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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 72hourswere 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 propoliswas 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 6o 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 anima lproduct, 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 (Umthong *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 Mititelu, 2004), and it is known that propolis content rich balsamic high efficient compounds (Kujunmajie *et al.,* 1999). Propolis possesses antioxidant, antimicrobial, antitumor and anti-inflammation properties, as well as the heap 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) (Meurman *et al.,* 2007). Yeast characterized as responsible for 80-95% of vaginal infections (Faro *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 ofpropolis have been obtained from the apiaries of Hosseinieh 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 Budrat and Shotipruk (2008) was followed in estimation of the total phenolic content in propolis samples of the study.

Estimation of flavonoids

Depending on thes tandard 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 isolation 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 (88.39 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 of70% 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 (Khacha-anada *et al.,* 2013), whereas80% ethanol was used in extraction of the total phenols from propolis collected from Iran (Yaghoubi *et al.,* 2007).

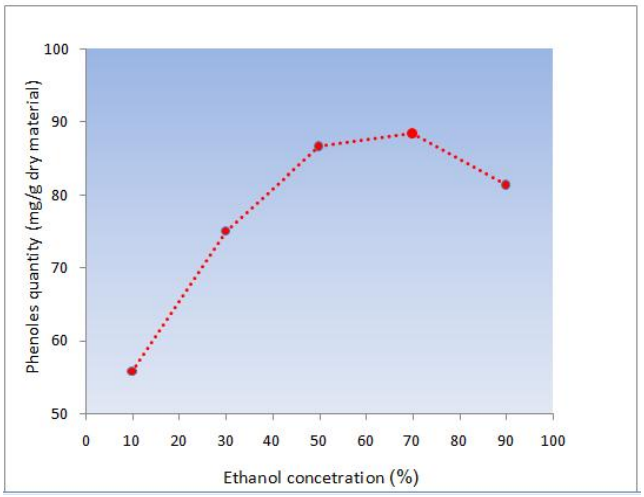

Figure 1. Effect of ethanol concentration in extraction of total phenols from propolis

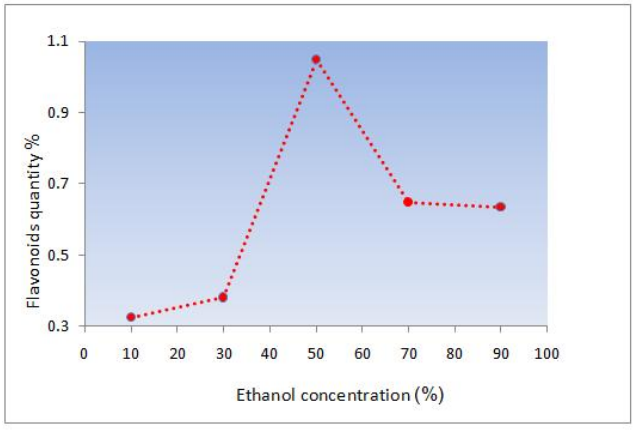

Figure 2. Effect of ethanol concentration in extraction of total flavonoids from propolis

The result from Figure (2) shows that the highest concentration off lavonoids (1.048%) was obtained when50% ethanol was used. The results of this study are consistent with those reported in the previous studies that included using a different concentrations (55-85%) of ethanol, which was found that the use of 71% ethanolis the most efficient in extraction of the total flavonoids from propolis (Margeratha *et al.,* 2012), 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 off lavonoids 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 off lavonoids 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 (Shouqin *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 stood the amount of extracted phenols 96.8 mg/g dry material, whereas the prolong of incubation periodto72, 96, and 120hours unhelpful for increasing the amount of extracted phenols, thisresult is consistent with what pointed Yaghoubi *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 (Rebaia *et al.,* 2014).

