



*Chemical Analysis and Antioxidant Activity of Four Propolis Samples
Collected from Different Regions of Lebanon*

*Lübnan'ın Farklı Bölgelerinden Toplanan Dört Propolis Örneğinin Kimyasal
Analizi ve Antioksidan Aktivitesi*

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Abstract

Propolis samples were collected from four different regions of Lebanon characterized by high biodiversity and high honey production. The samples were analyzed for their total phenolic contents (TPC), total flavonoid contents (TFC), chemical profiles, and antioxidant activity. The TPC were determined using Folin-Ciocalteu method while the TFC using the AlCl₃ method. The antioxidant activity of propolis was examined by two different methods, namely free radical scavenging assay and reducing ability. The chemical profiles of the samples were assessed by thin-layer

Özet

Propolis örnekleri yüksek miktarlarda bal üretimi ve biyolojik çeşitliliği ile karakterize edilen Lübnan'ın 4 farklı bölgesinden toplanmıştır. Örneklerin toplam fenolik içeriği, toplam flavonoid içeriği, kimyasal profilleri ve antioksidan aktiviteleri analiz edilmiştir. Fenolik madde içeriği Folin-Ciocalteu ve flavonoid içeriği AlCl₃ metodu ile belirlenmiştir. Propolisin antioksidan aktivitesi serbest radikal süpürme aktivitesi ve indirgeme gücü olarak incelenmiştir. Örneklerin kimyasal profilleri ince tabaka kromatografisi, UV-Vis ve gaz kromatografisi/kütle

chromatography (TLC), UV-Vis, and gas chromatography/mass spectrometry GC-MS analysis. Total phenolic content ranged from 53.35 ± 7.09 to 148.27 ± 15.08 mg gallic acid equivalents per gram (mg GAE/g), total flavonoid content ranged from 45.73 ± 2.8 to 134.5 ± 8.46 mg rutin equivalents per gram (mg RUE/g). GC/MS analysis revealed the presence of 9-octadecene and tetradecene as major compounds that have been previously reported to demonstrate antioxidant activity. In addition, Berqayel propolis sample showed high content of phenolic compounds and high antioxidant activity, while samples from Wadi Faara recorded poor chromatograms with the absence of most of the compounds present in Berqayel samples. The majority of propolis samples showed relatively interesting antioxidant activity, which was also correlated with TPC and TFC. These findings highlighted the effect of the beehive locations on the quality of Lebanese propolis in terms of chemical constituents and biological activities.

Keywords: Propolis, Antioxidant, Total phenolic content, Total flavonoid content, GC-MS analysis

Abbreviations: EEP, ethanolic extract of propolis; TPC, Total phenolic content; TFC, Total flavonoid content; TLC, Thin layer chromatography; GC-MS, Gas chromatography/mass spectrometry.

spektrometrisi ile belirlenmiştir. Toplam fenolik madde içerięi g başına 53.35 ± 7.09 ile 148.27 ± 15.08 mg gallik asit eődeęeri (mg GAE/g), flavonoid içerięi ise g başına 45.73 ± 2.8 ile 134.5 ± 8.46 mg rutin eődeęeri arasında deęişmektedir. GC/MS analizinde antioksidan aktiviteye sahip olduęu bilinen 9-oktadesen ve tetradesen bileőenlerinin varlıęı ortaya konulmuştur. Ek olarak, Berqayel propolisinin yüksek fenolik madde içerięi ve yüksek antioksidan aktiviteye sahip olduęu belirlenmiş, Wadi Faara'dan alınan numunelerin ise kromatogramları zayıf bulunmuş, Berqayel propolisinde bulunan birçok bileőeni içermedięi görülmüştür. Propolis örneklerinin çoęu toplam fenolik ve flavonoid madde içerięiyle korelasyon gösteren yüksek antioksidan aktiviteye sahiptir. Bu bulgular, kimyasal bileőenler ve biyolojik aktiviteler aęısından kovan lokasyonlarının Lübnan propolisinin kalitesi üzerindeki etkisini ortaya koymaktadır.

Anahtar kelimeler: Propolis, Antioksidan, Toplam fenolik içerięi, Toplam flavonoid içerięi, GC-MS analizi

1. INTRODUCTION

Propolis is a natural resinous product assembled by honey bees (*Apis mellifera* L.) from different sources of plant. It's used to make the protective shield at the entrance of beehives and its human use dates back to ancient times, when the product was employed in embalming bodies in Egypt (Soltani et al., 2017). In addition to antimicrobial activity of propolis, other biological and pharmacological properties have been demonstrated including antitumor, antibacterial, antioxidant, antifungal and other activities (Anjum et al., 2019).

Propolis is generally composed of 50 % resin and balm (including phenolic compounds), 30 % wax and fatty acids, 10 % essential oils, 5 % pollen and 5 % various organic and inorganic

compounds. The specific composition of propolis depends on the vegetation at the site of collection (Boisard, 2014).

Propolis showed the most potent antioxidant of all the bee products (Nakajima et al., 2009). Antioxidant activity of propolis was originated from their polyphenolic substances (Isla et al., 2005; Wang et al., 2016). Thus, propolis can be used for prevention and treatment of diseases related to the increase of oxidative stress such as cancer, aging, and cardiovascular diseases (Kocot et al., 2018).

