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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF HONEYBEE GLUE (PROPOLIS) COLLECTED FROM EGYPT AND SYRIA

Haggag, E.E.*; E. Nafea* and Wafa A. Yakoub**
1- Plant Protection Res. Inst., Agric. Res. Center ,Dokki, Giza.
2- Plant Protection Dept., Damascus University.

ABSTRACT

Some propolis samples collected from different regions of Egypt and Syria were in vitro investigated for chemical compositions and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Results indicated that the number of components in the propolis samples extracts were 15 compounds, with different percentages. The HPLC analysis indicated the presence of the following compounds:- Quercetin, Pinostrobin, chrysin, and Galangin as major Flavonoids, and Vallinin, Euganol, Cinnamic, Salicylic acid and phenol, Caffeic acid, Ferulic acid, β- ohbenzoic, Gallic acid, P- comaric and 3,5 diethoxy benzyl alcohol as Phenolic components. Results obtained indicated variable differences in percentages of the compounds in samples collected from both Egypt and Syria. Susceptibility to several ethanolic extracts of propolis was tested in reference strains of bacteria. Regarding the susceptibility of the tested bacteria strains, all propolis extracts tested showed great growth inhibition zone than control, but with variable degree. The main of inhibition zone of Syrian and Egyptian propolis against the three tested of bacteria strains were 19.33, 15.06, 15.02, 22.94, 21.39, and 18.73 mm, respectively. Further more results obtained indicated that gram positive bacteria was more sensitive to ethanolic propolis extract than gram negative bacteria.

INTRODUCTION

Propolis is a plant resin collected by honey bees from plants around their hives, used to maintain the hive environmental aseptic, strengthen, isolate and disinfect their nest. Popular buds are the main source of propolis, but in some cases other resinous plant can be considered an additional source of propolis. It has been used since ancient times in folk medicine in many parts of the world. The ancient Egyptian used it to embalm their dead sforcin *et al.* (2000). Nowadays propolis is commercially found in sprays, ointments, capsules, capillary lotions, and toothpastes because of its bacteriostatic activity and pharmacological properties.

The chemical composition of propolis, colour and aroma are changed according to geographical zone Metal, *et al.* (1975). Several studies have determined the activity of propolis against bacteria using different dilution and agar plate. Meresta and Meresta (1986) and Bankova (1997) found that the antibacterial activity of the hive product varied from region to other depending on its chemical composition. Moreover Alexandra *et al.* (2004) mentioned that using different solvents for propolis extract gave different compounds. kujumgiev *et al.* (1999) studied the antibacterial activity of propolis samples collected from different geographical zone and found that all samples were active against Gram positive bacteria .

Previous work showed that chemical composition and biological activity of propolis were differ and extremely complex and more than 180

constituent have been identified. In such cases chemical composition of propolis and its biological activity will bee changed . For this reason it is necessary to investigate the chemical composition of propolis from different countries. The comparison of data obtained will give information about the existence of additional plant sources of propolis in different countries and their biological activity.

Since many reports dealing with propolis from Arab countries are not available to most readers, this study was undertaken to:-

- Compare the chemical composition of propolis collected from different regions in Syria and Egypt.
- Comparing the sensitivity of some significant bacterial to propolis extract collected from the two countries.

MATERIALS AND METHODS

1. Sources of propolis:

Samples of propolis were obtained from the hives maintained and controlled by the technical stuff of the Department of Entomology of Faculty of Agriculture Damascus University, and plant protection Research, Honeybee Department in Egypt. Propolis was scraped from the top of combs using a sharp Knife. After screening propolis samples were carried out from the production site to the laboratories in polyethylene backets with tight filting lids and stored in the dark at 5°C. solution of propolis for testing were prepared aseptically and protected from bright light to prevent photo degradation. For its preparation crude propolis was dissolved by stirring it in ethanol 96% " MERCK" and submitted to filtration, according to the method of Boeru and Derevici (1978). After filtration the solvent were totally evaporated on a water bath at room temperature. The dray extracts were then redissolved in 70% ethanol in order to obtain solutions containing 10% (W/V) propolis extract.

2. Bacterial strains :

Both gram- positive and negative bacteria used in this investigation were applied from 24 h. cultures and suspended in sterile saline solution to obtain concentrations of approximately 18^{8} . The following species of bacteria tested were *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonus aeruginosa*. These species were obtained from microbiological Department, Agriculture College, Al Minia University.

