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**EVALUATION OF ANTIOXIDANT PROPERTIES OF PROPOLIS COLLECTED IN  
NORTH-WEST OF ALGERIA**

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**ABSTRACT**

Propolis is a natural resinous produced by honeybees collected from parts of plants, buds, and exudates. It possess many biological properties such as antitumor, antimicrobial, anti-inflammatory, and immunomodulatory effects, among others. In the present study, we quantify the potential of antioxidant activity, total phenolics, flavonoids and carotenoids contents of propolis collected in different areas in North West of Algeria. The results showed that among the solvents in use, the Ethanol 85% appears more effective in the extraction of total phenol, flavonoids and carotenoids. The same sample (with Ethanol 85%) exhibited the highest antioxidant activity followed by methanolic and aqueous extract of propolis.

**Keywords: Antioxidant activity, Carotenoids, Flavonoids, Propolis, Total phenol.**

**INTRODUCTION**

Propolis is a resinous substance collected by honey bees (*Apis mellifera*) from various tree buds which they use to coat hive parts and to seal cracks and crevices in the hive to protect

the entrance against intruders [1]. It is used in folk medicines in many regions of the world and has been reported to have many biological activities such as antibacterial [2,

3], anti-inflammatory [4, 5], and anticancer properties [6, 7]. Propolis has the advantage as a source of biologically active compounds because it has already been selected by bees for its biological activity and is collected from plants in nondestructive way [8]. Chemical composition of propolis depends on the specificity of the local flora at the site of collection and thus on the geographic and climatic characteristics of this place [9]. However, there are few reports focusing on the antioxidant activity, total phenol, flavonoids and carotenoids of propolis of the north west of Algeria. The aim of this study was to quantitatively analyze the phenolic, flavonoid and carotenoids contents, and antioxidant properties of propolis samples collected from different regions in north-western Algerian.

## MATERIALS AND METHODS

### Collection of propolis

Seven propolis samples were collected from beekeepers in different north-western localities of Algeria.

### Preparation of extract

Propolis was extracted using water, methanol (50%) and two concentrations of ethanol (50% and 85%) as solvents. The four extracts were prepared by adding 1g of the specimens of propolis to 10 ml of each solvent in test tube and shaking at room

temperature for one week. After extraction, the mixture was centrifuged at 2300g for 20 min to obtain the supernatants which were pooled, filtered and stored at 6 ° C.

### Determination of total polyphenols

Total polyphenols content was measured using Folin-Ciocalteu colorimetric method [10]. Aliquots of 0.1 ml of propolis sample extracts was placed into 10 mL test tubes, diluted to 1 mL with distilled water and followed by 0.2 mL of Folin-Denis reagent. After 1 min, 1mL of 6% (w/v) sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was added. The reaction was kept in the dark for 30 min and the absorbance was measured at 760 nm against the corresponding test and standard blank prepared in the same way without the Folin-Ciocalteu's phenol reagent.

Gallic acid at different concentrations was used as a reference for constructing a standard curve. The results were expressed as mg of gallic acid equivalent per 100g of crude propolis.

### Determination of total flavonoids

The total flavonoids contents in propolis extracts were determined by the method described by Marquele et al (2005) [11]. 1,5 mL of sample was added to 1,5 mL aluminum chloride methanolic (10%), and kept for 30 min in the dark at room

temperature. The absorbance was measured at 415nm.

Catechin was used as the reference standard, and flavonoid content was expressed as mg of catechin per 100g of crude propolis.

#### **Determination of carotenoids**

Total carotenoids (TC) were determined according to [12]. 0.1g of the sample was dissolved in 10 ml of solvent mixtures of cyclohexane, ethanol and acetone (3:2:1, v:v:v) and followed by 0.5 mL of potassium hydroxide (1 M). After 90 minutes of stirring, the solution was centrifuged at 2300g for 15 min at 15 ° C. The absorbance of the filtrate was measured at 470 nm. Contents of carotenoids were expressed in mg  $\beta$ -carotene per 100g of propolis using an external calibration prepared from the pure  $\beta$ -carotene.