In the present study, propolis extracts from four different Lebanese locations were prepared by maceration using ethanol/water, considered as green solvents. The effect of geographical origin on the phytochemical contents was assessed and results were compared for the antioxidant capacity in terms of free radical scavenging assay and reducing power assay. Moreover, the volatile compounds profile in the Berqayel sample was characterized using gas chromatography/mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

All chemicals used were of analytical grade. Methanol (MeOH), Ethanol (EtOH), chloroform, Folin-Ciocalteu, ascorbic acid, aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) and rutin were purchased from BDH (England). 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium carbonate anhydrous (Na_2CO_3), potassium ferricyanide, trichloroacetic acid and iron (III) chloride (FeCl_3), were purchased from Sigma Aldrich (USA). Samples were weighed using a RADWAG XA 82/220/2X laboratory balance. The absorbance values were measured using a VWR UV-6300PC double beam spectrophotometer and extracts were concentrated using HEIDOLPH (Germany) rotavapor apparatus.

2.2. Sample Collection

In this study, four Lebanese propolis samples were collected from four apiaries located in different Lebanese regions (Figure 1), more specifically from Berqayel ($34^\circ 28' 38.6''$ N, $36^\circ 2' 1.54''$ E, 350 m MSL), Debaal ($33^\circ 15' 02''$ N, $35^\circ 20' 56''$ E, 280 m MSL), Fakeha ($34^\circ 14' 44''$ N, $36^\circ 24' 21''$ E, 900 m MSL) and Wadi-Faara ($34^\circ 17' 22.0''$ N $36^\circ 18' 15.8''$ E, 2100 m MSL). The average annual temperature (AAT) in Berqayel and Debaal reaches 20°C , and the average precipitation (AP) ranges between 700 and 1,000 mm. whereas, the AAT in Fakeha is lower than that and reaches 16°C while the average precipitation drops to 400 mm in this

region. However, Wadi Faara experiences an Δ AAT less than 10 °C and the AP between 1,500 and 2,000 mm. The main process to collect the raw propolis samples was started by the initial preparation to separate it from extraneous macro impurities if present. The obtained samples were frozen at - 20 °C until analysis.

2.3. Obtaining the Ethanolic Extract of Propolis (EEP)

Each frozen brown to yellow propolis sample was chopped into small pieces and immediately homogenously pulverized. Then, one gram of each sample was macerated in 100 mL of ethanol 80 % for 48 hours at room temperature under magnetic stirring. The mixture was then filtered, and the filtrate was evaporated under reduced pressure to



Figure 1. Approximate geographical location of the samples of the propolis evaluated.

produce the ethanolic extract of propolis (EEP). Finally, the EEP was weighed and stored at +4 °C for further use.

2.4. Phytochemical Screening

The phytochemical screening was carried out by means of qualitative phytochemical tests based on color or precipitation reactions with the extract.

2.4.1. Test for Alkaloids

Extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Jaber et al., 2019).

2.4.2. Test for Phenols

A few drops of ferric chloride solution were added to 2 mL of the extract in a watch glass; the appearance of bluish green color indicated the presence of phenol (Yadav & Agarwala, 2011).

2.4.3. Test for Terpenoids (Salkowski test)

1 mL of each extract was mixed in 2 mL of chloroform, and concentrated H₂SO₄ (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids (Iqbal et al., 2015).

2.4.4. Test for Hydrolysable Tannins

A few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue-black coloration (Jaber et al., 2019).

2.4.5. Test for Quinons

1 mL of concentrated hydrochloric acid (HCl) was added to one mL of EEP. The presence of quinons is confirmed by the appearance of yellow color (Yadav & Agarwala, 2011).

2.4.6. Test for Flavonoids

1 mL of KOH is added to 1 mL of each extract. The color shifting to yellow indicates the presence of flavonoid (Morsy, 2014).

2.4.7. Test for Saponins

1 mL of extract was shaken with 2 mL of water. The persisting of foam for ten minutes indicates the absence of saponins (Jaber et al., 2019).

2.5. Determination of Total Phenolic Content (TPC)

The total polyphenol content in propolis extract was determined by using Folin-Ciocalteu method (Singleton et al., 1999) with some modifications. Briefly, 100 µL of the EEP extract was taken and mixed with 500 µL of aqueous Folin-Ciocalteu solution (10 %). After 5 min, 2 mL of sodium carbonate (7.5 %) were added. The obtained mixture was allowed to stand for 30 min in the dark. After which the absorbance was read at 760 nm in a spectrophotometer. The TPC in the extract was extrapolated from the calibration curve derived by repeating the same procedure for different concentrations of methanolic solutions of gallic acid (30-270 µg.mL⁻¹), and results were expressed in mg of gallic acid equivalents per g of propolis (mg GAE/g).

2.6. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminium chloride spectrophotometric assay (Barreca et al., 2016). Briefly, 1 mL of each EEP was mixed with 1 mL of 2 % aluminium chloride (AlCl₃) methanolic solution. After incubation for 30 min at room temperature, absorbance was measured at 410 nm. Rutin was used as the standard and a curve was constructed by preparing different dilutions (0 - 50 µg.mL⁻¹) using the same procedure for EEP. The blank sample consisted of 1 mL of extract solution in 1 mL methanol without AlCl₃. The amount of total flavonoids in the extracts was expressed as rutin equivalents (mg RUE/g).

2.7. In Vitro Antioxidant Capacity

In order to evaluate the *in vitro* antioxidant activity, two spectrophotometric methods were used: DPPH free radical scavenging assay and reducing power assay.

