# Antioxidant Activities of Propolis collected from Different Regions of Morocco

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**Abstract**— Propolis is a resinous substance collected by honeybees from various plant sources. The composition of propolis depends on time, vegetation, and the area of collection. The main objective of this study is to study the antioxidant Activities of Propolis from Different Regions of Morocco. To achieve this goal, the present work examined the antioxidant activity of propolis collected from 4 regions of Morocco: Agadir, Marrakech, Rabat and Settat. Ethanol extracts of propolis were prepared and evaluated for their antioxidant activities by; 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging. The investigation of the polyphenol and flavonoid contents was measured spectrophotometrically. 20 µg/ml of principal active extracted from each sample was analyzed. The percentage of DPPH degradation (I%) was different from region to other (Rabat: 87.96 %; Agadir: 76,66%; Settat: 65,77% and Marrakech: 49,46%). The total polyphenol contents was affected significantly by the collection site (Rabat: 241.66±, Agadir: 191.18±0.91, Settat: 127.22±2.61 and Marrakech: 77.89±1.91). The same finding was observed with flavonoids concentration, which was different from à site too other (Rabat: 91.48±1.47, Agadir: 70.93±2.26, Settat: 46.52±0.63 and Marrakech: 12.13±0.29). It was also observed that all samples of ethanolic extracts of propolis showed free radical-scavenging activity. The highest activities were found for samples from Rabat and Agadir. Also, a positive correlation between antioxidant activity and chemical composition (flavonoid and polyphenol) was detected.

**Index Terms**— Moroccan Propolis, Antioxidant activities, DPPH free radical scavenging.

## 1 INTRODUCTION

Over the past 30 years, propolis has become the subject of intense pharmacological and chemical studies to improve health and prevent disease [1, 2]. Propolis is a natural resin product of bees that has been used for centuries in traditional medicine worldwide [3, 4]. Propolis is characterised by a complex and diversified chemical composition, depending on the plant from which it is collected and many other factors [5], it is mainly constituted by resin (50%), wax (30%), essential oils (10%), pollen (5%), and other substances (5%), such as debris, minerals and organic compounds [1]. Among the many components of propolis, the most important role is attributed to the phenolic compounds, particularly phenolic acids and their esters. In ethanol extracts of propolis, the derivatives of both hydroxybenzoic and hydroxycinnamic acids have been detected and among them are the protocatechuic, gentisic, p-coumaric, ferulic and caffeic acids [6, 7, 8]. Numerous studies have shown that the composition of propolis depends on the region of origin and, above all, on vegetation which is located in the place of its origin. It means that the products differ from each other not only in composition, but also in properties they exhibit [7, 9, 10, 11].

The propolis has recently regained interest as a health food product in Taiwan, Brazil, Europe and the United States. It is used as nutritional additive contributing to the physical good being and to the prevention of certain serious illnesses such as cancer, diabetes, cardiovascular, and inflammatory diseases [12, 13]. However, in Morocco propolis remains a little-known product and less used in traditional therapy by appearing with honey which is widely known for its use. One of the few studies involving propolis extracts focused on the in vitro and in vivo evaluation of anti-cancer properties [14].

The aim of this work was to determine the polyphenolic and flavonoids profile and evaluation of antioxidant activity of ethanol extracts of propolis from different regions of Morocco.

## 2 MATERIALS AND METHODS

### 2.1 Origins of Propolis

Propolis was collected during July-August 2014 from different regions of Morocco.The regions were, 1) Agasdir, 2) Rabat, 3) Settat, 4) Marrakech. The collected propolis was kept dried in the dark until it was used.

### 2.2 Extract preparation


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To prepare ethanolic extract of propolis (EEP), samples of the propolis from each region (EEP1: Agadir, EEP2: Marrakesh, EEP3: RABAT, EEP4: Settat) were cut into small pieces and a mass of 10 g propolis and then extracted with 100 ml of 70% ethanol under agitation (150 rpm) at room temperature in the dark for 7 days. The ethanolic extract solution was left to sediment and the supernatant was centrifuged for 10 min at 2550×g and restored to its initial volume (100 ml) of 70% ethanol). A clear solution was stored at + 4 ° C, for use in case of need.

