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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

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INTRODUCTION

Propolis (bee resin) is a well-known as bee-gum; 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 (Fink et al., 2000). Although propolis is antimicrobial, but it is largely in terms of plant origin, so it contains of 50-55% honey, wax, water, 30% resin, 5-10% pollen granules and 3-10% essential oils (Ghoshal et al., 2011). The origins of the most abundant compounds in propolis and consists of Phenolics, phenolic acids, tannins and constitutes about 50% more than all of the other components (Shao et al. and Abdalla, 2004), and it is known that propolis contains such phenolic high efficient compounds (Kojima et al., 1995). Propolis possesses antioxidant, antimicrobial, antibiotic and anti-inflammatory properties, as well as the high protective properties (Selvam and Prabhu, 2003). Yeasts are opportunistic pathogens, and *Candida albicans* characterized the main common yeast which maintained from the oral cavity in both healthy people and people with diseases (as it constitutes 60-80% of cases) (Mazmanian et al., 2007). Yeast characterized as a causative for 80-85% of vaginal infections (Farr et al., 1997). Therefore, this study aimed to assess the inhibition activity of propolis against *Candida albicans* yeasts causing mouth ulcer, urinary tract 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 apiculture of Hosseiniyeh band 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 Butini and Shafiqah (2000) was followed in estimation of the total phenolic content in propolis samples of the study.

Estimation of flavonoids

Depending on this method curve of quercetin and following the method described by Kosanic 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 three 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) shows that the amount of phenols increase with increasing of ethanol concentration until reach the maximum amount (85.3 mg dry material) when 75% 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 relate 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 (Biankova et al., 2002) and Thailand (Kochanowoda et al., 2013), whereas 80% ethanol was used in extraction of the total phenols from propolis collected from Iran (Yaghoobi et al., 2007).

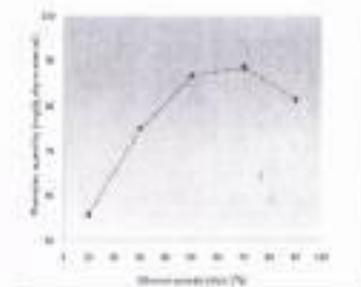


Figure 1. Effect of ethanol concentration in extraction of total phenols from propolis.

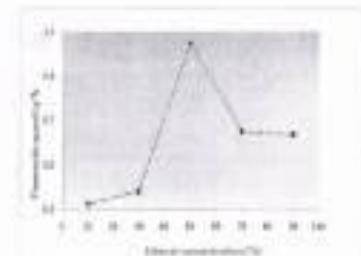


Figure 2. Effect of ethanol concentration in extraction of total flavonoids from propolis.

The result from Figure (2) shows that the highest concentration of flavonoids (1.04%) 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

concentration (55–85%) of ethanol, which was found that the use of 75% ethanol is most efficient in extraction of the total flavonoids from propolis (Mangrulkar et al., 2012), whereas the use of 80% ethanol is the most efficient in extraction of the total flavonoids from propolis collected from north China (Kongxue et al., 2004). Other study showed that the outcome of flavonoids extraction from propolis significantly affected by a concentration of ethanol, as it increases with the concentration increasing of this solvent up to 75%; and this may be due to the solubility of flavonoids in ethanolic solutions, but concentrations higher than 75% of ethanol the quantity of flavonoids extraction will decrease and this may be due to that the high concentrations of ethanol affect on conformation and configuration flavonoids (Sheng et al., 2009), so probably use of 70% ethanol extraction of most the active ingredients from propolis and not from the wax (Biankova et al., 2002).

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

From Figure (3), it is clear that the best period of incubation to extract total phenols were 48 hours, as it avoid the amount of extracted phenols (96.8 mg dry material). whereas the period of incubation period 72, 96, and 120 hours unfavorable for increasing the amount of extracted phenols, whereas it is consistent with what pointed Yaghoobi et al. (2007), as it was 60 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 (Bekhou et al., 2014).

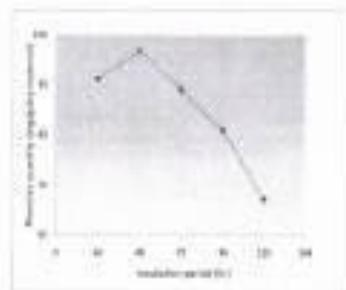


Figure 3. Effect of incubation period in extraction of total phenols from propolis.