2.7.1. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the extracts was evaluated with the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method developed by Blois (1958) (Kedare & Singh, 2011). The DPPH methanolic solution (0.032 mg.mL⁻¹) was prepared freshly. To 1 mL of aliquot of extract solution, 1 mL of DPPH• methanolic solution was added. The mixture was vortexed and then left to stand at room temperature for 30 min in the dark at room temperature. The absorbance was read at 520 nm using an UV-Vis spectrophotometer. Different concentrations of ascorbic acid (0.81 - 4.05 µg.mL⁻¹) have been produced for use as the positive control. A similar procedure was used for the blank, where the extract sample was replaced with methanol. The free radical scavenging capacity was expressed as the percentage inhibition of the radical oxidation and calculated using the following equation:

$$\% \text{ scavenging activity} = \frac{\text{Abs Control} - \text{Abs Extract}}{\text{Abs control}} \times 100$$

The results were expressed in IC₅₀ (The half-maximal inhibitory concentration) as the amount of antioxidant required to decrease the initial DPPH concentration by 50 %.

2.7.2. Reducing Ability

The reducing ability of EEP was determined according to the method reported by Oyaizu (Oyaizu, 1986). This assay is normally based on the blue coloration that develops due to the reduction of ferric iron to the ferrous. A serial dilution of extract solutions and ascorbic acid (0.7 - 0.05 g.mL⁻¹), were prepared in water. 200 µL of each extract solution was mixed with

200 μ L of 0.2 M phosphate buffer (pH 6.6) and 200 μ L of potassium ferricyanide (1 %). Reaction mixture was incubated at 50° C for 20 min. After cooling, 200 μ L of trichloroacetic acid (10 %) were added and mixture was centrifuged at 1000 rpm for 8 min. The upper layer (800 μ L) was mixed with 800 μ L of distilled water and 160 μ L of ferric chloride (0.1 %). After a 10 min reaction time, the spectrometric absorbance was recorded at 700 nm and compared with ascorbic acid as positive control. The absorbance values were plotted against the concentration, and a linear regression analysis was carried out. Higher absorbance readings indicate higher reducing power.

2.8. Measurement of the Absorption Spectra of Extracts

The UV- vis spectra of the propolis extract solutions were recorded from a mixture of 25 μ L of the extracts with 1750 μ L of ethanol (80 %). All the samples have the same concentration in mg solid propolis/mL. The obtained solutions were scanned at wavelengths range between 190 and 600 nm by UV-vis spectrophotometer. Quartz cuvettes of 1 cm optical path were used and the absorbance being recorded against an ethanol 80 % blank.

2.9. TLC and GC-MS Analysis

TLC analyses of EEP were performed on thin layer chromatographic (TLC) plates, composed of Silica gel 60 GF 254. The plate was developed in chamber previously saturated by eluent. Two systems of mobile phase were used: Petroleum ether / ethyl acetate 7:3 and cyclohexane / ethyl acetate 8:2. After drying, spots were investigated; visually and under UV 254/366 light. One microliter of EEP samples (Berqayel and Wadi Faara) was diluted (1:100) with hexane and injected into the Gas chromatography–mass spectrometry (GC-MS) system. GC SHIMADZU QP2010 system was used to analyze the volatile compounds in the propolis extracts (without derivatization). DB-5MS (5 % Diphenyl / 95 % Dimethylpolysiloxan) capillary column having (30 m length, 0.25 i.d., film thickness 0.28 μ m) and helium as carrier gas was used for compound separation. The oven temperature was programmed from 65 °C (2 min initial time) increased to 300 °C at 10 °C/min (isothermal for the final time) and the MS was operated in the electron impact mode at 70 eV ion source energy. Injection volume was 1 μ L and total run of one hour is performed. Data receipt and processing were performed using Shimadzu GC-MS solution software. The compounds were identified based on a comparison of their mass spectra with data in NIST (National Institute of Standards and Technologies, Mass Spectra Libraries).

2.10. Statistical Analysis

All determinations were conducted in triplicates ($n = 3$); the correlation coefficients (R^2) and the statistical mean \pm SD were calculated using Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

3. RESULTS and DISCUSSION

3.1. Phytochemical Screening

Table 1 shows the results of the phytochemical screening for different propolis samples. Different chemical profiles are observed between propolis samples from distinct geographic locations. The samples from Berqayel and Debaal were the richest in terms of potentially active constituents (phenols, alkaloids, hydrolysable tannins, flavonoids and terpenoids) while those from Wadi-Faara were the poorest.

Table 1. Phytochemical Screening of EEP.

Phytochemical Compounds	Debaal	Wadi-Faara	Fakeha	Berqayel
Flavonoid	+	+	+	+
Alkaloids	+	+	+	+
Hydrolysable Tannins	+	-	-	+
Terpenoids	+	-	+	+
Quinons	+	-	-	+
Phenols	+	+	+	+
Saponins	-	-	-	-

Note. +: detected, -: No detected

On the other hand, saponins were absent in all samples. Although, the later observation contradicts the study of Chamandi et al. (Chamandi et al., 2015) done on Lebanese propolis samples, it is in agreement with another study reported by Labyad et al. (Labyad et al., 2016). It is worth to point out here that the phytochemical composition (and subsequent biological activity) of propolis is highly dependent on the plant cover diversity as well as biotic and abiotic factors (Bueno-Silva et al., 2017; do Nascimento et al., 2019; Huang et al., 2014; Salatino et al., 2011; Savickas et al., 2005).

The phytochemicals detected have previously been shown to exhibit biological activity, such as antibacterial, antitumor and antihelminthic activity (Cowan, 1999; Rosli et al., 2016; Zeitoun et al., 2019).