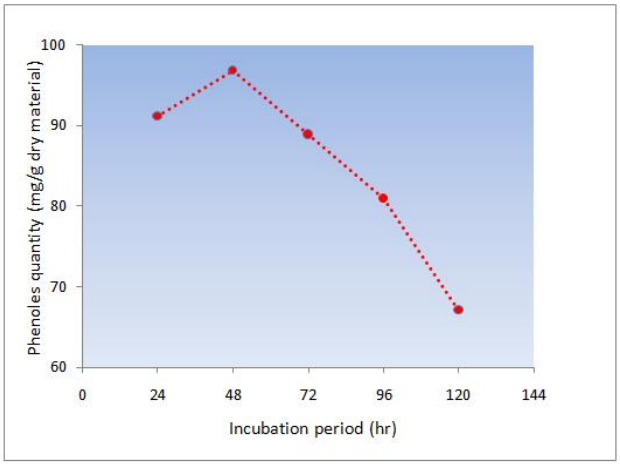

Figure 3. Effect of incubation period in extraction of total phenols from propolis

As shown in Figure (4) that emerges 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 to1.824%. 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 Pujirahaya *et al.* (2014) to the extraction of these compounds requires a longer timer each to 7days.

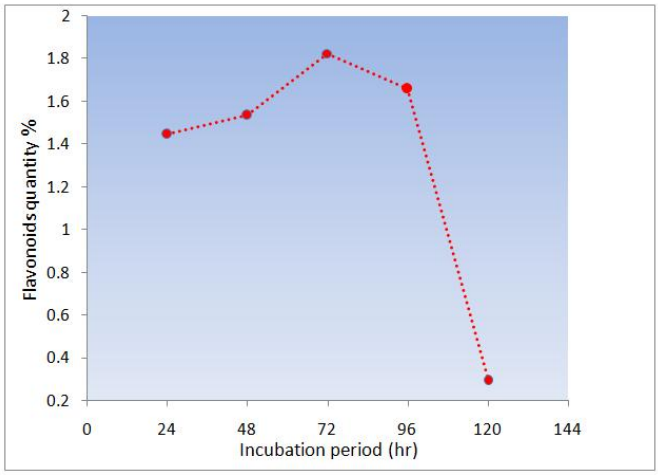

Figure 4. Effect of incubation period in extraction of total flvonoids from propolis

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, prolongthis 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 in hibition activity against all isolates of *C. albicans* yeast used in the this study, but with varying degrees and the difference in the in hibitionrate 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 ($P < 0.01$). *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 stood diameter of inhibition zone was 24.3 mm using inhibition concentration 25 mg/ml,

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

Type of sample	No. of isolate	Inhibition diameter (mm)								LSD of Cocn
		Nystatin Conc.	Extract concentration (mg/ml)							
		1 mg/ml	0.5	1	5	10	15	20	25	
Mouth swab	*C. albicans60*	F 0.11±2.9 a	H 0.0±0 a	G 0.0±1 E	E 0.3±10.6 a	D 0.4±13.3 a	C 0.3±15.6 a	B 0.5±19 a	A 0.4±24.3 a	
	C. albicans58	F 0.15±2.6 a	G 0.0±0 a	G 0.0±0 f	E 0.2±6.3 e	D 0.15±8.3 f	C 0.3±10.6 e	B 0.4±13.3 d	A 0.5±16.3 f	
	C. albicans55	F 0.2±2.5 a	H 0.0±0 a	G 0.1±1.7 d	E 0.3±5.3 f	D 0.25±7.3 g	C 0.31±8 g	B 0.3±10.3 F	A 0.2±13.3 i	
	C. albicans53	D 0.31±2.6 a	F 0.0±0 a	F 0.0±0 f	F 0.0±0 k	E 0.1±1.3 i	C 0.12±4.3 i	B 0.22±7.3 H	A 0.3±11.7 j	
	C. albicans28	F 0.23±2.2 a	G 0.0±0 a	G 0.0±0 f	E 0.3±4.3 gh	D 0.5±6 h	C 0.6±9 f	B 1.0±15 C	A 0.7±18 e	
	C. albicans26	F 0.41±2.6 a	G 0.0±0 a	G 0.0±0 f	E 0.6±7 d	D 0.3±8.3 f	C 0.8±13.7 b	B 0.5±15 C	A 0.61±19 d	
Vaginal swab	*C. albicans57*	F 0.51±2.8 a	G 0.0±0 a	G 0.0±0 f	E 0.57±9 b	D 0.33±9.7 de	C 0.3±11.3 D	B 0.3±12.7 D	A 0.2±15.3 G	0.467
	C. albicans56	F 0.12±2.5 a	G 0.0±0 a	G 0.0±0 f	E 0.37±3.3 i	D 0.56±7 g	C 0.3±10.3 e	B 0.54±13 D	A 1.7±15.3 G	
	C. albicans52	G 0.28±2.5 a	H 0.0±0 a	F 0.2±5.3 a	E 0.41±8.3 c	D 0.3±10.3 cd	C 0.1±13.3 bc	B 0.7±14.7 C	A 0.9±21.3 B	
	C. albicans50	F 0.43±2.6 a	G 0.0±0 a	G 0.0±0 f	E 0.22±8.3 c	D 0.3±10.3 cd	C 0.5±11.7 d	B 0.57±16 B	A 1.2±19.7 C	
	C. albicans25	G 0.18±2.7 a	H 0.0±0 a	F 0.55±5 b	E 0.59±10 a	D 0.8±10.7 bc	C 1.4±12.7 c	B 1.7±19.3 A	A 0.58±24 a	
Urine sample	*C. albicans29*	F 0.35±3.0 a	G 0.0±0 a	G 0.0±0 f	E 0.11±4.7 fg	D 0.32±5.7 h	C 0.35±7.3 h	B 0.56±9 G	A 0.6±15.7 fg	
	C. albicans27	F 0.58±3.0 a	G 0.0±0 a	G 0.0±0 f	E 0.31±3.7 hi	D 0.27±6.3 h	C 0.52±8 g	B 0.62±11 E	A 0.64±14 h	
	C. albicans24	E 0.42±2.9 a	G 0.0±0 a	G 0.0±0 f	F 0.14±1.3 j	D 0.59±11 b	C 0.53±13 c	B 0.64±16 B	A 0.96±20 c	
Skin swab	*C. albicans21*	F 0.38±2.8 a	G 0.0±0 a	F 0.17±3.3 C	E 0.25±7.7 c	D 0.34±9.3 e	C 0.4±10.3 e	B 0.6±14.7 C	A 0.59±19 d	
LSD of isolate		0.687								