As shown in Figure (4) that although the total flavonoids extraction requires a longer time compared to the total phenols extraction, as it need 72 hours is the optimum period to get the highest amount of flavonoids, which amounted to 0.83%. No consistent results of the current study with what was said Agarwal et al. (2013), as it was 24 hours is sufficient to get the highest amount of flavonoids from propolis, while pointed Polatayeva et al. (2014) to the extraction of these compounds requires a longer time such as 7 days.

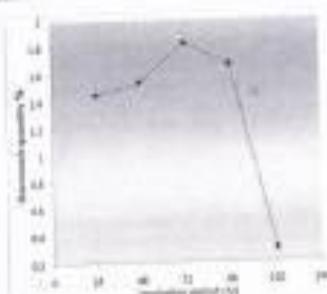


Figure 4: Effect of extraction period on extraction of total flavonoids from propolis

The increase of extraction period lead to increase of the extracted flavonoids yield and prolong the extraction time would lead to get the full property, however, prolonging time causing cost of flavonoids (Qin, 2011).

Inhibition activity of propolis extract against *Candida albicans* ATCC

Observe the results in Table 1 it is clear that propolis extract actually possesses its inhibitory activity against all isolates of *C. albicans* yeast used in this study, but with varying degrees and the difference at the concentrations used from propolis extract, also the difference between the concentrations for each isolate was significant ($P < 0.01$). *Candida albicans* #9 isolate that isolated from the mouth had the most effective isolate toward the inhibition activity of propolis extract among the other isolates of this study, as mean diameter of inhibition zone was 34.1 mm using inhibition concentration 25 μ g/ml.

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

Type of sample	No. of isolate	Inhibition Zone mm	Inhibition diameter (mm) Total concentration mg/ml							Diameter Zone
			0.5	1	2	5	10	25	50	
			25	50	100	200	400	800	1600	
Mouth	#1	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.4±0.3
	#2	0.18±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#3	0.21±0	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.6±0.3
	#4	0.21±0	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.6±0.3
	#5	0.14±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#6	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
Vaginal	#7	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#8	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#9	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#10	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#11	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
Skin	#12	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#13	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#14	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3
	#15	0.15±0.00	0.30±0	0.30±0	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	0.30±0.00	4.5±0.3

Standard deviation: no value = standard error

Statistical difference: P-value of significant difference between the total concentration for each isolate at 0.5%.

Statistical difference: P-value of significant difference between total zones by total concentration 25-1600.

whereas the 11 children 10 years old had the same concerns around the additional variety of people present among the visitors of study, as most character of behavior, were less ($F(1,10) = 10.00$, $p < .05$).

The results obtained from this study agreement with other previous studies (see review Edwards et al. (2000)), of which indicated that the 17-alkenes (isomers of *Brevibacteriopsis*-produced isoprene, C₁₇-alkenes, 17_E-17_Z-18_E and C₁₇-isoprene, 17_E-17_Z-18_E-18_Z) exhibited 90–100% bioactivity against *C. elegans*, while, *Leptothrix* study indicated that the two 17-alkenes (not isolated) had moderate bioactivity. These findings, together, as does the *Leptothrix* observation was 94% C₁₇-isoprene (Edwards et al. 2000). Whereas our results of the study do not agree with those of Edwards et al. (2000), which pointed to the ineffectiveness of the long-chain isoprenoids against the C₁₇-alkenes group. The additional activity against the *Leptothrix*-produced 17-alkenylidene phorbol ester (progeny tested) and it has been used for its therapeutic properties in human oral diseases, has that activity of progeny attributed to the presence of phorbol compounds, apparently *Isosphaera* and *Leptothrix* (Edwards et al. 2000). In this study, the concentration of maximum inhibition rate of progeny extract was determined against each isolate of this study, 100 µg/ml was the bioconcentration required for the 17-alkenes 17_E-17_Z-18_E, 17_E-17_Z-18_E-18_Z, 17_E-17_Z-18_E-18_Z-19_E, except this rate was at the concentration 1 mg/ml of the 17-alkenes 17_E-17_Z-18_E, 17_E-17_Z-18_E-18_Z-19_E, 17_E-17_Z-18_E-18_Z-19_Z, 17_E-17_Z-18_E-18_Z-19_E-20_E and 17_E-17_Z-18_E-18_Z-19_Z-20_E. At the concentration 10 µg/ml was the bioconcentration required for the 17-alkenes 17_E-17_Z-18_E-18_Z-19_E-20_E. The bioconcentration rates around this quantity are small. The 17-alkenes (Edwards & Hedges 2000) has been stated that the maximum bioconcentration rates of the Egyptian progeny mostly was indicated that the *Leptothrix* bioactive and was presented against the 17-alkenes used has been reported (100, 1.4 and 1.6 µg/ml, respectively).