3.2. Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to antioxidative activity. These compounds are very important constituents of plants and their radical scavenging ability due to their hydroxyl groups (Labiad et al., 2017). The propolis from Berqayel registered the greatest TPC (148.27 mg GAE/g) and TFC values (134.5 mg RUE/g) (Figure 2). These TPC and TFC values were about 3-fold of the lowest TPC and TFC values detected in Debaal region (53.35 mg GAE/g and 50.99 mg RUE/g, respectively), indicating the significant variations of TPC and TFC in propolis samples between the four Lebanese regions.

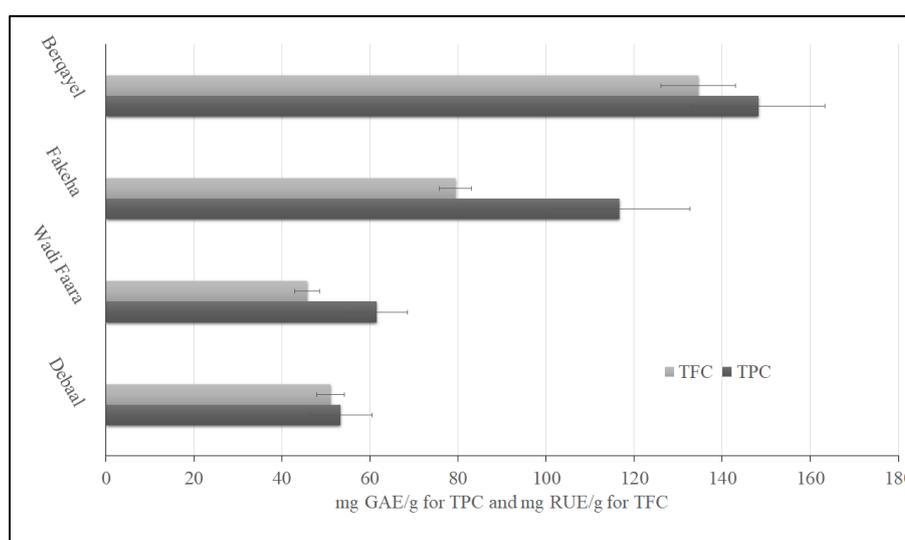


Figure 2. TPC and TFC of the four EEP. Values are the mean \pm SD of three replicates.

The TPC range (53.35–148.27 mg GAE/g) was comparable to that of Uruguayan propolis (Silva et al., 2011) and South African (Kumazawa et al., 2004); and, lower than others such as Brazilian propolis (277.81- 398.11 mg GAE/g) (Reis et al., 2019). The TFC contents of 50.9–134.5 mg RUE/g recorded in this study are in the same order with those reported in other propolis origins, using quercetin as standard in the colorimetric method instead of rutin. For instance, the TFC ranges of some Chinese propolis were 52.11-173.90 mg QE/g (Shi et al., 2012) and 08.3-188 mg QE/g (Ahn et al., 2007). Moreover, the TFC ranges of Brazilian and Canadian propolis were 42.00-108.02 mg QE/g (Reis et al., 2019) and 01.58-137.06 mg QE/g (Cottica et al., 2015), respectively. However, the values in this study are lower than those reported in Indonesia (Pratami et al., 2017).

3.3. Antioxidant Activity of Propolis Extracts

The presence of different secondary metabolites in EEP is an indication that the extract studied might have antioxidant capacity.

3.3.1. DPPH Assay

Free radicals produced in living systems and encountered exogenously, lead to various disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (Singh & Singh, 2008). The antioxidant activity was determined to be potent via two different assays, the DPPH assay and the reducing power assay. Antioxidant potency is usually associated with the content of phenolic compounds due to their extensive conjugated π -electron systems that facilitate the donation of electrons from the hydroxyl moieties to oxidizing radical species (Bittencourt et al., 2015). DPPH radical-scavenging activity has been widely used in propolis studies (Kumazawa et al., 2004).

Results in Figure 3 showed that *propolis from Berqayel and Fakeha* presented the highest free radical scavenging activity compared with vitamin C. The obtained results for DPPH are in agreement with the phenolics and flavonoids contents determined for each sample. Moreover, the found IC_{50} values are in good agreement with many previous works (Pratami et al., 2017; da Silva et al., 2019; Touzani et al., 2018). Nevertheless, the antioxidant activities of different Lebanese propolis are lower than those of Egyptian propolis (Ezzat et al., 2019).

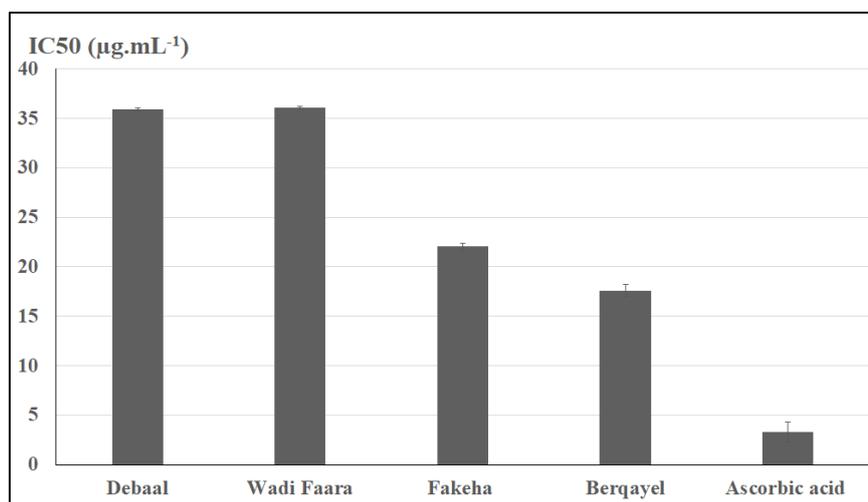


Figure 3. Comparison of 50 % radical inhibition by the different propolis extracts (Values are the mean \pm SD of three experiments)

