COMPARISON BETWEEN SOME PROPOLIS PHENOLIC COMPONENTS IN EGYPT

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Abstract

Propolis samples of different regions in Egypt, added to commercial Chinese propolis were tested to determine their phenolic compounds present in each using HPLC analysis and through Ethanolic and water extract methods. The results obtained revealed presence 12 phenolic compounds in the tested samples on more distributed between them with different concentsration and percent. The ellagic acid recorded the highest percent in the propolis Kfer- Elsheikh governorate (80%) followed by Qalubiasame collected from Kafer (60%), respectively. While with commercial Chinese it not recorded at all, and considers the very poor phenolic compounds,as well as it characterized by presence a higher percent of cinamicacid . The water propolis extract of old bee wax combs reveal presence a little amounts of twelve phenolic compounds, the highest of them was benzoic acid (60%).

INTRODUCTION

Propolis is a natural product derived from plant resins and collected by honey bees to seal the walls and entrance of the hive and contributes to protect the colony against different pathogens (Ghisalberti, 1979). Each region and colony seems to have its own preferred resin sources, which results in large variation of final composition. In Europe, honeybees preferably collect resins from leaf buds of *Populus* species (Krell,1996). The composition of propolis variety and depends on the environmental plants, differences in color, odor and chemical compounds are noticed relying on the source and the season of gathering (Souza *et al*., 2016). Phenolic compounds are a wide topping of plant accessories metabolites, showing a variety of structures including phenolic acids, flavonoids, lignans, quinones, tannins, coumarins and others (Huang *et al*., 2010).It's owns several biological properties such as antibiotic, antifungal, antiviral and have aanti-inflammatory activities (Santos *et al*., 2003). Furthermore, different compositions and amounts of the active substances are detected in separate samples of propolis (Bankova *et al*., 2002). The variety chemical compositions and biological activities of propolis are due to geographical location, plant sources and collecting season, therefore in ancient era, Egyptians, Greeks and Romans used propolis as a medication against some diseases (Sforcin and Bankova

2011). The therapeutic properties of propolis are due to its chemical composition with bioactive compounds; thus, researchers are interested tostudy it's chemical composition and biological properties (Bankova 2005). The volatile substances (aromatic oils) determine the flavor of propolis, and the variety of flavor depends on the geographical area and assortment of plants (Bankova, *et al*., 1994)). Furthermore, propolis has been found to contain phenolic acids (for example, phenolic derives of cinnamic and coumaric acids), characterized by very potent antimicrobial activity (Hegazi *et al*., 2000). The antimicrobial and anti-inflammatory activity of European propolis is associated with the presence of flavonoids, flavones, and phenolic acids and their derives (Bankova, 2005). Flavonoids, phenolic, diterpenoid acids, aromatic acids, and triterpenoids compounds are the major components of propolis (Kumazawa *et al*. 2008). The aim of this ivestigation is to determine the phenolic contents indefferent sources of propolis and the effect of this contents on its quality.

MATERIALS AND METHODS

This work was done in the year of (2017). Four propolis samples were collected from the apiaries of Kfer- Elsheikh and ElQalubia governorates, as well as commercial Chinese propolis added to propolis collected from oldbeewaxcombs. Ethanolic and water extract were done according to method of Iidenize *et al*., (2004) as follows; The Ethanolic extract was carried out by dissolving 10g. of the propolis samples in 100 ml of the Ethanol (80% v/v) for 7 days then filtered and dried . The propolis water extract was done only on the old wax combs.

Determination of propolis phenolic compounds by HPLC

a- Instrument used:

Agilent 1260 infinity HPLC Series (Agilent, USA), publication number 5991-3801 EN ,2014. equipped with quaternary pump, a Zorbax Eclipse plus C_{18} column 100mm x 4.6 mm i.d., (Agilent technologies, USA), operated at 25 C. The o5uz is achieved using a ternary linear elution gradient with (A) High Performance Liquid chromatography (HPLC) grade water 0.2 % H_3PO_4 (v/v),(B) methanol and (C) acetonitrile. The injected volume was 20 µL. Detection: VWD detector set at 284 nm. Environmental condition: Temperature: 23°C and humidity: 40%.

B- Test method;

1g of extract was propolis was soaked in50 ml methanol 80% overnight then centrifuged for 20 min at 4000 rpm. The supernatant evaporated till dryness then dissolved in 5 ml methanol UPLC grade filter thtough 0.45 um PTFE syringe filter.

