Available online on 15.01.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Polyphenol Contents and Antioxidant Activity of Ethanolic and Aqueous Algerian Propolis Extracts (Region of Serdj el ghoul)

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ABSTRACT

Aqueous and ethanolic extracts (EAP and EEP) of propolis sample collected from Serdj El Ghoul, region of Sétif (east of Algeria), were prepared and evaluated to identify their biological activities. Total phenolic contents were determined using Folin-Ciocalteu reagent and found to be 164,690 ± 0,044 (EAP), 155,078 ± 0,176 mg cafeic acid equivalent/ g of propolis. Flavonoids were evaluated by $AlCl_3$ method and shown to be 9,839± 0,006 (EAP), 55,758 ± 0,128 (EEP) mg quercetin equivalent/ g of propolis. The free radical scavenging potential of the extracts was determined by the DPPH method, the IC50 are estimated at 0.0865 (BHT), 0.0223 (EAP), 0.0194 (EEP) mg/mL. We can conclude that propolis contains molecules that are considered first class of antioxidants and can be used for therapeutic applications, knowing that antioxidants contribute very effectively to disease prevention such as cancer, and cardiovascular disease.

Keywords: Propolis, aqueous extract, ethanolic extract, Antioxidant

Article Info: Received 24 Oct 2019; Review Completed 04 Dec 2019; Accepted 12 Dec 2019; Available online 15 Jan 2020

Cite this article as:

Soltani E, Mokhnache K, Charef N, Polyphenol Contents and Antioxidant Activity of Ethanolic and Aqueous Algerian Propolis Extracts (Region of Serdj el ghoul), Journal of Drug Delivery and Therapeutics. 2020; 10(1):1-4
http://dx.doi.org/10.22270/jddt.v10i1.3797

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INTRODUCTION

Health and beauty are among the concerns of the man who continues to look for the best way to maintain them. His research has undergone considerable change in recent years. Several manufacturers such as pharmaceutical companies and the cosmetics industry have followed a new revolution: the return to nature. Thus, the soft medicine proposes treatments softer, less aggressive and especially more accepted by the patient and the cosmetology proposes preparations with natural products more appreciated and more sought after by the consumer [1]. Apitherapy is one of the natural care methods. It is based on hive products such as: honey, royal jelly, propolis ... etc. [1]. Propolis is defined as a natural remedy used since antiquity, and also is a plant resin. It is collected by bees and has a balsamic odor and a variable color depending on its plant origins. Indeed, propolis or bee glue is a complex mixture of several organic and inorganic compounds used by the bee as glue, coating and antibiotic [2]. Around the world, many studies are devoted to propolis, a major source of phenolic compounds, including flavonoids, various propolis substances harvested from different regions of the world have been identified, including phenolic acids, flavones, flavonols and flavanones mark their permanent presence (standard elements of propolis) [2].

This product of the hive is very valuable because of its antioxidant, anti-inflammatory, antibacterial, antiviral, anti-cancer and therapeutic properties related to its polyphenol and flavonoid composition. To these effects, propolis is extensively used in the food industry, medicine, cosmetology and veterinary medicine [3]. This study is carried out in order to be able to identify the chemical composition, the antioxidant properties, the anti-inflammatory activity and the bactericidal activity of extracts (ethanolic and aqueous) of the Algerian propolis typically from the wilaya of Sétif, and to exploit its virtues.

MATERIAL AND METHODS

The propolis sample (**Figure 1**) is the origin of Setif (Serdj El Ghoul region). It is harvested in December 2018, characterized by a brown color and a fragrant smell. The preservation of our sample was made cold in the refrigerator at 4 ° C until they were used.

Reagents

Sodium carbonate (Na2CO3); Aluminum Chloride (AlCl3); NaCl; Folin-Ciocalteu reagent; 1,1-diphenyl-2-picryl-hydrazyl (DPPH); Gallic acid; Quercetin, all come from Sigma-Aldrich. Other solvents obtained from Prolabo: Distilled water; Ethanol; Methanol; Acetone Dimethyl sulfoxide (DMSO).

Figure 1. Propolis sample

Preparation of extracts

Extraction is only a stage of transformation of the raw material (in our case it is propolis) into an extract. All the steps preceding or following the extraction must be precisely controlled for a final product of optimal quality. With this in mind, the cutting of propolis has been carried out so as to recover very fine pieces in order to optimize the extraction.