3.3.2. Reducing Ability

The transformation ability of compounds from Fe^{3+} / ferricyanide complex to Fe^{2+} /ferrous form acts as a potential indicator for the antioxidant activity of the extract (Do et al., 2014). In the FRAP assay, the yellow color test solution changes to green and blue depending on the reduction capacity of extracts. The complexing of metal ions by phenols typically induces a bathochromic displacement of their absorption bands in the UV-Visible range (Ghedadba et al., 2015). In Figure 4, and similarly to the radical scavenging activity, all the EEP showed concentration-dependent reducing power. The greatest reducing antioxidant power was recorded for propolis collected in Berqayel, while the lowest was found in the Wadi Faara extract. This is interpreted by the richness of Berqayel extract in phenolic compounds proved by the TPC and TFC results. Thus, it can be deduced from this test that polyphenols, especially flavonoids, play a very important role in the chelation of transition metals.

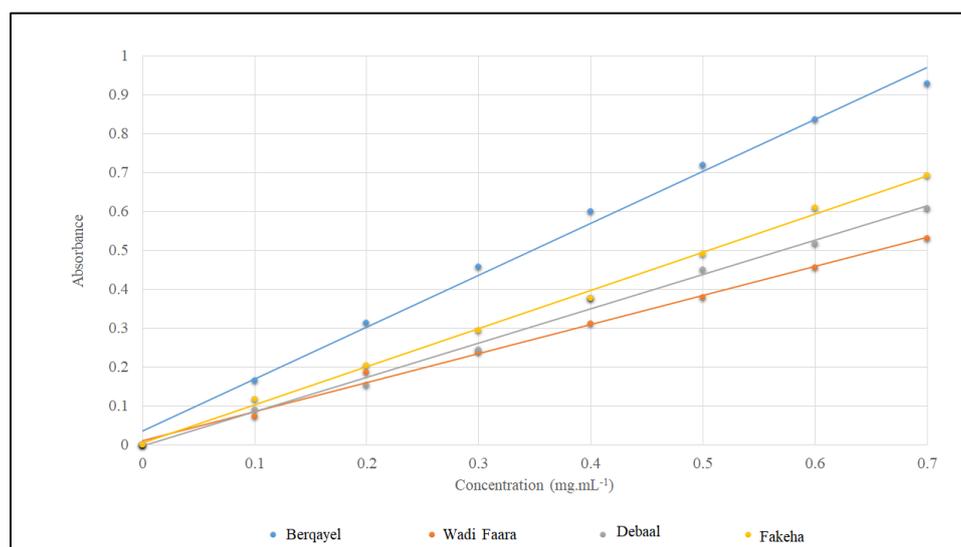


Figure 4. Reducing power assay of all extracts of expressed as absorbance at 700 nm (n = 3).

3.3.3. Correlation Between Antioxidant Activities, Phenolics and Flavonoids Contents of EEP

Several studies (Karou et al., 2005; Verzelloni et al., 2007) have shown the presence of good correlation between the total phenolic content and the antioxidant activity of the extracts, suggesting that phenolic compounds, particularly flavonoids are responsible for the antioxidant activity of the extracts. The results (Table 2) show a strong positive correlation between phenolics, flavonoids and antioxidant activity, this suggests that polyphenols, especially flavonoids may be responsible of the antioxidant activity.

Table 2. Correlation coefficient (R2) among TPC, TFC and antioxidant activity.

	DPPH method	FRAP method
TPC	0.9843	0.7744
TFC	0.8521	0.9675

Moreover, these correlations are consolidated by many previous works (Galeotti et al., 2018; Kumazawa et al., 2004; Wang et al., 2016) which showed that the high polyphenol and flavonoid contents are responsible for the highest antioxidant activity of propolis.

3.4. UV Spectrograms

The UV-vis spectra of EEP obtained from the four regions are illustrated in Figure 5. Two absorption bands were observed, the first between 190–250 nm, while the second is a broad band centered around 280–300 nm with a shoulder around 330 nm. They are similar to typical polyphenol spectra (Paganotti et al., 2014), indicating that the used extraction solution (EtOH: water, 80: 20, v/v) was able to recover the phenolic compounds.

