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RESEARCH ARTICLE

EXTRACTION OF SOME ACTIVE SUBSTANCES FROM PROPOLIS AND STUDYING ITS INHIBITION ACTIVITY AGAINST *CANDIDA ALBICANS* ISOLATED FROM PATIENTS

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ABSTRACT

Phenolic and flavonoids compounds were extracted from propolis obtained from Al-Hussainya district in Karbala province. Results revealed that incubation period 48 and 72hours were the optimum period for extraction total phenol and flavonoids, respectively. Where as the best ethanol concentrations for extraction of total phenol and flavonoids were 50% and 70 %, respectively. Inhibition activity of propolis was studied against 15 isolates of *Candida albicans* isolated from mouth, vagina, urine and skin of the patients. Results revealed presence of significant difference in the effect of propolis extract against the *C. albicans* isolates of this study. *C. albicans* isolates the isolate that isolated from mouth was the most sensitive isolate among the *C. albicans* isolates towards the propolis extract.

INTRODUCTION

Propolis defines as resin similar a wax known as bee glue. It's produced by honey bees from materials gathered from plants and mixed with honey wax and other compounds resulting from the metabolism of bees (Fokt *et al.*, 2010). Although propolis is animal product, but it is largely in terms of plant origin, as it consists of 50-55% balms, resins, gums, 30% wax, 5-10% pollen grain and 8-10% essential oils (Lindsberg *et al.*, 2011). The resins of the most abundant compounds in propolis and consists of flavonoids, phenolic acids, esters and constitute about 50% more than all of the other components (Stanciu and Mărghitaş, 2004), and it is known that propolis content rich balsamic high efficient components (Kujumgiev *et al.*, 1999). Propolis possesses antioxidant, antimicrobial, antitumor and anti-inflammation properties, as well as the hasp to protective properties (Selvan and Prabhu, 2010). Yeasts are opportunistic pathogens, and *Candida albicans* characterized the most common yeast which was isolated from the oral cavity in both healthy people and people with diseases (as it constitutes 60-80% of cases) (Mesrorian *et al.*, 2007). Yeast characterized as responsible for 80-95% of vaginal infections (Fare *et al.*, 1997).Therefore, this study aimed to assess the inhibition activity of propolis against *Candida albicans* yeasts causing mouth, skin, urinary tracts and vaginal infections.

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MATERIALS AND METHODS

Extraction of phenolic substances

Propolis has been grinding several times to get a very fine powder of it; the samples of propolis have been obtained from the apiaries of Hussainiah hand in holy Karbala province. The method described by Ahmed *et al.*, (1998) was followed in extraction of the phenolic substances in propolis, while the method described by Bullet and Shetpeak (2008) was followed in estimation of the total phenolic content in propolis samples of the study.

Estimation of flavonoids

Depending on the standard curve of quercetin and following the method described by Kosalec *et al.* (2005), the flavonoids content of propolis was estimated.

Inhibition activity of propolis against *Candida albicans* yeast

The inhibition activity of propolis extract was studied against fifteen isolates of *C. albicans* yeast; six isolates were isolated from mouth, five isolates were isolated from vagina, three isolates were isolated from urine and one isolate was isolated from skin. Agar well diffusion method was used to study inhibition activity of the propolis extract; therefore different concentrations of this extract ranged from 0.5-25mg/ml were prepared.

RESULTS AND DISCUSSION

Effect of ethanol concentration in extraction of total phenols and flavonoids from propolis

The Figure (1) show that the amount of phenols increase with increasing of ethanol concentration until reach the maximum amount (85.35 mg/g dry material) when 70% ethanol was used, and then the amount of phenols was decreased after that. The results obtained from this study are consistent with those reported in several studies which refers to use of 70% ethanol in extraction of the total phenols of propolis that collected from different regions of Bulgaria (Tylkowski *et al.*, 2010), as well as with the results of total phenols extraction of propolis from different regions of Italy, Switzerland (Bankova *et al.*, 2002) and Thailand (Kitcha-ounds *et al.*, 2013), whereas80% ethanol was used in extraction of the total phenols from propolis collected from Iran (Taghouti *et al.*, 2007).