The extraction was carried out by two solvents. The first is ethanol, and the second is distilled water. Two extracts were obtained, an ethanolic extract (EEP), and an aqueous extract (EAP).

Total polyphenol content

Total polyphenols are assayed as follows:

Using a micropipette, 100 µl of ethanolic extract and aqueous extract of propolis (1 mg / ml) were introduced into test tubes, followed by the addition of 500 µl of Folin-Ciocalteu reagent. (diluted 10 times in distilled H2O) in each tube. Stir vigorously and leave to act 4min before adding 400µl of 7.5% Na2CO3. After 2 hours of incubation at room temperature and protected from light, read the absorbances from the UV-visible spectrophotometer at 760nm.

White is prepared for each variety by replacing the Folin reagent with distilled water. 100 µl of each extract are introduced into the test tubes, 500 µl of distilled H_2O are added, after 4 min 400 µl of Na_2CO_3 are added. All measurements are made in triplicate.

Determination of total flavonoids

The aluminum trichloride ($AlCl_3$) method cited by Djeridane et al [4], and Boudiaf [5], is used to quantify the flavonoids in our extracts.

1ml of each extract and the standard (dissolved in methanol) with the appropriate dilutions was added to an equal volume of a solution of $AlCl_3$ (2% in methanol).

The mixture was vigorously stirred and the absorbance at 430 nm was read after 10 minutes of incubation [6].

Antioxidant activity

For carrying out this test, the DPPH is solubilized in methanol (0.004%). 1250 µl of this solution are added to 50 µl of the extract in solution in methanol (or 50 µl of HE in DMSO) at different concentrations, then, the tubes are placed in the dark for 30 minutes. The absorbance was measured at 517 nm. The positive control is represented by a solution of an antioxidant which is BHT, rutin, and quercetin [7]. All operations are performed in triplicate.

Inhibition percentage was calculated using the following formulae:

$$\text{Scavenging activity (\%)} = 100x(A0 - A1) / A0$$

A_0 is the absorbance of the blank and A_1 the absorbance of extracts

RESULTS AND DISCUSSION

Ethanolic extraction

The operation of the extraction of propolis with ethanol (EEP) made it possible to obtain a dry residue of crude extract with a yield of 18.2%.

Aqueous extraction

The yield of propolis extract after decoction (EAP) was estimated at 7.4%.

This variation depends on the extraction solvent and the origin of the propolis, which proves once again the complexity of the propolis composition. The extraction yields of propolis by distilled water and ethanol are shown in **Table 1**. These extracts contain all the compounds that can be extracted by water or ethanol, these compounds can be flavonoids, phenolic compounds, etc. the yield was determined relative to 10 g of crude propolis. The results were expressed as a percentage by weight (w / w).

Table1. Colors and yields of investigated extracts

Extract	Color	Yields
EEP	Brown	18.2 %
EAP	Beige	7.4 %

It's very clear from **Table 1** that the yield of the ethanolic extract (18.2%) is higher than the aqueous extract EAP (7.4%), the difference of these values can be related to several factors such as the solvent used and extraction methods.

In the case of our study, the yield obtained with ethanol is lower compared to that obtained by Paviani et al [8], and Biscaia and Ferreira [9], who used maceration extraction to obtain yields. in the order of 39.45% and 46.00%, respectively. Indeed, this rate can vary from one sample to another in the same region. This has been demonstrated for Brazilian propolis, where rates ranging from 40.7% to 73.9% were obtained with 70% ethanol. Moreover this rate increases with the increase of the maceration time, however the qualitative composition remains the same [10].

Regarding the water, the latter being polar, the yields are very low. These results are similar to those found in the literature. Indeed, a study on the aqueous extraction indicated yields of the order of 3.2%, 7.7% and 9.6%, respectively for the propolis of Peru, China and Brazil [11]. The extraction with water has shown a lower yield because the water has an affinity with the polar compounds, the hydroxyl group (-OH) turns the water into a bad solvent for the organic compounds.

From studies on propolis extraction, it can be deduced that the different extraction yields and concentrations were probably related to propolis characteristics, such as season and area of harvest, species of bees, regional flora, storage conditions as well as climatic conditions and the choice of solvent.

Phytochemical analysis

Total polyphenol content

determination of total polyphenols performed according to the method of Singleton and Rossi (1965) with the use of Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent test was used to estimate the total phenolic content of propolis extracts. The curve shows a linearity of the absorbance as a function of the concentrations (**Figure 2**). The concentrations of the total polyphenols are calculated from the regression equation of the calibration range established with gallic acid of type: y = 0.0086 x + 0.07 knowing that R^2 = 0.9981.

