Assiut Journal of Agricultural Sciences 54 (4) 2023 (212-220) Website: http://ajas.journals.ekb.eg/
ISSN: 1110-0486 / EISSN: 2356-9840 E-mail: ajas@aun.edu.eg

(Original Article)

Characteristics of Commercial Propolis Samples Collected from Upper Egypt Region

Mohamed O.M. Omar[1]; Salah H. Rateb[1*]; Mahmoud S. O. Mabrok[2] and Mervat A. Shaker[2]

[1]Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt.
[2]Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt.

*Corresponding author email: salah.hashem@agr.aun.edu.eg
DOI: 10.21608/AJAS.2023.210262.1253

Abstract

Egyptian propolis has recently become a subject of increasing attention for biologist and chemists. To estimate the propolis quality, twenty-three samples collected from Upper Egypt region during autumn and spring seasons were chemically evaluated.

Results indicated that propolis samples significantly varied in its quality according to the seasons and localities. Chemical tests of impurity, wax content and oxidation speed rate were determined. Total of active substances as polyphenolic compounds ranged from 49.5% to 59.3% in autumn and from 49.5% to 57.6% in spring. The time of oxidation speed was negatively correlated with presence of active compounds in every sample. Oxidation speed was considered one judge on propolis quality.

Additionally, propolis samples contamination with lead as a major contaminant was determined. The level of lead contamination was lower than the international permissible limits. The results of present study are consistent with the idea that Egyptian propolis is a promising subject for further studies.

Keywords: *Honeybee ، Propolis ، Polyphenolic compounds.*

Introduction

Honeybees use propolis as a material to glue hive parts that are more or less moveable or plug any hole. Bees collect propolis from various plant sources in the northern temperate zone, particularly from species of poplar, birch, and elm. Little is known about sources of propolis in the tropics (Ghisalberti, 1979; Greenaway *et al.*, 1990).

Propolis has a complex chemical composition which has not yet been fully determined. In general, propolis has been found to contain resins, waxes and mechanical impurities. It has also been stated that flavones, flavonoles and flavonones are the main components of propolis. Terpenes isovanillin and aromatic unsaturated acids, caffeic and ferric acid, which are characterized by their biological activity, have also been identified (König, 1985; Walker and Crane,

Received: 22 May 2023/ Accepted: 1 October 2023/ Published online: 5 October 2023

1987; Greenaway *et al*., 1990; Burdock, 1998; Sforcin, 2007; Huang *et al*., 2014; Guendouz *et al*., 2019; Kim *et al*., 2021; and Sunil *et al*., 2021).

Studies on propolis chemical analysis indicated that propolis composition varies according to geographical region as well as to season of collection (Vakhonina and Dushkova, 1978; Omar, 1989; and 1994). Bankova (2005) reported that composition of propolis is quite complicated and more compounds have been identified from time to time. In Egypt, propolis needs to determine chemical standardization that guarantees its quality, safety and efficacy.

The aim of the present investigation was to clarify the quality of commercial propolis produced under Upper Egypt conditions.

Materials and Methods

The present work was carried out at Laboratories of Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt during 2021, 2022 seasons.

Collection of propolis samples

Twenty-three apiaries located in five governorates (Assiut, Sohag, Qena, Luxor and Aswan) at Upper Egypt region were co-operated for obtaining propolis samples during November 2021 and March 2022.

The Egyptian beekeepers are using scraping propolis from inner cover and frame runners of hive. Approximately 15-20 gm. were collected from every apiary. All samples were kept under dark condition at -5°C until chemical analysis.

Propolis quality determination

To determine the parameters of quality, some procedures well be used for determining quality control of crude propolis.

a. Impurities and insoluble matter

This determination of impurities depends on the capacity of crude propolis to dissolve in a chloroform-acetone mixture (2:1) which permits the quantitative determination of the amorphous fraction of propolis (Popravko, 1978). One gram of fine ground propolis was used for every sample.

b. Wax determination

Fine ground propolis was processed in hot ethyl alcohol (65°C) in 1:10 solution until the extract was colourless. The extract was then filtered, at high temperature, to remove the impurities and the insoluble matter, and the filtrate was diluted with water to a 70% concentration and then cooled to 5°C. By this operation, waxes were eliminated. The extracts were filtered through Whatman paper (No. 4) and washed with alcohol several times and dried at 40°C until a constant weight was reached (Ushkalova and Murykhinch, 1978).

c. Total phenolic substances and flavonoids

The total phenolic substances and flavonoids and other active compounds were determined as percentage by subtraction with total wax and insoluble residues.

d. Time of oxidation speed

After calculation the total percentages and active compounds in tested propolis samples, twenty-three samples from different localities and seasons were arranged into three groups according to the percentages of active compounds as follows:

G1: Samples that contain more than 60% active compounds.