RESULTS AND DISCUSSION

The Ethanolicproplis extract c0llected from kafr Elshiekh showed present 11phenolic compounds. The ellagic acid recorded The higest percent of them (53.45%), while same from Qalubia governorate recorded seven phenols The highest of them was the ellagic acid too (80.60%) at same time proplis from kschataerized by presence a benzoic and vanillic acids whis percent (20%,20.33%),respectively . In contrast the water extract old wax combs showed high benzoic acid contration (61.5%) followed with benzoic 1.2 diop (10.5%), and caffeine (9%), Its notcable to not that water extract old wax combs showed little amoung of 12 phenolic compounds in comparisonTo same of kafer Elshik and Qulubia governoments. The benzoic acid recorder the higher percent (61.5%). The commercial chinesepropolisrecorded only five phenolic compounds with a little amounts of them and consider the most propolis of the phenolic components. The Cinnamic acid showed the highest percent of them (92.41%).

It could be summarized that the following results; Propolis samples of different regions of Egypt, added to commercial Chinese propolis were tested to determine their phenolic compounds present in each using HPLC analysis and through ethanolic and water extract methods. The results obtained revealed presence 12 phenolic compounds in the tested samples on more distributed between them with different concentration and percent. The ellagic acid recorded the highest percent in the propolis Kfer- Elsheikh governorate (80%) followed by Qalubia same collected from Kafer (60%), respectively. While with commercial Chinese it not recorded at all, and considers the very poor phenolic compounds, as well as it characterized by presence a higher percent of cinamicacid. The water propolis extract of old bee wax combs reveal presence a little amounts of twelve phenolic compounds, the highest of them was benzoic acid (60%).

From results obtained it could be decided that Egyptian propolis was the best content phenolic compound. compared with the commercial chinesepropolis. In addition that water extract of old bee wax combs contains all phenolic compound tested by a little amounts. It can be advice to gone both of Ethanolic and water extracts because they detected all tested phenolic compounds.

Table 1. Phenolic compounds concentration of four kinds of extract propolis (KP, QP, CP and WP) determinate by HPLC.

Phenolic compounds	Ethanolic extract						Water extract	
	KP		QP		CP		WP	
	mg/100 g	R%	mg/100g	R%	mg/100g	R%	mg/100g	R%
Catechol:*Benzene-1,2-diol $C_6H_6O_2$	4.57	0.28	ND	-	ND	-	9.55	10.55
Benzoic acid :*Benzenecarboxylic acid $C_7H_6O_2$	332.60	20.07	12.88	4.83	ND	-	55.80	61.62
p-Hydroxy benzoic acid :*4-Hydroxybenzoic acid $C_7H_6O_3$	32.82	1.98	5.01	1.88	ND	-	ND	-
Salicylic acid :*2-hydroxybenzoic acid $C_7H_6O_3$	ND	-	ND	-	8.92	6.07	0.80	0.88
Gallic acid: *3,4,5-trihydroxy benzoic acid $C_7H_6O_5$	0.86	0.05	ND	-	0.77	0.52	1.27	1.40
Vanillin: *4-Hydroxy-3-methoxybenzaldehyde $C_8H_8O_3$	23.64	1.43	6.03	2.26	ND	-	ND	-
Vanillic acid :*4-Hydroxy-3-methoxybenzoic acid $C_8H_8O_4$	337.06	20.34	23.60	8.85	0.85	0.578	ND	-
Caffeine: *1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione $C_8H_{10}N_4O_2$	3.25	0.20	1.42	0.53	ND	-	8.16	9.01
Cinnamic acid: * (*E*)-3-phenylprop-2-enoic acid $C_9H_8O_2$	0.29	0.02	ND	-	135.9	92.42	0.07	0.08
o-Coumaric acid: * (*E*)-3-(2-hydroxyphenyl) -2-propenoic acid $C_9H_8O_3$	ND	-	ND	-	ND	-	0.21	0.23
p-Coumaric acid: *3-(4-hydroxyphenyl)-2-proponic acid $C_9H_8O_3$	ND	-	ND	-	ND	-	3.62	3.99
Caffeic acid: * 3-(3,4-dihydroxyphenyl)-2-propenoic acid $C_9H_8O_4$	ND	-	ND	-	ND	-	3.49	3.85
Syringic acid : * 4-Hydroxy-3,5-dimethoxybenzoic acid $C_9H_{10}O_5$	35.46	2.14	2.78	1.05	ND	-	2.51	2.77
Ferulic acid: *(*E*)-3-(4-hydroxy-3-methoxy-phenyl) -2- propenoic acid $C_{10}H_{10}O_4$	0.83	0.07	ND	-	0.61	0.42	0.82	0.90
Ellagic acid :* 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione. $C_{14}H_6O_8$	885.91	53.46	214.92	80.60	ND	-	4.26	4.70
Rutin:*2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6)[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl)oxan-2-yl]oxy}-4*H*-chromen-4-one. $C_{27}H_{30}O_{16}$	ND	-	ND	-	ND	-	ND	-
Total Phenolic	1657.29		266.64		147.03		90.56	