3. Media :

3.1 -LB medium (Luria – Bertani medium) (Atlas, R.M., 1997) :

It was used for cultivation and maintenance of *E. coli*

3.2 -Micrococcus medium, (Atlas, R.M. ,1997):

It was used for cultivation and maintenance of *Staphylococcus aureus*

3.3 -Beef extract Agar, (Atlas, R.M.,1997) :

It was used for cultivation and maintenance of *Pseudomonas aeruginosa.*

The data were statistically analyzed using the multivariant analysis (Anova) and least significant differences.

4. Preparation of Propolis Extract:

Preparation of propolis extract was carried out according to the method of Boeru and Derevici (1978).

Determination of phenolic compound in honey samples:

Preparing of 10 % propolis solution:

One g of propolis was dissolved in 10ml ethyl alcohol 70%, and then kept in closed glass tubes for analysis.

Estimation weight % of phenolic compounds:

The scanning of identified phenolic compounds extracted in propolis samples by (HPLC) analysis are estimation of weight % for these compound was calculated using the relation, (William, 1991).

HPLC Identification:

Identification of phenolic compounds of propolis samples was performed by a HPLC (JASCO), using a hypersil C_{18} reversed- phase column (250 X 4.66 mm) with 5 µm particle size.

Injection by means of a Rheodyne injection valve with 50 µl fixed loop was used. A constant flow rate of 1 ml min^{-1} was used with two mobile phases (A) 0.5 % acetic acid in distilled water at pH 2.65; and solvent (B) 0.5 % acetic acid in 99.5 % acetonitrile . The elution gradient was linear starting with (A) and ending with (B) over 35 min, using a µv detector set at wavelength 254 nm. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standards mixture chromatogram. The concentration of individual compound was calculated on the basis of the peak area measurements, and then converted to µg phenolic g^{-1}dry weight. All chemicals and solvents used were in HPLC spectral grade. Twenty standard phenolic compounds were obtained from Sigma (St, Louis , USA) and from Merck-Schuchard + (Munich، Germany) chemical companies (Soliman, 2002).

5. Antibacterial Activity test

A concentrations 10 % of each source of propolis in 70 % ethanol were prepared and kept in a refrigerator at 4°C . The antibacterial activity of propolis was determined by the paper-disc plate method described by Anon (1982). Antibacterial activity was determined by measuring the diameter of inhibition zones around the discs to the nearest 1 mm. Three replicates were prepared for each experiment. Blanks in case of propolis were carried out using filter paper discs impregnated with the solvent (70% ethyl alcohol) and dried before being similarly tested. All blanks gave no antibacterial effect against any of the test organisms.

RESULTS AND DISSCUSION

1. Chemical composition:

Propolis composition have been recently become the subject of investigations, In order to determine its therapeutic application especially the Flavonoids and phenolic compounds, that considered the most biological active component used in folk medicine. it was taken into consideration that propolis is a complex of compounds, so more than 180 propolis compounds have been identified by gas chromatography mass spectrometry. In this investigation the following compound have been detected by (HPLC) device

using the most commonly solvent Ethanolic for propolis extract preparations and the available standard compounds.

Results obtained in Table (1) and chromatogramic in Fig. (1) indicated variable differences among composition of propolis samples collected from different regions of Syria and Egypt. HPLC analysis successfully provided the presence of 15 chemical compounds in the six samples with relative variables, and several incompletely identified derivatives of these substances.

As well as other determinations, the major Flavonoids that were isolated from the Egyptian and Syrian propolis were Quercetin, Pinostrobin, chrysin, and Galangin with percentages ranged from 2.49 to 72.16, 0.35 to 7.83, 36.26 to 620.91, 19.812 to 180.20, 15.31, 0.80 to 5.23, 6.88, and 0.16 to 2.1 mg/100 gm, respectively. These compounds were isolated in all Egyptian samples, but the third and fourth compounds were identified in one sample of Syrian propolis only.

On the other hand, the major phenolic compounds obtained in samples were Vanillin, benzoic acid, cinnamic acid, Ferulic acid, Caffeic acid, Eugenol, Gallic acid, phenol, and benzyl alcohol, with percentages from 2.5 to 14.44, 5.4 to 12.86, 0.28, 0.19 to 37.7, 101.15 to 706.29, 124.72 to 218.16, 1.52 to 38.8, 0.0, 39.99 to 649.12, 5.43 to 75.7, 5.36 to 5.78, 0.33 to 4.36, 0.0, 0.0 to 1.64, 29.42 to 419.84, 29.68 to 289.68, 0.0 to 0.63, 0.0 to 0.63, 0.0 to 6.039 mg/100 gm in Syrian and Egyptian samples, respectively. In contrast Gallic acid was absent in all tested samples except one sample from Egypt, while Ferulic acid was not identified in Egyptian propolis samples too. Therefore Tables (3 &4) clearly showed the chemical names of these identified Flavonoids and phenolic compounds.