G2: Samples that contain 50-59% active compounds.

G3: Samples that contain 40-59% active compounds.

The propolis quality of each group was evaluated using criterion of oxidation speed time (in second) as determined by Vakhonina and Dushkova (1978). A simple modification of this method was done as follows:

Row propolis, 200 mg from each sample, finely ground was placed in flask (250 ml) with 5 ml rectified ethyl alcohol. After one hour, 100 ml of boiled distilled water was added, and then filtered through filter paper. Then, 2 ml of the filtrate or diluted solution was pipetted into a 50 ml glass beaker, 1 ml 20% sulphuric acid was added, and the mixture stirred for 1 min. A drop of 0.1 N potassium permanganate solution was pipetted into the acidified solution, and the time (in second) was taken for the decolorization of solution. The temperature of the solution was adjusted between 18 and 22°C. The speed of oxidation for all samples in every group was determined.

e. Level of lead contamination

Toxic contaminants are considered one of the major concerns regarding quality control of propolis. To evaluate propolis contamination with heavy metals, lead concentration was determined. One mixed sample from every governorates was prepared for analysis. Lead determination was carried out in Central Laboratory of Chemical Analysis, Faculty of Agriculture, Assiut University. The element was determined using ICP (iCAP 6200) according to Issac and Johnson (1985) Method.

Statistical Analysis

Data of all treatments were statistically analyzed using Statistic 8.1 software (Statistix 8.1 (2002) and subjected to either independent Sample T-test or the analysis of variance. Means were separated by LSD test (least significant difference test) as a significant level of $P<0.05$ (Steel and Torrie, 1980).

Results and Discussion

The data presented in Tables 1, 2 and 3 show percentages of main contents of propolis samples collected from Upper Egypt regions during Autumn (2021)

and Spring (2022) seasons. The values of propolis samples varied significantly according to the locality and season of propolis collection. Data in Table (1) show that the impurities percentages of autumn samples ranged between 8 to 12%. However, the percentages of waxes varied from 28.3% to 42.4%. After calculation of propolis contents, the total polyphenolic and active substances percentages ranged between 49.5% to 59.3% and the values differed significantly among the governorates (Table 1). The same trend was observed in data obtained during spring season, 2022 (Table 2). Data of general means of propolis chemical composition of two seasons were recorded in Table (3). The means of impurities, waxes and polyphenolic compounds slightly differed between the two seasons and insignificant differences were observed.

Table 1. Chemical composition of propolis samples collected from some Upper Egypt regions, Autumn 2021

Governorate	Propolis contents (mean ± SD) %		
	Impurities	Wax	Total of poly phenolic comp.
Assiut	12.000 ± 1.500 a	29.889 ± 5.159 d	58.111 ± 3.859 a
Sohag	9.667 ± 3.041 bc	31.000 ± 8.775 cd	59.333 ± 9.473 a
Qena	8.000 ± 1.871 c	42.444 ± 8.353 a	49.556 ± 8.575 b
Luxor	10.333 ± 2.549 ab	40.111 ± 5.667 ab	49.556 ± 4.035 b
Aswan	11.222 ± 1.302 ab	35.222 ± 1.922 bc	53.556 ± 1.878 ab

Means with followed by the same letter are not significantly differed at 5% level.

Table 2. Chemical composition of propolis samples collected from some Upper Egypt regions, Spring 2022

Governorate	Propolis contents (mean ± SD) %		
	Impurities	Wax	Total of poly phenolic comp.
Assiut	8.500 ± 1.378 a	42.000 ± 2.366 a	49.500 ± 2.665 c
Sohag	10.667 ± 3.141 a	32.167 ± 1.472 c	57.167 ± 4.167 a
Qena	9.667 ± 1.211 a	35.167 ± 3.189 c	55.333 ± 3.502 ab
Luxor	10.66 ± 1.966 a	38.667 ± 3.011 b	50.667 ± 4.844 bc
Aswan	-	-	-

No samples collected from Aswan governorate.
Means with followed by the same letter are not significantly differed at 5% level.