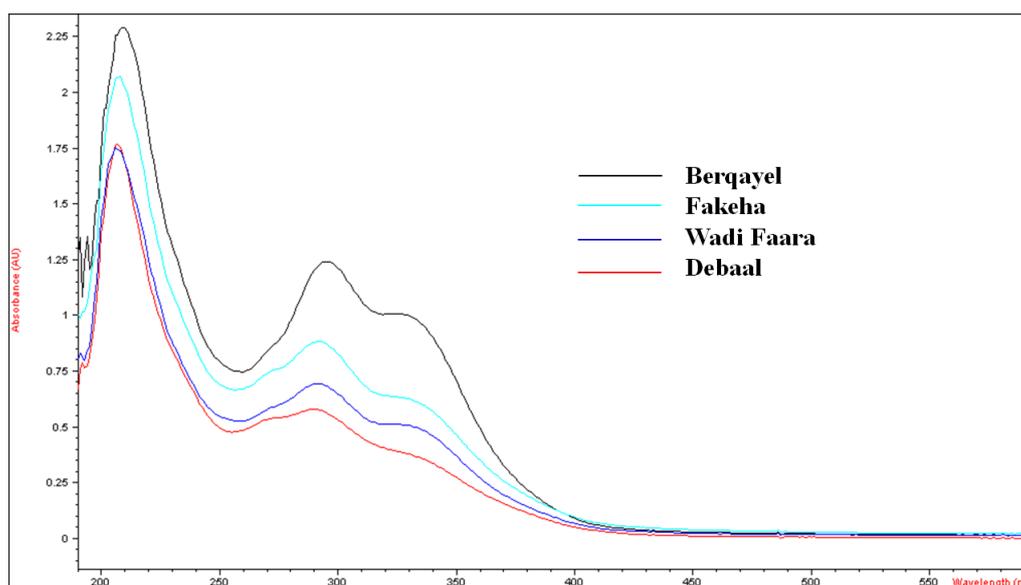


Figure 5. UV absorption spectra of Lebanese propolis extracts.

Furthermore, the spectral profiles were identical, suggesting a homogeneous chemical composition among the samples, despite their collecting regions. The Argentine Food Code (CAA) establishes in its physical and chemical requirements that the UV-Vis spectrogram of propolis should have a maximum absorption between 270 nm and 315 nm, regardless of the profile obtained (Isla et al., 2005; Maldonado et al., 2020). In this study, the CAA requirement was met in all propolis samples from different locations. By comparison with reported studies, UV-Vis spectra profile was similar to the Argentinian (Maldonado et al., 2020), Romanian (Moț

et al., 2011), and Brazilian propolis with a slightly bathochromic shifting (Tomazzoli et al., 2015).

3.5. TLC and GC-MS Analysis

The current study displays the presence of different compounds as TLC experiment separates numbers of spots. TLC analysis of the EEP samples from the four regions showed common chromatographic plates with slight differences. Some compounds such as pinocembrin, pinostrobin and phenylethyl cafeate can be identified by comparing their retention factors with those of previous works (Boisard, 2014). The obtained results are in agreement with those obtained by UV-Vis analysis, i.e. the main compounds are present in all samples and differences are mainly quantitative not qualitative.

The EEP were analyzed by GC-MS (Figure 6) to detect volatile, small and non-thermolabile metabolites. The analysis was done for the two samples the one who give the high antioxidant effect (Berqayel) and the low effect (Wadi Faara). The EEP from Berqayel was found to have small metabolites, mostly hydrocarbons. Compounds that were identified to be present in large amounts include; 1-tetradecene (23.84 %), 9-octadecene (32.57 %) and isodecene (14.9 %) (Table 3). GC-MS analysis of Wadi Faara extract revealed a poor chromatogram in term of peaks and the absence of most of the compounds founded in Berqayel sample.

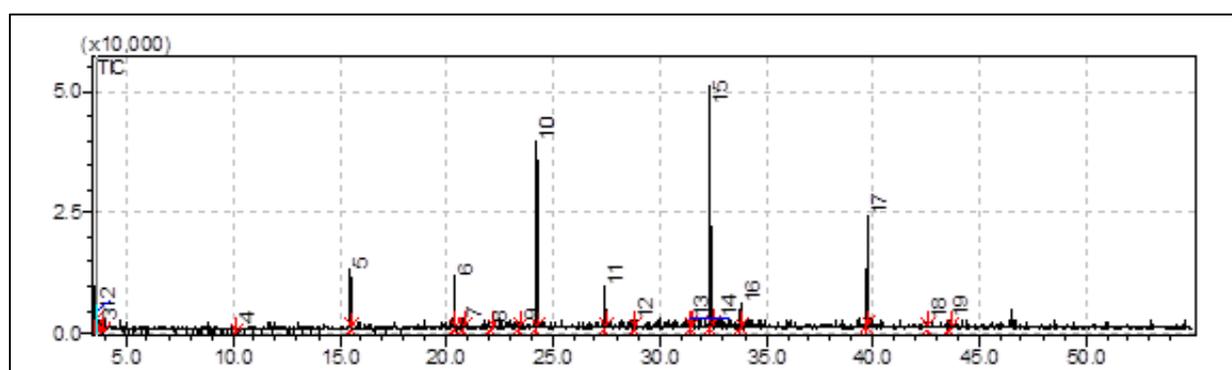


Figure 6. GC-MS profile of propolis ethanolic extract from Berqayel.

Table 3. GC/MS analysis of main constituents of EEP.

Compound	R.Time	M.W.	Molecular formula	Area(%)
anhydride-propanoic acid	10.067	130	(CH ₃ CH ₂ CO) ₂ O	1.31
2-Ethylhexanol	15.508	130	C ₈ H ₁₈ O	5.87
Dodecamethylcylohexasiloxane	20.383	444	C ₁₂ H ₃₆ O ₆ Si ₆	6.58
5-hexen-3-one	20.808	98	C ₆ H ₁₀ O	0.47
tert-pentane	23.417	72	C ₅ H ₁₂	0.38
1-tetradecene	24.217	196	C ₁₄ H ₂₈	23.84
Unknown	27.433	--	--	5.24
Isocyanomethane	28.817	40	C ₂ H ₃ N	0.56

propane,1-3,diamine	31.425	74	C ₃ H ₁₀ N ₂	0.36
9-octadecene	32.358	252	C ₁₈ H ₃₆	32.57
Unknown	33.833	--	--	2.69
Isodecene	39.758	140	C ₁₀ H ₂₀	14.92

According to literature, the compound, octadecene has been reported to have anticancer, anti-inflammatory and antioxidant activities (Gautam et al., 2018). Moreover, tetradecene is another compound reported to possess antioxidant abilities (Manoj et al., 2012; Tiloke et al., 2018). Thus, the presence of these compounds can explain the antioxidant capacity of EEP from Berqayel propolis observed in this study.