#### **Determination of antioxidant activity using FRAP assay**

Ferric reducing antioxidant power (FRAP) assay was performed according to the method explained by [13]. 0.5 ml of different amounts of the propolis was added with 1.25 mL 0.2 M phosphate buffer at pH 6.6 and 1.25 mL of 1%(w/v) potassium ferricyanide solution. The mixture was then incubated at 50C° for 20 min. Later 2.5 ml of 10% (w/v) trichloroacetic acid was added, and the mixture was centrifuged for 10 min

at 2500g. Aliquot of 2.5 ml from the mixture supernatant layer was diluted with 2.5 mL distilled water. The mixture was then added with 0.5 ml of 0.1% (w/v) of ferric chloride solution, and the absorbance was read at 700 nm.

Increased absorbance of the reaction mixture indicates increased reducing power. Reducing power (PR) was expressed as acid gallic equivalents antioxidant capacity (GEAC).

#### **Statistical analysis**

All the experiments were conducted in triplicate. Values are expressed as mean ( $\pm$  standard deviation).

## **RESULTS AND DISCUSSION**

#### **Total polyphenols**

The total concentrations of polyphenolic compounds in water, ethanol and methanol extracts are summarized in Figure 1. The amount total of phenolics varied in different samples and ranged from 183 to 532 mg/100g. From the results reported in figure, it is discriminated that the ethanol 85% is the best solvent of extraction and the extract from Graia2 (P 4) show the highest polyphenol concentrations (532 mg / 100g), where as the aqueous extracts from Bou-Hanifia (P3) had the lowest concentrations (183 mg / 100g). The polyphenol contents in our samples are higher with those reported by

[14] (85 - 228 mg / 100g), and those obtained by [15] (211 mg / 100 g) and are lower than those reported by [16] (171.72 - 5351.22 mg / 100g).

Buratti et al.(2007) [17] reported very low values (2-181 mg / 100g), this difference in polyphenol concentrations of propolis could be due to the variations in the preferred regional plants visited by honeybees.

### Total flavonoids

Total flavonoids contents of each extract were also measured and the results which varied between the samples are displayed in figure 2. These results were ranged between 121 and 331 mg/100. Like that of the polyphenol, the variation of flavonoid concentrations of propolis is mainly attributable to the difference in the preferred regional plants collected by honeybees [18].