Table (1): Major Flavonoids and phenolic compound Isolated from Syrian and Egyptian samples (2005)

Common name	Samples tested					
	S1	S2	S3	E1	E2	E3
Galliç	0	0	0	0	1.637985	0
B-oh benzoic	0	0.280102	0	0.19377	37.69587	11.69973
Caffeic	462.3771	649.1191	39.99812	16.42892	75.65007	5.427326
Phenol	114.5026	29.42093	4198.416	29.68448	289.6837	42.30805
P-comaric	0	184.128	0	124.543	17.43191	0.169451
Salicylic	6.308386	162.5641	42.95019	5.27948	56.32417	36.26206
Ferulic	38.77559	1.525128	34.83615	0	0	0
Cinnamic	101.146	706.2853	115.5323	124.7186	218.1604	141.7112
Quercetin	2.487824	72.15969	6.894007	0.354843	1.106767	7.82547
Euganol	5.775807	7.256134	5.360143	0.331253	4.357674	0.473628
Chrysin	0	0	15.31335	0.801949	5.231192	5.117655
Galangin	0	6.887134	0	0.159185	0.200045	2.01993
Pinostrobin	620.9058	340.2694	36.258	0	19.81199	180.1994
Vanillin	2.495082	14.43936	5.35022	2.952611	12.86135	0.541211
3,5di ethoxy benzyl	0.063437	0	0.060555	0.026614	0.039106	0
	mg/100gm	mg/100gm	mg/100gm	mg/100gm	mg/100gm	mg/100gm

S1, S2, S3 : Samples from Syria. E1, E2, E3: Samples from Egypt.

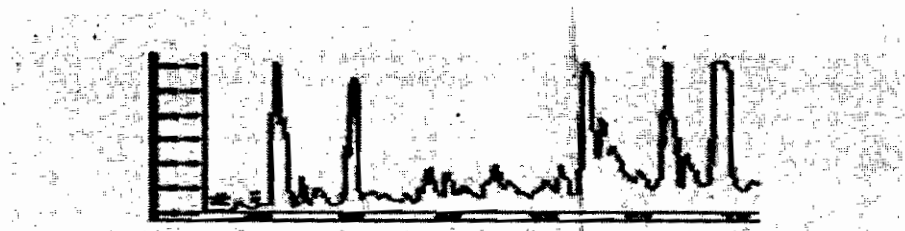

Fig (1) :Phenolic compounds of Egyption propolis.

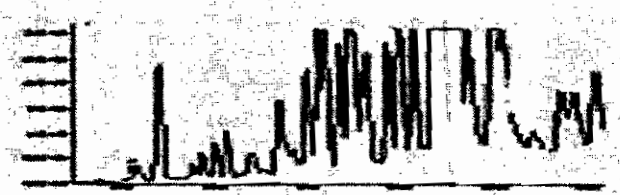

Fig (2) :Phenolic compounds of Syrian propolis.

It was interesting to mention that these differences in propolis composition could be found in samples from different geographical regions, since local flora influence its chemical composition and probably its biological activity. These results are in the same range as those reported by Mohanny (2005) who found that the major phenolic compounds of Egyptian propolis collected during spring season were phenol and P-coumaric, while the minor was gallic and Ferulic acid. He added that there was a great differences in phenolic compounds of propolis collected in different seasons of Egypt.

Hegazi and Abd El-Hady (2002) investigated the chemical composition of European propolis and found that benzyle Ferulate and galangin were predominant in German propolis, Benzyl caffate was the major compound in French and Austrian propolis. Bankova *et al.* (1997) found that Bulgarian propolis contain more than 50% polyphenolic accompanied by terpenoids. He found also similarity in chemical composition and biological activity of Bulgarian, Manajolian and Albanian propolis.