Table 3. means of chemical composition of propolis samples collected from some Upper Egypt governorates during Autumn (2021) and Spring (2022) season

Season	Propolis contents (mean ± SD) %		
	Impurities	Wax	Total of poly phenolic compounds
Autumn 2021	10.2440 ± 0.3695	35.422 ± 1.1813	54.002 ± 1.0892
Spring 2022	9.8750 ± 0.4354	37.000 ± 0.9149	53.167 ± 0.9921

According to the chemical tests carried out by Omar (1994) on propolis samples collected by scraping different parts of bee hives, the results revealed that bee wax and impurities of every propolis sample indicate its quality. Also, he found that propolis sample collected from internal walls contain a high wax percentage (54.7%) while sample scraped from hive bottom board exhibited the highest impurities (24.1%). When Negri *et al*. (2000) compared the composition of propolis wax and comb waxes which were produced by the same colony no

difference was found to allow a distinction. Suggesting a common region for both wax sources. Salatino and Salatino, 2021 reported that the wax content is highly variable in propolis.

The contents of total polyphenolic substance in two seasons in the present study are considered as an important index for evaluating the propolis quality. Zhang *et al*. (2014) used the total polyphenolic compounds and flavonoids percentages in propolis as a criterion to evaluate the quality of temperate propolis. Shan *et al*. (2011) noticed that a direct correlation between harvesting method and wax concentration in Romanian propolis samples. Therefore, the propolis samples collected by the means of hive tools had significantly higher amount of wax.

After determination of polyphenolic compound percentages (Tables 1, 2 and 3), the criterion of oxidation speed time (in sec.) was done as described by Vakhonina and Dushkova (1978) to assurance the level of propolis quality. Data in table (4) presented the values of oxidation speed for all propolis samples collected from some governorates of Upper Egypt during the two seasons of study. The results show that the faster speed of oxidation time was recorded for Autumn samples collected from Assiut governorate. However, the longest time of oxidation speed (6.05 sec.) was recorded in Assiut samples which were collected during Spring season. This result was associated with the highest percentage of bee waxes 42.4%. The present results are in agreement with Burdock (1998), Sforcin (2007) findings, they reported that propolis contains diversity of compounds depending on plant origin, basically is composed of 55% vegetable resins and balsam, 30% bee wax 10% essential oil and 5% pollen.

Table 4. Oxidation speed of propolis samples collected from some Upper Egypt regions during Autumn (2021) and Spring (2022)

Governorate	Speed of oxidation mean ± SD (time in sec.)	
	Autumn, 2021	**Spring, 2022**
Assiut	3.97 ± 0.403 a	6.05 ± 0.308 b
Sohag	4.11 ± 1.467 a	3.92 ± 0.646 a
Qena	5.96 ± 1.812 b	5.05 ± 0.647 b
Luxor	5.52 ± 0.415 b	5.95 ± 1.778 b
Aswan	4.37 ± 0.218 a	-
General mean	**4.78**	**5.24**

According to the time of oxidation rate one can judge on propolis quality. Faster speed of oxidation time means high quality of propolis. Silici and Kutluca (2005) reported that the chemical composition of propolis is susceptible to the geographical location, botanical origin and method of collection.

After determination of oxidation speed time of all propolis samples collected from different localities, the values were arranged in to three groups according to the percentage of polyphenolic compound (Table 5). The results indicated that a negative relationship between oxidation speed time and total polyphenolic compounds was found in all groups. The present results proved that the first group which contains high percentage (60% and more) from active substance, the potassium permanganate solution decolorized faster within 3.45 sec. in the

suspension of 200 mg propolis/100 ml distilled water. In the third group which contains a range from 40 to 49.9 active compounds, the time needed for oxidation increased to 7.3 sec. These results indicated that the rate of oxidation speed varies according to the purity of propolis. Vakhonina and Dushkova (1978) recorded that the rate of oxidation speed of propolis is related also to the presence of number from non- saturated acids specific to honey bee workers and to the glandular secretion which are added to propolis. The biological activity of propolis is associated with percentage of flavonoids, flavones and phenolic acids and their derivates.