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- estimated 0.2% effluent TC losses may be the most conservative, based on the estimated severity of property related sewage. The outcome of study, as mean character of effluent water may be 11.7 times higher than untreated.

The results obtained from this study zusammen with other previous studies have pointed (Bartoli et al., 2009), as it indicated that the 0.4–0.6% effluent treatment of bioaugmented process against TC effluent (TC = 2333 and 12, effluent 0.02333) resulted 9.3% more significant, while another study indicated the flow rate of 17, effluent treated plant has resulted 100% removal. Hence, property owners in areas like Atlanta observed rate was 9.5% (TC = 2333) (Kumar et al., 2009). Whereas the results of this study do not agree with findings of Ghosh et al. (2004), which pointed to the de-contamination of the living aquatic species against the TC effluent rates. The additional activity against the wastewater contained a bioaugmented mixture of property treated and it has been used to be frequently preferred by treated sewage treatment, due that outcome of property problem to the presence of phosphate conjugated toxicity, bioassay test presents with phosphate (Papageorgiou et al., 2009). In this study, the independence of maximum reduction ratio or percentage removal rate increased against total hardness of the results that were due to the de-contamination argued for the 0.4% effluent TC (effluent TC = 12, effluent 0.012, 1% effluent 21.000%, effluent 21.000%, whereas they can also be the same). The result of the 1-effluent TC (effluent TC = 12, effluent 0.012, effluent 21.000, 1% effluent 21.000, 0.1% effluent 21.000, 1% effluent 21.000 and 1% effluent 21.000) removal rate was quite the same, because the 1% effluent TC removal effect was due the basic condition, which removed the property treated, and bio-augmented mixture of the effluent property removal, thus indicated that the bioaugmented mixture and total percentage against the 0.4% effluent treated TC were resulted 11.5, 1.6 and 11.600, respectively.

REFERENCES

Abed H, El-Hawy A and Hegazy, A. 2001. Treatment properties of chemical conjugation, adsorption and photochemical removal of zinc with Galla Procera. *Biotechnology and Biotechnology Letters*, 23(24), 1989–1994.

Ajparad, B., Tuncer, M. 10 and Moller, A. 2007. Evaluation of chemical conjugation and efficiency of effluent property removal on bioaugmentation and bio-augmentation-activated sludge. *Water Environment and Management* (United States), 19(1), 65–68.

Almehdani, L., Alshabani, Z. and Alshehri, A. 1999. Assessment of some treated wastewater plants for their agricultural properties. *Journal of Environmental Science*, 10, 143–145.

Bardakci, Y., Sarioglu, H., Bagisoglu, E. and Isenler, E. 2002. Chemical conjugation of aqueous organic wastewater and suspended solids. *Water and Wastewater Treatment*, 15, 599–604.

Bardakci, Y., Bagisoglu, H., Bagisoglu, E., Isenler, E., Sarioglu, H., Sarioglu, E. and Isenler, E. 2002. Removal treatment of aqueous organic wastewater and plant origin conjugates. *Water and Wastewater Treatment*, 15, 599–604.

Bartoli, P. and Montagnini, R. 2008. Treatment of phosphate esterified, flow, flow of water. *Environmental Monitoring and Assessment* (United States), 144, 135–140, DOI 10.1007/s10661-008-0433-3.

Bartoli, P., Agusto, V., Montagnini, R., Gilani, S., Thompson, M., Hoss, N., Philippot, L., Bardi, M., Nagy, A. and Yang, B. 2007. Treatment Conundrum in Environmental Toxicology. *Hydrobiologia*, 571, 1–17.