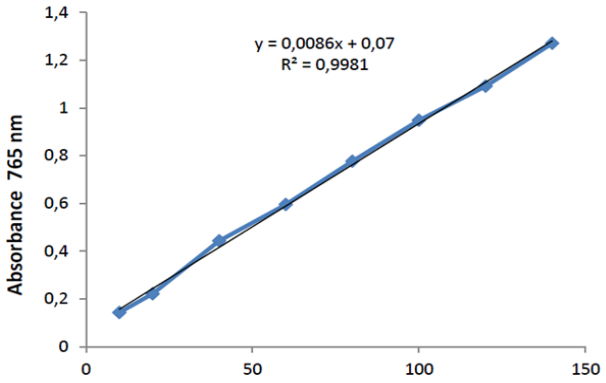

Figure 2: Gallic acid curve for the determination of total polyphenols.

These results of the colorimetric analysis of the total polyphenol compounds of the propolis extracts are summarized in **Table 2**.

Table 2. Total polyphenol content of EAP and EEP extracts

Extracts	EAP	EEP
Polyphenols[a]	164.690±0.044	155.078±0.176

[a] µg gallic acid equivalent per mg of extract (µg EAG / mg extract).

Given the results obtained, we note that the polyphenol content is slightly higher for the aqueous extract, This can be explained by the presence of many phenolic compounds, relatively polar, water-soluble. The investigations made on the determination of the total polyphenol content of bee propolis are numerous, and the results are different from one study to another, we can mention a few:

The total polyphenol content of Brazilian propolis is of the order of 232 (± 22.3) mg EAG / g [12]. This value is large compared to that found in our aqueous extract.

The study on propolis in Portugal shows total polyphenol contents ranging from 151.00 (± 0.01) to 329.00 (± 0.01) mg EAG / g propolis respectively for the Fundao region and Terminal [13]. Which means that the propolis of Portugal is rich in polyphenols compared with our ethanolic propolis.

Work on Iranian propolis [14] has reported phenolic compound contents in the range of 8.46% (± 0.03), 7.11% (± 0.19) and 3.08% (± 0.02) of crude propolis for Teheran, Isfahane and Khorasan respectively, these values are lower than those found in the present study (155.078 ± 0.176 mg EAG / g).

Total flavonoid content

Flavonoids were assayed using the aluminum trichloride ($AlCl_3$) method and the standard was quercetin. The content of flavonoids is expressed in milligram equivalent of quercetin per gram of fresh material (mg EQ / g of propolis). The flavonoid levels of the aqueous and ethanolic extracts of propolis were obtained from the calibration curve (**Figure 2**) which follows an equation of type: y = 0.028x + 0.010 knowing that R2 = 0.9983.

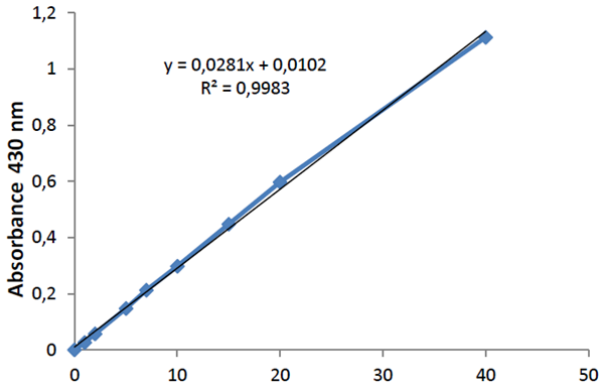

Figure 2. Calibration curve of quercetin for total flavonoid assay.

The results of the total flavonoid content of the propolis extracts are shown in **Table 3**.

Table 3. Total flavonoid content of EAP and EEP extracts.

Extracts	EAP	EEP
Flavonoids[b]	9.839±0.006	55.758±0.128

[b] µg equivalent of quercetin per mg of extract (µg EQ / mg of extract).

It can be deduced that the flavonoid content of the ethanolic extract has a high value in flavonoids, whereas the flavonoid content of the aqueous extract has a very low value. It is clear that ethanol is the solvent that allows for a higher total flavonoid yield.

The total flavonoid content detected in the ethanolic extracts of Iranian propolis was 77.9 (± 0.39), 31.1 (± 0.08) and 12.2 (± 0.33) mg EQ / g of propolis. Respectively, for Teheran, Isfahane and Khorsan samples [14].

