of inhibition (mm)	(MPE) Concetration ug/ml	Average diameter of inhibition (mm)	(MGAPE) Concentration ug/ml	The inhibitory Mm
50	R	50	R	50	R
125	2.0	125	R	125	R
250	2.26	250	R	250	R
375	2.3	375	R	375	R
500	2.55	500	R	500	R
750	3.05	750	R	750	R
1000	3.4	1000	R	1000	R
1500	3.6	1500	R	1500	R

Table 2: The synergistic effect of combination (fluconazole 50% + MPE 50%) against *C.albicans*.

Concentration ug/ml	Average diameter of inhibition (mm)	(MPE) Concentrationug /ml	Average diameter of inhibition (mm)	Combination(fluconazole + MPE) (50%+50%) ug/ml	Average diameter of inhibition (mm)	A Synergistic Percent of combination %
125	1.8	125	R	250	1.85	2.7
250	2.26	250	R	500	2.3	1.8
375	2.3	375	R	750	2.76	16.7
500	2.55	500	R	1000	3.0	15
750	3.05	750	R	1500	3.8	19.8

Table 3: The synergistic effect of combination (fluconazole 50% + MGAPE 50%) against *C. albicans*.

Fluconazole Concentration ug/ml	Average diameter of inhibition (mm)	(MPE) Concentration ug/ml	Average diameter of inhibition (mm)	Combination (fluconazole +MGAPE) (50%+50%) ug/ml	Average diameter of inhibition (mm)	A Synergistic Percent of combination %
125	1.8	125	R	250	1.85	2.7
250	2.26	250	R	500	2.4	5.9
375	2.3	375	R	750	2.55	9.9
500	2.55	500	R	1000	2.8	9.0
750	3.05	750	R	1500	3.5	12.9

Early appropriate therapy may alter the course of fungal infections especially, in immunodeficient or immunosuppressed patients. Therefore, early determination of an organism's drug susceptibility (Hadley *et al*., 2002 ; Hosphental *et al*., 2004). In studying, the inhibitory range of (MIC) of fluconazole against (*C.albicans, C. parapsilosis* and *C. tropicalis*) was between (0.25 – 64) ug/ml (Fahriye *et al*., 2013). In this study, fluconazole didn't show inhibatory effect against *C.albicans* at concentration 50 ug/ml, the starting effect of fluconazole was at concentration (125) ug /ml. About (MPE) and (MGAPE) each one alone, I think the few concentrations used for the study were the same as the concentrations of fluconazole Which prevented any inhibitory effect against *C.albicans*.

The content of both propolis and green apple peels of phenolic compounds is due to the synergistic effect with fluconazole drug, Where propolis contains many phenolic compounds such as (Flavanone, Naringenin, Flavone, 3-Hydroxyflavone, 6-Hydroxyflavone, Morin, Chrysin, Quercetin, Galangin, Apigenin ..ect), (Marica *et al.*, 2004). Also green apple peels contains phenolic compounds, in study; the compounds most commonly in apple peels consists (catechin, procyanidin, epicatechin and phloridzin), (Escarpa and Gonzalez, 1998). Da Silva *et al*., (2004) agree with me when reported, Three combinations formed by the flavonoids (+)-catechin hydrated, hydrated quercetin, and (-)-epigallocatechingallate at a fixed concentration with fluconazole were tested. Flavonoids alone had no antifungal activity within the concentration range tested, but when they were used as a cotreatment with fluconazole, there was significant synergistic activity.The fact that the (MPE) is more synergisticthan (MGAPE) is due to Propolis, a large complex mixture of compounds (Ricardo *et al*., 2015). In the end, studies about propolis and green apple peel bioactivity must start with chemical profiling of the extracts since that information is essential to

have detailed and consistent comparative data between each type of biological activity and chemical data. This information allows extrapolating the possible activity and mechanism of action.

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