### 2.3 Total phenolic content

Total polyphenol contents in extract were determined by the Folin–Ciocalteu using the method of Gulcin et al [15]. With minor modification. Hydro-alcoholic extracts (25 µL) were mixed with 125 µL of Folin-Ciocalteu reagent (0.2 N) and 100 µL of 7.5% Na2CO3, and the absorbance was measured at 760 nm after 2 h of incubation at room. The total polyphenol content was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample [16,17].

### 2.4 Flavonoid content

The amounts of flavones in extracts were determined according to the method of Miguel et al. [18]. with minor modification.
An amount of 100 µL of Al2Cl3 (20%) was added to 100 µL of extract, and after 1 h at room temperature the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalents (mg QE/g) using a calibration curve.

### 2.5 Diphenyl-1-picrylhydrazyl (DPPH) free radical

Scavenging activity Scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assayed following the method of Hatano et al. [19] with some modification. Solutions with different extract concentrations were prepared and 25 µL of each solution was added to 150 µL of DPPH solution (63.4 µM) and 125 µL of 96% ethanol. The mixture was left to stand for 1 h in the dark at room temperature. The absorbance was recorded at 517 nm. Control solution contained only ethanol and DPPH. Results were expressed as percentage decrease with respect to control values [20]. EEP samples were evaluated at a final concentration of 20 µg/ ml, and ascorbic acid (AC) at the same concentration was used as the reference samples.
The percentage inhibition was calculated by the equation:
%inhibition (I %) = Abs DPPH- Abs Sample/Abs Dpph.

### 2.6-Statistical Analysis

The statistical analyses were performed using JMP SAS 11.0.0 (SAS Institute Inc., Cory, NC, USA) program.
A factorial design ANOVA analyzed firstly the data of polyphenol and flavonoid contains and secondly, I% in each propolis extract. The statistical model included the fixed effect of propolis extract. When statistically significant differences were detected, the Tukey's post hoc, was used to compare the means and standard errors, considering the significance level of $P < 0.05$. Data are expressed as the mean ± SE.
Spearman test correlation test was used, coefficient was calculated to evaluate the relationship between polyphenol, flavonoid contains and I%. Differences between variables were considered significant, at least ($P < 0.05$).

## 3 Results

### 3.1. Total polyphenols and flavonoid contents

Total polyphenol and flavonoid contents of propolis extract samples from the four regions of morocco were investigated (Table 1).
Results showed that there was a significant difference ($P<0.05$). The amounts of total phenolics and flavonoids found in propolis extracts changed according to the place where samples were collected. The highest polyphenol was recorded in propolis extract of Rabat (241.66±2.84) , followed by extract of Agadir (191.18±0.91), then extract of Settat (127.22±2.61) and finally, extract of Marrakesh showed the lowest values (77.89±1.91). The highest concentration of flavonoids was found in a sample from Rabat (91.48±1.47mg/g) and the lowest was in a sample from Marrakesh (12.13±0.29 mg/g) (Table1).

TABLE 1
TOTAL PHENOLIC AND FLAVONOID CONTENTS OF PROPOLIS SAMPLES

| Extract Ethanolic of propolis | Total phenolic content mg GAE/g) | Total flavonoids content (mg QE/g) |
|---|---|---|
| EEP1 | 191.18±0.91[b] | 70.93±2.26 [b] |
| EEP2 | 77.89±1.91 [d] | 12.13±0.29 [d] |
| EEP3 | 241.66±2.84[a] | 91.48±1.47 [a] |
| EEP4 | 127.22±2.61[c] | 46.52±0.63 [c] |

Value is mean±SE. Means with different superscript letters within a column are significantly different at P<0.05

### 3.2 Antioxidant activity

Propolis from Rabat and Agadir were among the best DPPH scavengers;
Propolis from Marrakesh had the poorest capacity for scavenging DPPH free radicals, followed by samples from Settat (Fig 1).

It is noticed that there is a strong correlation between poly-

phenols and flavonoids of propolis in the four regions of Morocco. A highly significant correlation ($P < 0.001$) was observed between polyphenol, flavonoid contents and the antioxidant activity (Table 2).