The work carried out on the ethanol sample from Brazil indicates a flavonoid content of about 43 (± 0.1) mg EQ / g of crude propolis. This value is lower than that found in the presence study (55.758 ± 0.128mg EQ / g) [15].

The total flavonoid content in the ethanol extracts of propolis was 116 (± 9.3), 147 (± 9.3) and 168 (± 6.4) mg EQ / g propolis respectively for samples from Chile. , China and Uruguay [12], these values are higher than those found in the present study (55.758 ± 0.128 mg EQ/g).

The work carried out on the aqueous sample from Thailand indicates a flavonoid content of about 2.5 (± 0.8) mg EQ / g of propolis [15], this value is low compared to the quantity of Flavonoids contains in our aqueous extract (9.839 ± 0.006 mg EQ/g).

Antioxidant activity

The antioxidant activity was evaluated by measuring the trapping power of the radical DPPH• extracts of propolis. It is a synthetic radical [16]. When a solution of DPPH • is mixed with a substance that can give a hydrogen atom, this gives rise to the reduced form with a loss of the violet color. The discoloration will be directly proportional to the number of protons captured and can be followed by reading the absorbance of the reaction medium at 517 nm. It makes it possible to evaluate the DPPH reduction rate and thus provides a practical means of measuring the antioxidant

power of the extracts studied. Many studies have established relationships between the chemical structures of flavonoids and their anti-knocking ability [17]. The inhibition of the fading of the DPPH• radical is a function of the concentration of the various extracts used and of the control BHT (reference antioxidant).

Antioxidant activity of the extracts is expressed in IC50, this parameter has been used by several groups of researchers to present their results, and it defines the effective concentration of the substrate which causes the loss of 50% of the activity of the radical (color)

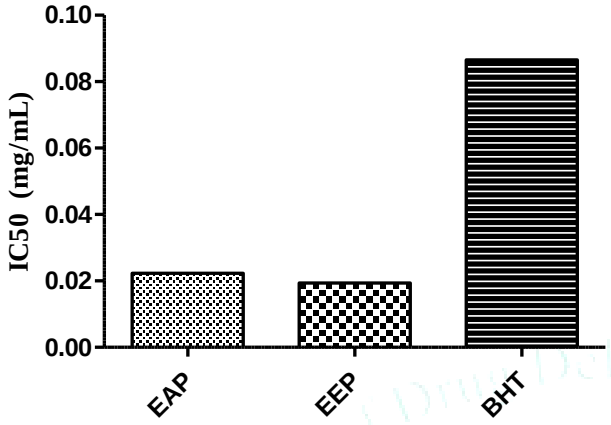

Figure 3. IC50 values of extracts and BHT.

All extracts have an anti-free radical power towards DPPH•, plus the value of the IC50 is small plus the extract is considered a powerful antioxidant. The results shown in **figure 3** show that the antiradical activities of propolis extracts are quite important. It is also noted that the antiradical power increases with increasing concentration.

As shown in the (**Figure 3**), it is noted that the antioxidant activity of the aqueous and ethanolic extracts is greater than that of BHT. These results can be explained by the existence of a molecule with a stronger electron donor reducing potential in EPP and EAP. On the other hand, it is observed that the ethanolic extract of propolis had a higher activity than that of the aqueous extract. The ethanolic extracts of propolis from Portugal have shown that the IC50 values obtained are of the order of 0.006 and 0.025 mg / ml respectively for the Bornes and Funddao propolis [13]. It is observed that the ethanolic extract of Setif propolis had a higher activity than that of Funddao, on the other hand the propolis of Bornes had a greater antiradical power than our sample analyzed.

CONCLUSION

The extracts tested were obtained from the Serdj El Ghoul region (Daïra de Babor) Wilaya of Sétif (east of Algeria) by extraction, using two solvents (distilled water and ethanol). The evaluation of the total polyphenol contents reveals the presence of moderately important quantities of. Similarly, we have determined the flavonoids which lead us to conclude that propolis contains a considerable amount of flavonoids. The antiradical potential of the extracts was determined by the DPPH method whose results show that these extracts have a good activity. We can conclude that propolis contains molecules that are considered first class of antioxidants and can be used for therapeutic applications, knowing that antioxidants contribute very effectively to disease prevention such as cancer, and cardiovascular disease.

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