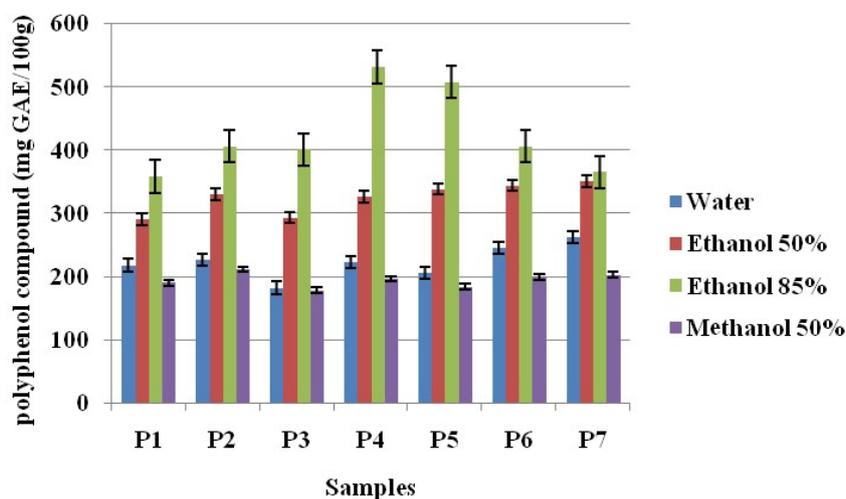


Figure1: Total polyphenol content in propolis sample

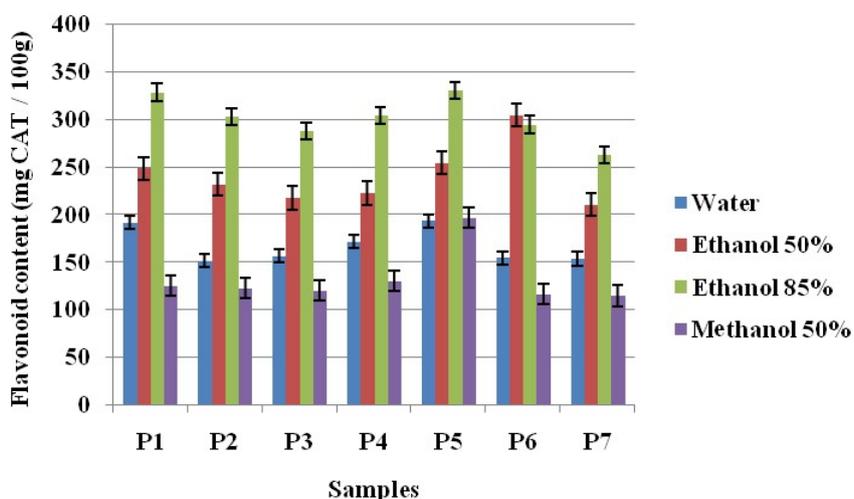


Figure 2: Total flavonoids contents in propolis samples.

In this study, ethanol extract (85%) contains the high concentration of total flavonoids 331mg / 100g compared to other extracts (Fig.2), followed by ethanol extract (50%) and water extract, while methanol extract (50%) contains the lowest. These results are lower than those obtained by [19, 20].

Choi et al (2006) mentioned 48-78 mg EQ/g range of flavonoids in the propolis from different regions of South Korea [21]. Mohammadzadeh et al (2007) obtained 12-78 mg EQ/g from that collected from different Iranian regions [19]. According to Isla et al. the flavonoid content of propolis from Argentina is between 14 and 62 mg EQ/g [22]. In China, Ahn et al. (2007) demonstrated that the content of flavonoids varied between 8 and 188 mg EQ/g [23].

The flavonoids content used as criterion for differentiation between propolis was suggested by some authors, like Kumazawa et al. [24]. Tosi et al.(2006) reported 7.87 % total flavonoids content for Pampeana provinve propolis and 3.61 % for Chaqueña province samples. Flavonoids have an enormous property in the biological activity in propolis [25].

#### **Carotenoid contents**

The carotenoid contents were shown in figure3. The determination of carotenoid showed significantly carotenoid contents

ranging from 26 to 121 mg / 100 g of  $\beta$ -carotene equivalent / 100 g of propolis.

The sample (P 2) Graia (1) had the highest level of carotenoids, while the sample (P 4) Graia (2) had the lowest. The other samples showed intermediate values (64; 82; 88; 98; 113mg / 100g) (Fig.3). These results are higher less than those reported by [24] (25.82 to 44.37 mg / 100g).

The carotenoids content in propolis can be influenced by the culture conditions and maturity of fruits, foraged by bees [26, 27].

#### **Determination of antioxidant activity**

Propolis is a natural product and has a powerful antioxidant capacity which is due to its high concentration of phenolics and antioxidant compounds [28]. The methylation degree and hydroxylation of phenolic compounds may also be involved in this activity [29]. Gülçin et al. (2005) and other authors say that the reducing power of propolis is also a supporting feature for its antioxidant activity [30]. The FRAP (ferric ion reducing antioxidant power) assay takes advantage of an electron transfer reaction in which a ferric salt is used as an oxidant [31]. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity

of a compound may serve as a significant indicator of its antioxidant activity [32, 33].

The results of the FRAP test of the different extracts of propolis is given in Fig 4.