4. CONCLUSION

In this work, we focused on the contents and the levels of TPC, TFC and antioxidant for EEP obtained by maceration of propolis from different geographical origins in Lebanon. Phytochemical screening shows the existence of different secondary metabolites: flavonoids, polyphenols, alkaloids, tannins, quinons and terpenes, while saponins are absent. The quantitative determination of polyphenol and flavonoids clearly shows the richness of propolis by these compounds. Moreover, it has shown that the propolis from Berqayel had the greatest TPC of 148.27 mg GAE/g and TFC value of 134.5 mg RU/g propolis. On the other hand, the study of the antioxidant activity by evaluating their anti-free radical and reducing power revealed the good potential of Berqayel and Fakeha extracts to scavenge the DPPH radical and to reduce iron from the Fe³⁺ to the Fe²⁺ form. In addition, the antioxidant activity was also correlated with the total polyphenol and flavonoid content. Results of UV-VIS and TLC showed, similar profiles in comparison to previous works with no small differences among them. Finally, GC-MS was employed to identify the volatile compound in Wadi Faara and Berqayel samples. The chromatogram from Berqayel contain mostly hydrocarbons e.g. 1-tetradecene (23.84 %), 9-octadecene (32.57 %) and isodecene (14.9 %). While, the chromatogram obtained from Wadi Faara extract revealed the absence of most of these compounds.

Therefore, our results demonstrated that propolis samples from different Lebanese locations have different biological activity. However, further studies are needed to support our results, to evaluate another biological potential of these propolis extracts and to identify the extract biomolecules.

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REFERENCES

- Ahn, M. R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., & Nakayama, T. (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chemistry* 101(4), 1383-1392. <https://doi.org/10.1016/j.foodchem.2006.03.045>.
- Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., Bashir, M. A., Tahir, M., Ansari, M. J., Ghramh, H. A., Adgaba, N., & Dash, C. K. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences*, 26(7), 1695–1703. <https://doi.org/10.1016/j.sjbs.2018.08.013>.
- Barreca, D., Laganà, G., Leuzzi, U., Smeriglio, A., Trombetta, D., & Bellocco, E. (2016). Evaluation of the nutraceutical, antioxidant and cytoprotective properties of ripe pistachio (*Pistacia vera* L., variety Bronte) hulls. *Food Chemistry*, 196, 493–502. <https://doi.org/10.1016/j.foodchem.2015.09.077>.
- Bittencourt, M. L. F., Ribeiro, P. R., Franco, R. L. P., Hilhorst, H. W. M., de Castro, R. D., & Fernandez, L. G. (2015). Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. *Food Research International*, 76, 449–457. <https://doi.org/10.1016/j.foodres.2015.07.008>.
- Blois, M. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 181, 1199–1200. <https://doi.org/10.1038/1811199a0>.
- Boisard, S. (2014). *Caractérisation chimique et valorisation biologique d'extraits de propolis* [Phd thesis], University of Angers. (Thesis No. 1426)
- Bueno-Silva, B., Marsola, A., Ikegaki, M., Alencar, S. M., & Rosalen, P. L. (2017). The effect of seasons on Brazilian red propolis and its botanical source: Chemical composition and antibacterial activity. *Natural Product Research*, 31(11), 1318–1324. <https://doi.org/10.1080/14786419.2016.1239088>.

- Chamandi, G., Olama, Z., & Holail, H. (2015). Antimicrobial effect of Propolis From different Geographic Origins in Lebanon. *Int.J.Curr.Microbiol.App.Sci*, 4(4), 328–342.
- Cottica, S. M., Sabik, H., Antoine, C., Fortin, J., Graveline, N., Visentainer, J. V., & Britten, M. (2015). Characterization of Canadian propolis fractions obtained from two-step sequential extraction. *LWT - Food Science and Technology*, 60(1), 609–614. <https://doi.org/10.1016/j.lwt.2014.08.045>.
- Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, 12(4), 564–582. <https://doi.org/10.1128/CMR.12.4.564>.
- do Nascimento, T. G., dos Santos Arruda, R. E., da Cruz Almeida, E. T., dos Santos Oliveira, J. M., Basílio-Júnior, I. D., Celerino de Moraes Porto, I. C., Rodrigues Sabino, A., Tonholo, J., Gray, A., Ebel, R. E., Clements, C., Zhang, T., & Watson, D. G. (2019). Comprehensive multivariate correlations between climatic effect, metabolite-profile, antioxidant capacity and antibacterial activity of Brazilian red propolis metabolites during seasonal study. *Scientific Reports*, 9(1), 18293. <https://doi.org/10.1038/s41598-019-54591-3>.
- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>.
- Ezzat, S. M., Khattaby, A. M., Abdelmageed, S., & Abd Elaal, M. A. (2019). Cytotoxicity, antioxidant, anti-inflammatory activity, and GC-MS analysis of Egyptian propolis. *Comparative Clinical Pathology*, 28(6), 1589–1598. <https://doi.org/10.1007/s00580-019-02971-6>.
- Galeotti, F., Maccari, F., Fachini, A., & Volpi, N. (2018). Chemical Composition and Antioxidant Activity of Propolis Prepared in Different Forms and in Different Solvents Useful for Finished Products. *Foods*, 7(3), 41-51. <https://doi.org/10.3390/foods7030041>.
- Gautam, V., Kohli, S. K., Arora, S., Bhardwaj, R., Kazi, M., Ahmad, A., Raish, M., Ganaie, M. A., & Ahmad, P. (2018). Antioxidant and Antimutagenic Activities of Different Fractions from the Leaves of *Rhododendron arboreum* Sm. And Their GC-MS Profiling. *Molecules: A Journal of Synthetic Chemistry and Natural Product Chemistry*, 23(9), 2239-2251. <https://doi.org/10.3390/molecules23092239>.