Table 5. Relationship between polyphenolic compounds level and oxidation speed time of propolis samples collected from some Upper Egypt regions

Type of samples	Total of flavonoids comp. %	Time of oxidation speed (sec.)	Simple correlation (r)	
	Mean ± SE (rang.)	Mean ± SE (rang.)	Value (r)	P-value
Group I > 60%	62.660±0.9436 a (60.30-66.30)	3.45±0.2902 a (2.88-3.68)	-0.9371	0.0187
Group II 50-59.9%	54.064±0.6362 b (50.30-58.50)	4.89±0.1937 b (3.70-5.89)	-0.8995	0.0005
Group III 40-49.9%	46.64±0.7975 c (43.7-48.7)	7.30±0.2453 c (6.21-8.28)	-0.9304	0.0024

The relationship between speed of oxidation and active propolis components was calculated statistically using the simple correlation. Recorded data in table (6) show a highly significant negative correlation between the speed of oxidation and total phenolic compounds.

Table 6. Simple correlation coefficients (r) between speed of oxidation and the major active propolis components

Season	(r)
Autumn 2021	-0.8885
Spring 2022	-0.9082

The commercial propolis introduced from producing countries for medicinal and food uses. Some standards were determined as an indicator of its purity. Heavy metals determination is one of the main standards for propolis quality assurance.

Results in Table (7) show the lead contamination level in propolis samples collected from the studied five governorates. These values ranged between 3.37 and 4.77 mg/kg in mixed propolis samples. All determined lead concentrations in the tested Egyptian samples were under the international limit. Fearnley (2005) suggested that we can use the lead contamination as an indicator for environmental pollution. Toxic contaminants are one of the major concerns regarding quality control of propolis. Heavy metals may accumulate in dangerous amounts in propolis. Also, Alcici (1996) reported that propolis can contaminate from their sources as painting hives and wrapping samples in newspaper or plastic bags.

Table 7. Lead contamination level of propolis samples collected from some Upper Egypt governorates

Governorate	Mean of Pb (mg/kg.)
Assiut	4.55 b
Sohag	3.37 d
Qena	4.01 c
Luxor	4.77 a
Aswan	3.48 d
General mean	**4.036**

The results of the present study are consistent with the idea that Egyptian propolis is a promising subject for further studies and still a lot of work to be done to achieve a reliable standardization of Egyptian propolis.

It can be concluded from the present results that in corporation of propolis, can sequently, detail of their chemical analysis well be needed to determine the Egyptian propolis fingerprint.

References

Alcici, N.M.F. (1996). Heavy metals in propolis, practical and simple procedure or reduce the lead level in the Brazilian proplis. In Mizrahi A. Lensky Y. (eds) Bracticts. Planum Press: New York USA pp. 231-239.

Bankova, V. (2005). Recent trends and important development in propolis research. Evidence Based Complementary and Alternative Medicine, 2: 29-32.

Burdock, G.A. (1998). Review of the biological properties and toxicity of bee propolis (Propolis). Food and Chemical Toxicology, 35 (4): 347-363.

El-Guendouz, S., Lyoussi, B. and Miguel, M.G.C. (2019). Insight on propolis from Mediterranean countries: Chemical composition, biological activities and application fields. Chem. Biodiv., 16(7): 1-35

Fearnley, J. (2005). Bee propolis natural healing from the hive. Souvenir Press London, 172 pp.

Ghisalberti, E. (1979). Propolis a review. Bee World, 60 (2): 59-84.

Greenaway, W.; Scayasbrook, T. and Whatley, F.R. (1990). The analysis of bud exudates of Populus x euramericana, and of propolis, by gas chromatography-mass spectrometry. Proc. R. Soc. London B 232: 249-272.

Huang, S., Zhang, C.P., Wang, K., Li, G.Q., Hu, F.L. (2014). Recent advances in the chemical composition of propolis. Molecules, 19(12):19610–19632.

Issac, R.A. and Johnson, W.A. (1985). Elemental Analysis of Plant Tissue by Plasma Emission Spectroscopy: Collaborative Study. J. Assoc. Off. Anal. Chem., 68 (3): 499.

Kim, S.K.; Woo, S.D., and chang, J.S. (2021). Biological properties of propolis isolated from honey bee. Journal of Life Science, 31 (7): 686-697

König, B. (1985). Plant sources of propolis. Bee World, 66: 136-139.

Negri, G.; Marcucci, C.; Salatino, A. and Salatino, M.L.F. (2000). Comb and propolis wax as from Brazil. J. Braz. Chem. Soc., 11: 453-457.