Numbers: Inhibition diameter rate (mm) ± standard error. *

***Horizontal different large letters**: Persistence of significant differences between the extract concentrations for each isolate at ($P < 0.01$).

***Vertical various small letters**: Persistence of significant differences between yeast isolates for each concentration at ($P < 0.01$).

whereas the *C. albicans* 53 isolate was the less sensitive isolate toward the inhibition activity of propolis extract among the isolates of study, as stood diameter of inhibition zone was 11.7mmatthe same concentration.

The results obtained from this study consistent with what previous studies have pointed Rezende *et al.* (2006), as it indicated that the in hibition diameter of Brazilianpropolis extract against C. *albicans* FT 2010 and *C. albicans* ATCC10231 reached 9, 15 mm respectively, while another study indicated that they east *C. albicans* yeast showed less sensitive toward Slovak propolis extract, as stood the inhibition diameter was only 3.75 mm (Kacaniova *et al.,* 2009). Whereas the results of this study do not agree with finding of Hendi *et al.* (2011), which pointed to the in effectiveness of the Iraqi prpolis extract against the *C. albicans* yeast. The inhibition activity against the microorganism considered a fundamental property of propolis extract and it has been used for its therapeutic properties by human for many centuries, the vital activity of propolis attributed to the presence of phenolic compounds specially flavonoids and phenolic acids (Paviania *et al.,* 2010). In this study, the concentration of minimum inhibition rate to propoplis extract was determine against yeast isolates of this study, this rate was at the concentration1 mg/ml for the *C. albicans* 60, *C. albicans* 55, *C. albicans* 52, *C. albicans* 25 and *C. albicans* 21 isolates, while this rate was at the concentration 5 mg/ml of the *C. albicans*58,*C. albicans* 57, *C. albicans* 56, *C. albicans* 50, *C. albicans* 29, *C. albicans* 28, *C. albicans* 27, *C. albicans* 26 *and C. albicans* 24, while this rate was at the concentration 10 mg/ml for the *C. albicans* 53 isolate which was the least sensitive isolate toward the propolis extract. Abd El-Hadyand Hegazi, (2001) has been found that the minimum inhibition concentration value of the Egyptian propolis extracts that collected from the Dahkalia, Ismailia and east provinces against the *C. albicans* yeast has been reached 1.32, 1.4 and3.38mg/ml, respectively.

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