Similarly, it could be concluded that the availability in propolis samples composition may be correlated with the local flora diversity around bee hives and other factors influencing the similarity in chemical composition of samples collected from Syria and Egypt. In spite of the above reasons, the local flora could hardly influence the composition of volatile constituents of propolis. Santos *et al.* (2003) mentioned that the composition of propolis a resinous hive product collected by honey bees from various plant sources depend on various factor such as season, and vegetation of the area. Alexandra *et al.* (2004) reported that propolis was submitted to extraction using several solvents, resulting in extracts with different composition.

Table (2): The antibacterial activity of Egyptian and Syrian propolis at concentration (10%) against some bacterial strain

Results are represented as mm. Results are means of 3 replects

Strain of bacteria	Type of Propolis	
	Egyptian propolis	Syrian propolis
Styph. aureus	19.33	22.94
P. aeruginosa	15.06	21.39
E .coli	15.02	18.73

Table (3): Major Flavonoids identified from Syrian and Egyptian propolis samples.

No.	Common name	Chemical name
1.	Chrysin	3,7 dihroxy Flavone
2.	Galangin	3,5,7 trihroxy Flavone
3.	Querctin	3,3,4,5,7 Pentahydroxy Flavone
4.	Pinostrobin	5, hidroxy-7-methoxy Flavone

Concerning data obtained it could be concluded that these results confirmed the variable composition of this honeybee glue product collected from different regions of Syria and Egypt during (2005).

2- Antibacterial activity:

Results presented in Table (4) and presented chromatographically in Fig. (3 & 4)demonstrated that all ethanolic extract of both Egyptian and Syrian propolis at concentration 10% has greater growth inhibition zone, comparing with the control agar disks ethanol in which these was no visible growth of bacteria on the surface of agar culture was noticed. The mean diameter of these inhibition zones as means of three replicates were 19.33, 15.06, 22.94, 21.39, and 18.73 mm of Egyptian and Syrian propolis against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Eschericia coli*, respectively. By using a multiple comparison test (Anova), it showed non significant difference in bacterial activity among propolis samples collected from different regions of Syria and Egypt.

Table (4): Major phenolics identified from Syrian and Egyptian propolis samples.

No.	Common name	Chemical name
1.	Vahillin	4, hydroxy-3, methyl benzaldehyde
2.	Cinnamic acid	3, phenyl- 2, propenoic acid
3.	Caffic acid	3, (4, dihydroxy-3 methoxy phenyl) 2- propenoic
4.	Ferulic acid	3, (4 hydroxy-3, methoxy phenyl) 2- proponic acid
5.	Euganol	2, methoxy-4 (2- propenyl) phenol
6.	Benzoic acid	
7.		3,5 diethoxy benzyl
8.	P-comaric	3- (4- hydroxy phenyl) prop-2-enoic acid
9.	Salicylic	
10.	Phenol	
11.	Galic acid	

Fig. (3): **Bacterial growth in treated media with Syrian propolis.**

In this work the evidence that propolis samples does not induce the same activity against tested bacteria strain, come from the large number of chemical components which justify propolis biological activities. On the other hand, *Staphylococcus aureus* as gram-positive bacteria was the greatest sensitivity for both propolis extract prepared from Syria and Egypt.

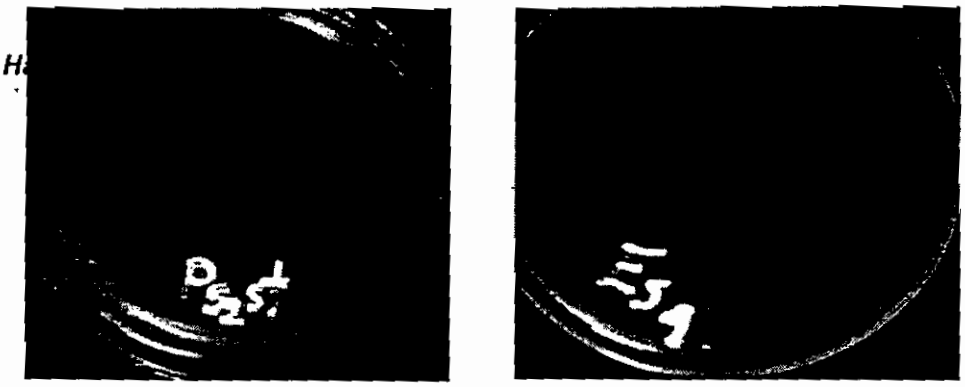

Fig. (4): Bacterial growth in treated media with Egyptian propolis.

Fig. (5): Bacterial growth in untreated media "control".

