

COMPARISON OF ETHANOLIC EXTRACT YIELD, pH, POLYPHENOLS AND FLAVONOIDS IN ALGERIAN PROPOLIS COLLECTED FROM VARIOUS GEOGRAPHIC REGIONS

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ABSTRACT

The present work aims to evaluate the yield, pH, the total polyphenols and flavonoids contents in propolis collected from different ecosystems in Algeria. Ten samples of propolis, issued from different floral and geographical origins, have been collected by beekeepers between March and April 2015 and were classified according to the geographical regions (Est, West, Center and South) the samples were labeled from P1 to P10 and kept in dark at 4°C until use. The determination of polyphenols and total flavonoids contents was carried out in a specialized laboratory in Belgium. In this study, the propolis collected from the southern and western regions had the highest concentrations of polyphenols 99,98; 71,6 and 76,1 Gallic acid/g of propolis. The same result was obtained for flavonoids with 54,4; 38,7 and 42,9 mg Quercetin/g propolis. The higher yield, after ethanolic extraction of the propolis, was obtained from the southern region sample (P10) with a rate of 39,32%. Our study showed that the richest propolis samples of Algeria, in total flavonoid and total polyphenol contents were the ones collected from the southern (P10) and the western region (P6 and P8).

Keywords: Algerian propolis; yield; pH; ethanolic extract.

INTRODUCTION

Propolis is a sticky resinous substance collected by bees on certain parts of plants, essentially leaves, flowers and bud exudates removed mixed with bee secretions and wax [1]. Based on plants origin, several chemical types of propolis can be distinguished, European, North American, non-tropical regions of Asia, Birch propolis from Russia, Mediterranean propolis, Brazilia Green

Propolis, Cuba and Venezuela Red propolis, Pacific propolis (Okinawa, Taiwan) and Canary propolis [2,3]. As a natural product of the beehive and for thousands years, propolis extracts have been used internally and externally as a healing agent in traditional medicine [4]. The propolis chemical composition depends on its floral origin, geographical conditions and region climatic characteristics [5,6]. It is composed of 50% vegetable resin and balm, 30% wax,

10% essential and aromatic oils, 5% pollen and 5% of several substances including organic debris [7].

Propolis use has great effect on human health and is used for its antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory activity [8], other properties were reported as local anesthetic effect, reduction of spasms, healing of gastric ulcers, strengthening of capillaries [9] stimulation of bone integration [10,11] and cartilage formation [12,13]. The antimicrobial activity of propolis against Gram-positive bacteria and yeasts was also reported [14]. This work aimed to quantify the polyphenols and flavonoids in different propolis samples collected from different Algerian ecosystems.

MATERIALS AND METHODS

Propolis Samples

In order to select the richest propolis in polyphenols and flavonoids, ten samples of Algerian propolis from different floral and geographical origins have been collected by beekeepers between March and April 2015 and were classified according to the geographical regions (Est, West, Center and South). The samples were labeled from P1

to P10 and kept in dark at 4°C until use (Table 1).

The polyphenols concentrations were determined using the Folin Ciocalteu method, however flavonoids concentrations were determined by the Woisky and Salatino method. Analysis was performed at a specialized laboratory CARI ASPL (Boltzmann Building Place Croix du Sud, 1 box L7.04.01B-1348 Louvain-La-Neuve, Belgium).

Ethanolic Extract of Propolis

The extraction of useful polyphenols was carried out in the Laboratory of food technology and biochemistry of the life Sciences Faculty of Tiaret. The 10 samples of propolis were purified by traditional method that consists of mixing 10 g of the raw propolis with 100 ml of ethanol at 70% in flasks with periodic stirring using an electromagnetic stirrer for a duration of 15 days in the dark [4,5,15]. The filtration of the ethanolic solution of the raw propolis was ensured using Whatman paper, the filtrate was put into boxes of moldings the evaporation of the ethanol was made in a drying oven at 45°C, the propolis extracts were obtained by scratching and kept in darkness at 4°C.

Table 1. Propolis samples classified according the harvesting region

Label	Harvesting region	Altitude	Longitude
P1	SOUK AHRAS (Bir bouhouche)	36° 0'50,52"N	7°25'48,08"E
P2	SOUK AHRAS (HNANACHA)	36°15'40,69"N	7°47'16,29"E
P3	MILA (AHMED RACHEDI)	36°20'39,48"N	6° 7'53,37"E
P4	TIPAZA	36°37'4,36"N	2°23'28,45"E
P5	BLIDA	36°28'32,85"N	2°49'47,13"E
P6	TIARET (BOUCHAKIF)	35°21'23,00"N	1°30'48,96"E
P9	RELIZANE	35°44'2,17"N	0°33'31,96"E
P8	TIARET (OUALED BOUGHADOU)	35°22'32,12"N	1°18'54,17"E
P7	LAGHOUAT (EL kheneg)	33°44'43,42"N	2°47'43,48"E
P10	LAGHOUAT (HAMDIA agricultural zone)	33°48'0,46"N	2°52'10,96"E

Determination of the Propolis pH

Small pieces of the ten propolis samples were mixed with distilled water three times. The volume of each sample was heated in beakers in a water bath for half an hour, stirring occasionally with a stick. After filtration, the resulting aqueous solution was poured into small beakers to determine their pH using a pH-meter with immersing the electrode completely in the solution [16].