*IUPAC name: (International Union of Pure and Applied Chemistry) ND: not determined KP: Kfer- Elsheikh
QP: Qalubiapropolis WP: old wax propolis CP: Chinese propolis

The findings of various studies confirm that chemical composition of propolis depends on trees and plants available to the bees, on the season in which it is collected, on the geographical area, and other factors (Kartal *et al*., 2002). The variety temperate zone and plant sources propolis is generally referred as poplar propolis because mainly produced from the bud exudates of Populus trees (Popova *et al*., 2004). Birch propolis is found specifically in Russia and is different from poplar propolis (Christov *et al*., 2006). Various forms of Brazilian propolis are available: green propolis is derived from *Baccharisdracunculifornia* (Righi *et al*., 2011). while brown propolis comes from Copaifera species (Sawaya *et al*., 2006) and red propolis is obtained from *Dalbergiaecastophyllum* L. (Piccinelli *et al*., 2011). Different compositions and amounts of the active substances are detected in separate samples of propolis (Bankova *et al*., 2002). Obviously, the chemical compositions of propolis samples vary between different samples (Rushdi *et al*., 2014). composition of phenolic constituents were different in the three kinds of ethanolic extract propolis and Egyptian propolis were contained more phenolic compounds than in the Chinese propolis and old wax comb extract (Kamel *et al*., 2013). Chemical compound differences of propolis are thus easily comprehended as it is a complex mixture of compounds gleaned from various plants and processed by salivary enzymes of bees. Therefore, composition of propolis depends of the plants, the seasons resins are collected, and the bee species. This chemical variety brought a crucial question of standardization, even bee do not change its chemical composition (Bankova *et al*., 2000). Propolis sample analyses from differences parts of the world have been collectively consists of over 300 different chemical compounds (Huang *et al*., 2014). It has been possible to identify several families of chemically active compounds through various, technics such as mass spectroscopy, nuclear magnetic resonance, gas chroma-tography coupled with mass spectroscopy, but not to define a minimal common composition with clear concentrations of the various compounds. Phenolic compounds include various acids such as cinnamic, p-coumaric, chicoric, caffeic and fulric acids (Bankova, 2005).

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مقارنة بين بعض مركبات البروبوليس الفينولية فى مصر

أيمن محمد محمد غنيه[1] ، نجلاء الأحمدي غزاله[1] ، محمدعلي إبراهيم عبدالعظيم ، أحمد رمضان مزيد[1] وسمير هاشم رمضان[2]

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تم اختبار عينات من البروبوليس جمعت من مناطق مختلفة من مصر اضافة الى بروبوليس تجارى صينى وذلك لتحديد المركبات الفينولية الموجودة فى كل منها وذلك باستخدام جهاز التحليل الكروماتوجرافى (HPLC) ومن خلال المستخلص الكحولى والمائى أظهرت النتائج وجود 12 مركب فينولى على الأكثر فى العينات المختبرة موزعة بينهم بتركيزات ونسب مختلفة. أظهر مركب ellagic acid وجوده بنسبة عالية فى البروبوليس المجموع من محافظة كفر الشيخ (80%) يليه البروبوليس المجموع من محافظة القليوبية (60%) على التوالى. بينما أظهر البروبوليس التجارى الصينى أنه لم يسجل اية نسبة من ذلك المركب والذى يعتبر فى نفس الوقت من أفقر أنواع البروبوليس المختبرة فى محتواها من المركبات الفينولية الإ أنه يتميزبوجود نسبة عالية من مركب ال Cinamic acid (90%) . فى حين أن مستخلص البروبوليس المائى لشمع النحل المجموع من أقراص النحل القديم أثبت وجود كميات قليلة من ال 12 مركب فينولى المسجلة وكان أعلاهم هومركب Benzoic acid(60%).

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