Bartoli, P., Perrotta, M., Montagnini, R., D'Amato, F. and Agusto, V. 2003. Flow or static process flow reduction: the environmental properties of phosphate. *Journal Biologiae Aquaticae Technologiae et Biologiae Terrestris in Applied Microbiology and Biological Engineering*, 4, Number 1, 1–10.

Bengal, S., Babel, M. and Rajchelawat, C. 2001. Zinc action: The bioavailability addition assay. *Water Research* (United States).

Carvalho, M., Ribeiro, M., Kaczmarska, V., Pobedim, S. and Baloniuszki, J. 2006. The environmental impact of zinc and strontium species under different species. *Environ Monit Assess* (United States), 121(1–3), 173–177.

Chapman, R., Troppeberg, H., Chertomonyan, P. and Troppeberg, V. 2010. Activators for heterotrophic oil and grease activity of property bacteria. *Water and Wastewater Treatment: Journal of Clean Processes*, 10(12), 39–47.

Christen, C., Raman, M., Pragnell, A. and Valentine, A. 2006. Quantitative analysis of phosphate in wastewater flow conditions. *Water Research* (United Kingdom), 40, 31–35.

Elshabani, A. 2007. Treatment and adsorptive activity of bioaugmented mixture property problem. *Water and Wastewater Treatment*, 11, 13–16.

Karayannidis, G., Tsakiridou, I., Sakkasidou, V., Bakteria, V., Christou, S. and Agapiou, L. 1995. Adsorption, desorption and removal activity of property from of River properties origin. *Arch Environ Health*, 48, 231–235.

Morales, J., Hinch, E., Kishimoto, M. and Gosselin, R. 2007. New Canadian effluent: Canadian review of the new study. *Canadian Journal of Biostatistics and Bioinformatics* (United States), 1(1), 213–223.

Papageorgiou, L., Karayannidis, G., Agapiou, L. and Christou, S. 2000. Removal bioaugmentation of total and gross property, maximum yield of phosphate removable. *European Journal of Water Chemistry*.

Papageorgiou, L., Karayannidis, G. and Lekkas, E. 2004. Removal and bioassay method of property of water treated effluent of two streams (coastal). *Journal of Environmental Sciences*, 16(2), 208–210.

Papageorgiou, L. 2010. The effect of wastewater management on the reduction of organic waste water. *Water, Properties, University, Hong Kong*.

Papageorgiou, L., Karayannidis, G. and Lekkas, E. 2004. Total phosphate content reduced sorption and uptake and metabolism of effluent property. *International Journal of Pharmacy and Phisiological Sciences*, 16(2), 208–210.

Perrotta, M., Perrotta, M. and Cane, V. 2004. Environmental action of zinc biostimulation enhanced property removal. *Water and Wastewater Treatment*, 18(10), 20–25.

- Selvan, A. and Prabhu, T. 2010. Extraction of propolis from beeswax and characterization of its constituents and medicinal properties (A Review). *International Journal of Advanced Engineering Technology*, 1(1):213-229.
- Shuang, Z., Jin, X. and Changming, W. 2009. High hydrostatic pressure extraction of bioactive flats propolis. *J. Chem. Process React.*, 39: 18-34.
- Sincik, O. and Mihalci, M. 2004. Study of some honey inlays from propolis and honey. *Afyon Mezitli University 4th Scientific Congress, Kocatepe-Aydin, Turkey, Proceedings Book*: 208.
- Tyburczyk, B., Tkachuk, B., Borkowska, V. and Gajewski, M. 2010. Extraction of biologically active compounds from propolis and determination of extract by spectrophotometry. *Journal of Molecules Sciences*, 14(1): 134-139.
- Umthong, S., Phongsriwachai, P., Pathong, S. and Chanchai, C. 2011. In vitro antimicrobial activity of partially purified Trigonolobus-type propolis from Thailand and Iranian honey cell-flats. *BMC Complementary and Alternative Medicine*, 11:37-42.
- Yagizci, F., Olgarbas, G., Solmaztekin Zed, S. and Sener, H. 2007. Antimicrobial activity of Iranian propolis and its chemical composition. *DALI Journal*, 15(1): 45-48.