Omar, M.O.M. (1989). Some characteristics of propolis from upper Egypt. Proc. 4 int. Conf. Apic. Trop. Climates, Cairo, 88-92.

Omar, M.O.M. (1994). Quantity and quality of propolis collected from different places of honey bee hives. Assiut J. of Agric. Sci., 25 (1): 127-137.

Popravko, S.A. (1978). Chemical composition of propolis, its origin and standardization A remarkable hive product: Propolis, Apimondia. Publ. House, Bucharest, pp. 15-18.

Salatino, A.; Salatino, M.L.F. (2021). Scientific note: Often quoted, but not factual data about propolis composition. Apidologie, 52(2): 312–314.

Sforcin, J.M. (2007). Propolis and the immune system: a review. Journal of Ethnophamacology, 113 (1): 1-14.

Silici, S. and Kutluca, S. (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honey bees in the same region. J. Ethnopharmacol., 99: 69-73.

Shan, L.; Marghitas, L.Al. and Dezmirean, D. (2011). Quality criteria for propolis standardization. Animal Science and Biotechnologies, 44 (2): 137-140.

Statistix 8.1 (2003). User's Manual. Analytical Software, Tallahassee. Florida USA.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York.

Sunil, L.S., Vignesh, S. And R. Meenatchi (2021). Physicochemical properties and antimicrobial activity of Indian propolis. The Pharma Innovation Journal, 10(10): 1660-1664

Ushkalova, V.N. and Murykhlich, T.P. (1978). Antioxidant value of propolis. In A remarkable hive product. Propolis Bucharest, Romania, Apimondia Publishing House.

Vakhonina, T.V. and Dushkova, E.S. (1978). Quality of propolis. Pp. 236-241. In a remarkable hive product: propolis. Bucharest, Romania: Apimondia Publishing House.

Walker, P. and Crane, E. (1987). Constituents of propolis. Apidologie 18: 327-334.

Zhang, C.; Huang, S.; Wei, W.; Ping, S.; Shen, X.; Li, Y.; Hu, F. (2014). Development of High-Performance Liquid Chromatographic for Quality and Authenticity Control of Chinese Propolis. J. Food Sci., 79: C1315-C1322.

خصائص عينات بروبوليس تجاري (صمغ نحل) جمعت من منطقة مصر العليا

محمد عمر محمد عمر[1]، صلاح حفني راتب[1]*، محمود سيد عمر مبروك[2]، مرفت عطاالله شاكر[2]

[1]قسم وقاية النبات، كلية الزراعة، جامعة أسيوط، مصر.
[2]معهد بحوث وقاية النبات، مركز البحوث الزراعية، مصر.

الملخص

لقد أصبح البروبوليس المصري حديثاً موضع اهتمام الكيميائيين والبيولوجيين. وفي هذه الدراسة قد تم جمع ثلاثة وعشرون عينة من البروبوليس المصري من خمس محافظات في منطقة مصر العليا خلال موسمي الخريف والربيع ليتم تقييمها كيميائياً.

دلت نتائج البحث على وجود اختلافاً معنوياً في جودة البروبوليس تبعاً للموسم ولمنطقة الجمع. حيث تم تقدير درجة النقاوة ومكونات الشمع ومعدل سرعة الأكسدة.

وقد وجد أن نسبة المواد الفعالة (المركبات الفينولية) تراوحت من 49.5% إلى 59.3% في العينات المجموعة خلال موسم الخريف، بينما تراوحت نسبتها من 49.5% إلي 57.6% في عينات موسم الربيع. كما سجلت النتائج أيضاً أن وقت سرعة الأكسدة قد ارتبط ارتباطاً سالباً مع وجود المركبات الفعالة في كل عينة، حيث تعتبر سرعة الأكسدة هي أحد المعايير الهامة لتقدير جودة البروبوليس. وبالإضافة إلى النتائج السابقة فقد تم تقدير درجة تلوث عينات البروبوليس بعنصر الرصاص للتعبير عن درجة نقاء البروبوليس، حيث أظهرت النتائج أن نسبة تلوث العينات بعنصر الرصاص كانت أقل من الحدود المنصوص عليها في المواصفات القياسية المعتمدة دولياً.

وتعطي نتائج الدراسة الحالية انطباعاً على أن البروبوليس المصري يعتبر موضوعاً مهماً يحتاج إلى المزيد من الدراسات المستقبلية.