Determination of the Propolis Yielding

The yield of propolis extraction for each region can be obtained with the following equation [9]: $\text{Yield} = (\text{PE} / \text{PM}) \times 100\%$ (PE: weight of propolis extract (g); PM: weight of raw propolis (g)).

For each parameter, mean and standard deviation values were determined and a statistical analysis using SPSS IMB 20 And the ANOVA test was made.

RESULTS AND DISCUSSION

The results of our study for the ten samples collected are presented in the Tables 2 and 3. The propolis concentrations of total polyphenols was the highest in the P10 sample issued from the southern region of Laghouat with 99,98 mg Gallic Acid/g of propolis, followed by P6 and P8 from the western region of Tiaret with respectively 76,1 and 71,6 mg Gallic Acid/g of propolis. We recorded higher levels for flavonoids in the same samples P10, P6 and P8 with the values about 54,4; 42,9 and 38,7 mg Quercetin/g of propolis respectively. Many studies were performed to evaluate the total polyphenols and flavonoids of propolis, the total polyphenol reported for Ghardaia's propolis is about of 4,93 mg Gallic Acid/g of propolis and about 1,94 mg Quercetin/g of propolis total flavonoids [17], this values are

lower than those found in this study with 99,98 mg Gallic Acid/g propolis and 54,4 mg Quercetin/g of propolis for polyphenols and flavonoids respectively.

In this study, the propolis harvested from the southern and the western regions showed the highest significantly ($p < 0,05$) values for both polyphenols and flavonoids as the levels detected in Tiaret's propolis were 71,6 and 76,1 mg Gallic Acid/g of propolis for total polyphenols and 38,7 and 42,9 mg Quercetin/g of propolis for total flavonoids respectively for the samples Ouled boughadou (P8) and Bouchakif (P6). However, the total polyphenol content of the propolis of Annaba's region called (Elbouni) was reported to be about 100,90 mg Gallic Acid/g propolis is of the order of 58,99 mg Quercetin/g of propolis of total flavonoids [18], which is in agreement with our results for the region of Souk Ahras and Mila, with the values for the total polyphenols about 19,2; 49,7 and 14,2 mg Gallic Acid/g of propolis and total flavonoids 6,5; 23,3 and 5,6 mg Quercetin/g of propolis respectively. However, the values reported by other researchers for the region of Khenchla [17] with 14,23 mg Gallic Acid/g of propolis for total polyphenols and 3,45 mg Quercetin/g of propolis for total flavonoid are close to ours for the Mila region and inferior to the Souk Ahras's ones.

Propolis is an apicultural product from plant origin, its chemical composition and its biological activity depend on the specificity of the local flora, the season of harvest and the species of bees. The arid and semi-arid climate plays an important role in the transhumance of bees, and long days give bees the opportunity to harvest and visit different local flora [19]. The propolis harvested in the central and eastern regions of Algeria was reported containing various high concentrations of polyphenolic

Table 2. Mean±SD values for polyphenols and flavonoids in the propolis collected

Samples	Polyphenols (mg/g of propolis)	Flavonoids (mg/g of propolis)
P1	19,00±0,71 ^a	6,50±0,71 ^a
P2	49,70±2,12 ^b	23,25±1,06 ^b
P3	14,20±4,81 ^{ac}	5,60±0,42 ^{ac}
P4	35,25±5,59 ^d	15,95±0,07 ^d
P5	39,55±4,03 ^d	15,90±0,85 ^d
P6	76,10±0,71 ^e	42,85±0,49 ^e
P7	7,30±1,41 ^c	2,00±0,14 ^f
P8	71,55±1,48 ^e	38,70±0,28 ^g
P9	20,25±4,45 ^{ac}	5,80±0,14 ^{ac}
P10	99,80±8,06 ^f	54,50±1,41 ^h

Means followed by the same letter in the column are not significantly different ($P > 0,05$)

Table 3. The values of propolis pH and yield after ethanolic extraction

Samples	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
pH	4,2	4,7	4,8	4,5	4,3	4,7	4,7	4,0	4,5	4,3
Yield (%)	18,50	20,43	15,92	14,11	29,22	28,01	31,27	36,38	13,37	39,32

compounds [20], whereas in our study, in the regions of center Blida and Tipaza the values for total polyphenols were respectively 35,3 and 39,6 mg Gallic Acid/g of propolis and 16 and 15,9 mg Quercetin/g of propolis for total flavonoids. We also observed that the lowest value was recorded for the Laghouat's region that seems to be due to the poor conservation of the product at the beekeeper.

In this work, the values of pH in the range of 4 means that all propolis are acidic in nature. This acidity is due to its composition in aromatic and aliphatic acid [21]. The ethanolic extraction of the Algerian propolis from the four regions revealed an average yield of $24,74 \pm 9,42\%$ and the highest value was noted for the propolis of the southern region with $35,59 \pm 5,26\%$, these results are higher than the values reported for the London's propolis with $18,00 \pm 1,82\%$ [9] and similar to $32,42$ to $39,36\%$ reported for Algerian propolis [21].

CONCLUSION

Our study showed that the southern Algerian propolis collected from the region of Laghouat was richest in flavonoids and polyphenols, followed by the one collected from the western region of Tiaret. This work confirmed also that Algerian propolis had an acidic pH.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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