It was noticed also from the present result that the inhibition zone correlated with the large number of chemical composition which justify propolis biological activities. This bacterial activity of propolis samples confirm results of Vallanueva (1964) who stated that microbial activity of propolis due to galangin compound. In addition Metal, *et al.* (1975) found that Ferulic acid and methyl benzoic were the major propolis substances cause antibacterial and antiviruses activities. In contrast these compounds were identified in samples collected from different parts of Syria and Egypt, by HPLC analysis.

The present results go in line with Kujumgiev *et al.* (1999) who reported that propolis samples collected from different geographic origins has antibacterial activity against *Eschericia coll* and *Staphylococcus aureus* and all gram-positive bacteria strains, in spite of the differences in their chemical compositions. Santos *et al.* (2003) stated that phenolic constituents and the combination between this compounds are essential for the biological activity of propolis. He also studied propolis collected in the dry and rainy seasons and found that there was non significant differences in phenolic and flavonoids compounds, so there was no effect of seasonality on the inhibitory activity of propolis. In contrast this result go in line with those of Sforcin *et al.* (2000) who reported that differences in propolis extracts could be found in samples from different geographical regions, since local flora influence its chemical composition and probably its biological properties. Therefore, results presented confirm those of literature which emphasized the lower sensitiveness of gram-negative bacteria strains compared to gram-positive ones. On the other hand, Fernandes *et al.* (1999) stated that since variation in the susceptibility to propolis among several microorganisms have been reported, but their specific mechanism of action not clearly explained whether the cell structure and permeability to such compounds or even specific targets in the cell enzymes are involved in microbial susceptibility. Moreover, tests of Chinese and Japanese propolis relating different inhibition zone from 6.0 to 9.0 mm. So on the whole one cannot conclude if the variation in results of propolis biological activity was due to methods employed or actually correlated to the activity of the propolis samples tested.

Regarding the present result it could be concluded that there was non significant differences in chemical composition and an efficient antibacterial action of propolis collected from different geographical regions in Syria and Egypt mainly against gram-positive bacteria.

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التركيب الكيماوي و النشاط المضاد للبكتيريا للبر وبوليس المجموع من مصر و سوريا

السيد إبراهيم حجاج*،عماد نافع*و وفاء أسعد يعقوب**

*** معهد بحوث وقاية النباتات، مركز البحوث الزراعية ، الدقي، مصر**

**** قسم وقاية النبات ، كلية الزراعة، جامعة دمشق**

أجريت هذه الدراسة بهدف إجراء دراسة مقارنة بين البروبوليس السوري والمصري الذي تم جمعه خلال عام (٢٠٠٥) ، وذلك من حيث التركيب الكيميائي باستخدام جهاز (HPLC).
وكذا دراسة تأثير البروبوليس كمضاد بكتيري ضد بعض السلالات البكتيرية مثل *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

- وقد أظهرت نتائج التحليل وجود أربعة مركبات فلافونية هي كيورستين، باينوستروبين، كريزين والجلانجين بينما عدد المركبات الفينولية إحدى عشر مركبا وهى فالينين، ايوجانول، سينماميك، سلسيلك، كافيك، فيروليك، ب-بزويك، جاليك، ب-كومارك، ٣، ٥، ديثوكسى بنزيل، فينول وبنسب منوية مختلفة بالإضافة إلى بعض المركبات الأخرى التي لم تعرف لعدم توفر المركبات الاستاندرد اللازمة لتعريفها .
- كما أوضحت النتائج وجود مركبات فينولية مشتركة بين البروبوليس المصري و السوري وهي : الفينول، الكافيك، السلسيلك، السينماميك، الكورستين، ايوجانول و الفانيلين. كما وجد أن الجاليك أسيد موجود في عينة واحدة فى البروبوليس المصرى فقط أما الفيروليك فغير موجود فى عينات البروبوليس المصري وموجود فى العينات السورية، أما ب-كومارك يوجد فى البروبوليس المصري وفى عينة واحدة من البروبوليس السوري وذلك لاختلاف المناطق الجغرافية والفلورا حول المناحل.
- أظهرت الاختبارات البيولوجية أن كلا النوعين من البر وبوليس المصري والسوري ذو فعالية جيدة في تثبيط نمو السلالات البكتيرية المستخدمة فى الدراسة ولا توجد فروق معنوية بينهما مما يدل على أن اختلاف التركيب الكيميائي لأنواع البروبوليس واختلاف مصادره الجغرافية لا يؤثر على كفاءته الحيوية.