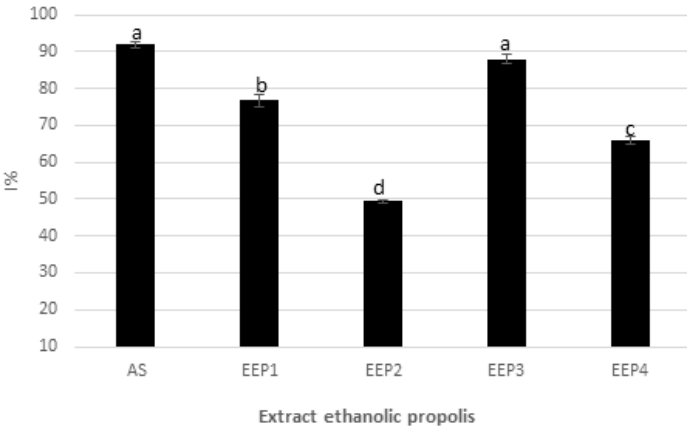


Figure 1: DPPH free radical scavenging activity of the different propolis extracts (I%)
Value is mean±SE. Means with different superscript letters (a,b,c,d) within a barrs are significantly different at P<0.05

TABLE 2
CORRELATION BETWEEN POLYPHENOL, FLAVONOID CONTENTS AND I%

| Extract Ethanolic of propolis | Total phenolic content mg GAE/g) | Total flavonoids content (mg QE/g) |
|---|---|---|
| EEP1 | 191.18±0.91[b] | 70.93±2.26 [b] |
| EEP2 | 77.89±1.91 [d] | 12.13±0.29 [d] |
| EEP3 | 241.66±2.84[a] | 91.48±1.47 [a] |
| EEP4 | 127.22±2.61[c] | 46.52±0.63 [c] |

Correlation are significant at $P < 0.05$
I%: The percentage inhibition; FC: Flavonoid content ((mg QE/g); PC: Polyphenol contents (mgGAE/g)

## 4. DISCUSSION

In the present study, the total polyphenol and flavonoid contents were evaluated and according to our result, different regions of Morocco produced propolis containing different concentrations ($p<0.05$) of polyphenols, ranging from a minimal value of 77.89 mg/g GAE for propolis from Marrakech to a maximal value of 241.66 mg/g GAE for propolis from Rabat (Table 1). The highest concentration of flavonoids was found also in a sample from Rabat (70.93mg/g QE) and the lowest was from a sample from Marrakech (46.52 mg/g QE).

It is evident that the quantitative differences in those compounds in propolis samples harvested in different regions. Some author's studing propolis from different areas also found quantitative differences in total phenols and flavonoid contents. Data in the literature showed a larger variability in polyphenol contents from different areas of China: 43–302 mg/g, India: 159–269 mg/g, Iran: 31–187 mg/g, Portugal: 151–329 mg/g and Algeria: 55-279 mg/ g. A larger variability in flavonoid contents was shown in propolis collected in different regions of Iran ranged from 12 to 78 mg/g. the flavonoid content of propolis from China is between 8 and 188 mg/g of propolis. Propolis from Greece and Cyprus contained flavonoids at levels from 8.8 to 182.6 mg/g and flavonoid content of propolis from Algeria ranged between 10-69 mg/g. [18].

The results of the free radical scavenging effect of the four propolis samples, and positive control (vitamin C ) in DPPH free radical system were determined (Fig. 1).

The model system of scavenging DPPH free radical is a simple method for evaluating the antioxidant activity of compounds. It is accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability. [21,22]

We evaluated various EEP and the reference samples (ascorbic acid) at the final concentration of 20 µg/ml.

In this study, the antioxidants quantification in propolis gave values significantly different between the samples ($p<0.05$).As shown in Fig. 2, EEP samples from Rabat and Agadir ,had strong DPPH free radical scavenging activities of over 87.96 and 76,66% respectively.EEP from Marrakech was less important capacity for scavenging DPPH free radicals with a value of 65,77%, followed by samples from Settat with 49,46%. These variations are attributed to the floral source, and geographic origin. [23, 24].

Referring to the results found by Kumazawa et al [25], we can consider that the propolis collected from Rabat and Agadir are the best in terms of antioxidant activity compared to the results found in different countries(Australia, China, Hungary, and New Zealand), and this can be explained by the climax of the Rabat region

A positive correlation between the antioxidant activity and concentrations of flavonoids and polyphenols are exposed in this study. Similar results have been reported by several studies, including the work of Balasundrum et al. [26] and Beretta et al. [27]

## CONCLUSION

According to our results it can be concluded that the Moroccan propolis has a strong antioxidant activity especially in the region of Rabat and Agadir. In Morocco almost, all beekeepers produce only honey. While, it can be considered that the Moroccan propolis has a very important value among the other products harvested from the hive and that it must be exploited more and more in the therapy and in the industry.

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