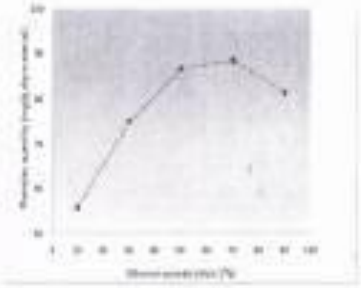

Figure 1. Effect of ethanol concentration in extraction of total phenols from propolis

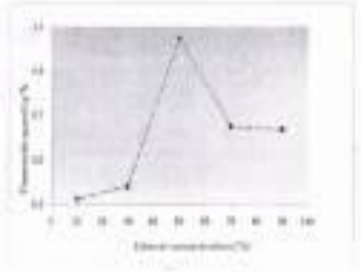

Figure 2. Effect of ethanol concentration in extraction of total flavonoids from propolis

The result from Figure (2) shows that the highest concentration of flavonoids (1.048%) was obtained when 70% ethanol was used. The results of this study are consistent with those reported in the previous studies that included using a different concentrations (35-85%) of ethanol, which was found that the use of 71% ethanol is the most efficient in extraction of the total flavonoids from propolis (Margaritis *et al.*, 2013), whereas the use of 80% ethanol is the most efficient in extraction of the total flavonoids from propolis collected from north Croatia (Kosalec *et al.*, 2004). Other study showed that the outcome of flavonoids extraction from propolis significantly affected by a concentration of ethanol, as it increase with the concentration increasing of this solvent up to 75%and this may be due to the solubility of flavonoids in ethanolic solutions, but atconcentrations higher than 75%of ethanol the outcome of flavonoids extraction will decrease and this may be due to that the high concentrations of ethanol affect on conformation and configuration flavonoids (Shengjie *et al.*, 2005), so preferably use of 70% ethanolin extraction of most the active ingredients from propolis and not from the wax (Bankova *et al.*, 1992).

Effect of incubation period in extraction of total phenols and flavonoids from propolis

Of note Figure (3), it is clear that the best period of incubation to extract total phenols were 48 hours, as it reach the amount of extracted phenols 96.8 mg/g dry material, whereas the prolong of incubation period(s)72, 96, and 120hours enhelpful for increasing the amount of extracted phenols, thisresult is consistent with what pointed Taghouti *et al.* (2007), as it was 48 hours of incubation period is sufficient to extract the total phenols from Iranian propolis. While this result does not agree with what was stated in other studies as it was 24 hours sufficient to extract the total phenols from Bulgarian propolis (Tylkowski *et al.*, 2010), as well as the Algerian propolis (Bchata *et al.*, 2014).

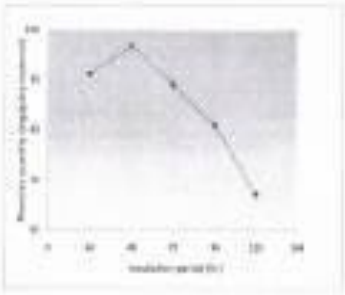

Figure 3. Effect of incubation period in extraction of total phenols from propolis

As shown in Figure (4) that amongst the total flavonoids extraction requires a longer time compared to the total phenols extraction, as it was72 hours is the optimum period to get the highest amount offlavonoids, which amounted to1.024%. No consistent results of the current study with what was said Agarwal *et al.* (2012), as it was 24 hours is sufficient to get the highest amount offlavonoids from propolis, while pointed Pujirahayu *et al.* (2014) to the extraction of these compounds requires a longer time such is 7days.

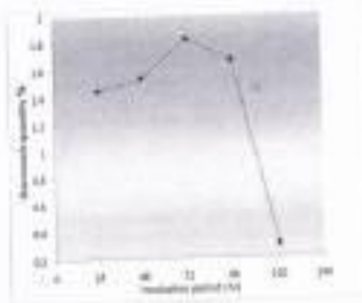

Figure 4. Effect of incubation period in extraction of total flavonoids from propolis

The increase of extraction period lead to increase of the extracted flavonoids yield and prolong the extraction time could lead to get the fullpurity, however, prolonging time causing crash of flavonoids (Qun, 2010).

Inhibition activity of propolis extract against *Candida albicans* yeast

Observe the results in Table (1) it is clear that propolis extract actually possesses to inhibition activity against all isolates of *C. albicans* yeast used in the this study, but with varying degrees and the difference in the inhibitionrate among isolates was significant (p <0.01) for each concentration used from propolis extract, also the difference between the concentrations for each isolate was significant (P <0.05). *Candidaalbicans* 60 isolate that isolated from the mouth was the mostsensitive isolate toward the inhibition activity of propolis extract among the other isolates of this study, as mean diameter of inhibition zone was 24.1 mm using inhibition concentration 25 mg/ml.

Table 1. Inhibition activity of propolis extract against *Candida albicans* isolates

Type of sample	No. of isolate	Positive Cont. mg/ml	0.5	1	5	10	15	20	25	LSD of Conc.
Mouth swab	C. albicans60	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	C. albicans48	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Vaginal swab	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Urine sample	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Skin swab	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
LSD of isolate										

Numbers: inhibition diameter (mean ± standard error)
Horizontal different large letters: the presence of significant differences between the studied concentrations for each isolate at (P <0.05)
Vertical various small letters: the presence of significant differences between yeast isolates for each concentration at (P <0.01)

[illegible]

[illegible]

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