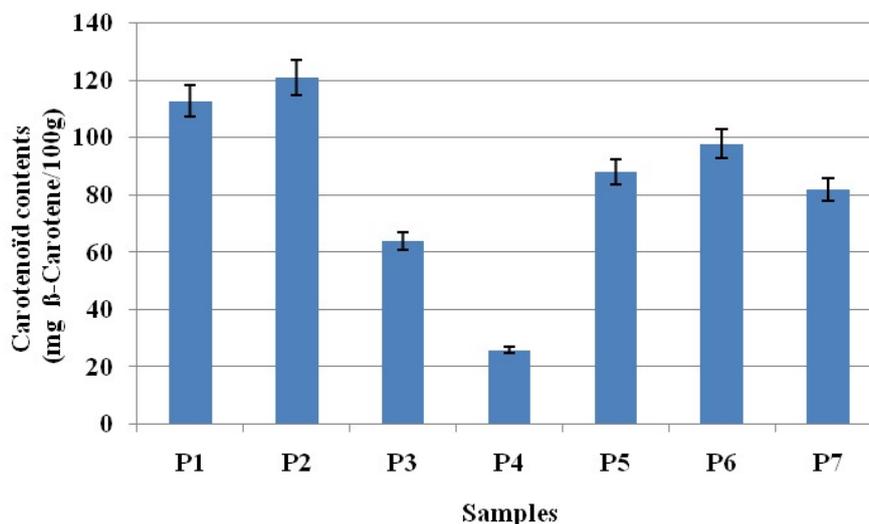


Figure 3: Carotenoid contents (mg / 100g).

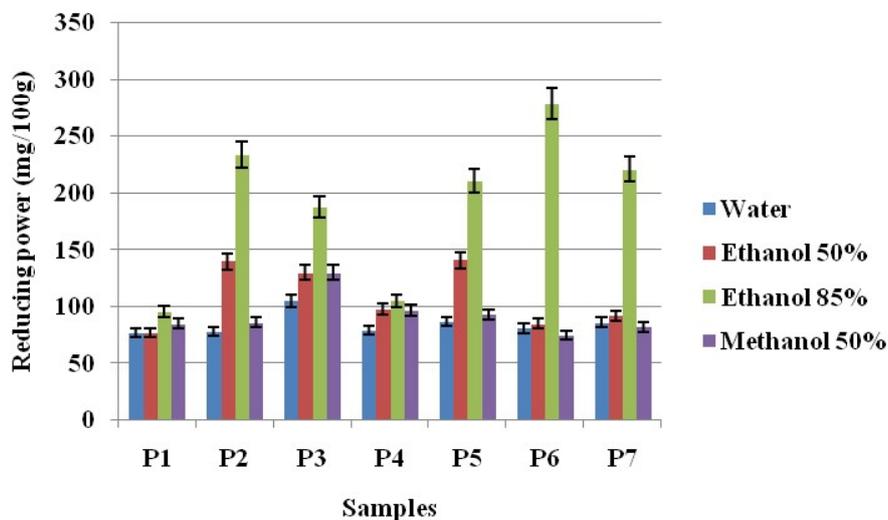


Figure 4: Reducing power variation in function of the concentration of propolis.

The FRAP values expressed as acid Gallic equivalents; the results showed that the extracts possessed reducing activity in the range of 75 -279 mg/100g.

The extract from Zmaecha obtained with ethanol 85% showed the highest antioxidant

capacity by FRAP with values of 279 mg/100g. The strongest antioxidant, based on the FRAP assay, was propolis p6. Samples 1 showed lowest reducing power. Results shown here indicate that ethanol 85% solvent exhibits comparatively high reducing power

for all samples were the methanolic and water extract of propolis showed lowest reducing power.

## CONCLUSION

In this study, the antioxidant activity of propolis collected from different regions in north-west of Algeria was investigated. The chemical composition of the samples is heterogeneous. The ethanol extract (85%) give a better results of total polyphenol, flavonoids, and carotenoids. Propolis samples have strong antioxidant activities, and the highest was found in sample P6. The optimization of extraction conditions is important to obtain from propolis rich extracts in phenolic compounds and have power antioxidant capacity. This research concludes that propolis could contribute as a highly significant bioresource of antioxidants to be used in food and pharmaceutical industry.

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