- Ghedadba, N., Hambaba, L., Ammar, A., Mohamed-Cherif, A., Bousselsela, H., & M. Oueld-Mokhtar, S. (2015). Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de Marrubium deserti de Noé. *Phytothérapie*, 13, 118–129. <https://doi.org/10.1007/s10298-015-0944-4>.
- 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. <https://doi.org/10.3390/molecules191219610>.
- Iqbal, E., Salim, K. A., & Lim, L. B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniiothalamus velutinus (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University - Science*, 27(3), 224–232. (<https://doi.org/10.1016/j.jksus.2015.02.003>).
- Isla, M. I., Paredes-Guzman, J. F., Nieva-Moreno, M. I., Koo, H., & Park, Y. K. (2005). Some Chemical Composition and Biological Activity of Northern Argentine Propolis. *Journal of Agricultural and Food Chemistry*, 53(4), 1166–1172. <https://doi.org/10.1021/jf040130h>.
- Jaber, A., Edmond, C., Ibrahim, G., & Lamis, A.-H. (2019). Phytochemical study and antioxidant activity of extract from the leaves of lebanese datura metel l. *European Journal of Pharmaceutical and Medical Research*, 6(8), 65–71.
- Karou, D., Dicko, M. H., Simpore, J., & Traore, A. S. (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *African Journal of Biotechnology*, 4(8), 823–828. <https://doi.org/10.4314/ajb.v4i8.15190>.
- Kedare, S., & Singh, R. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48, 412–422. <https://doi.org/10.1007/s13197-011-0251-1>.
- Kocot, J., Kielczykowska, M., Luchowska-Kocot, D., Kurzepa, J., & Musik, I. (2018). Antioxidant Potential of Propolis, Bee Pollen, and Royal Jelly: Possible Medical Application. *Oxidative Medicine and Cellular Longevity*, 2018, 7074209. <https://doi.org/10.1155/2018/7074209>.
- Kumazawa, S., Hamasaka, T., & Nakayama, T. (2004). Antioxidant activity of propolis of various geographic origins. *Food Chemistry*, 84(3), 329–339. [https://doi.org/10.1016/S0308-8146\(03\)00216-4](https://doi.org/10.1016/S0308-8146(03)00216-4).

- Labiad, M. H., Harhar, H., Ghanimi, A., & Tabyaoui, M. (2017). *Phytochemical Screening and Antioxidant Activity of Moroccan Thymus satureioides Extracts. Journal of Materials and Environmental Sciences, 8(6), 2132-2139.*
- Labyad, N., Dorro, B., Elmarbet, N. S., Aluonsy, M. M., & Kahmasi, M. (2016). Phytochemical antioxidant and antimicrobial study of Libyan propolis ethanolic extract. *International Journal of Herbal Medicine, 4(5), 01–04.*
- Maldonado, L., Marcinkevicius, K., Borelli, R., Gennari, G., Salomón, V., Isla, M. I., Vera, N., & Borelli, V. (2020). Differentiation of argentine propolis from different species of bees and geographical origins by UV spectroscopy and chemometric analysis. *Journal of the Saudi Society of Agricultural Sciences, 19(3), 185–191.* <https://doi.org/10.1016/j.jssas.2018.09.003>.
- Manoj, G., Manohar, S. H., & Murthy, H. N. (2012). Chemical constituents, antioxidant and antimicrobial activity of essential oil of *Pogostemon paniculatus* (Willd.). *Natural Product Research, 26(22), 2152–2154.* <https://doi.org/10.1080/14786419.2011.633082>.
- Moț, A. C., Silaghi-Dumitrescu, R., & Sârbu, C. (2011). Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV–vis spectroscopic data. *Journal of Food Composition and Analysis, 24(4), 516–522.* <https://doi.org/10.1016/j.jfca.2010.11.006>.
- Morsy, N. (2014) Phytochemical analysis of biologically active constituents of medicinal plants *Main Group Chemistry, 13(2014), 7–21.* <https://doi.org/10.3233/MGC-130117>.
- Nakajima, Y., Tsuruma, K., Shimazawa, M., Mishima, S., & Hara, H. (2009). Comparison of bee products based on assays of antioxidant capacities. *BMC Complementary and Alternative Medicine, 9(4).* <https://doi.org/10.1186/1472-6882-9-4>.
- Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics, 44(6), 307–315.*
- Paganotti, R. S. N., Rezende, J. C. T. de, & Barbeira, P. J. S. (2014). Discrimination Between Producing Regions of Brazilian Propolis by UV-VIS Spectroscopy and Partial Least Squares Discriminant Analysis. *Current Analytical Chemistry, 10(2), 537-544.* <https://doi.org/10.2174/15734110113099990030>.

Pratami, D. K., Mun'im, A., Sundowo, A., & Sahlan, M. (2017). Phytochemical Profile and Antioxidant Activity of Propolis Ethanolic Extract from Tetragonula Bee. *Pharmacognosy Journal, 10*(1), 128–135. <https://doi.org/10.5530/pj.2018.1.23>.

Reis, J. H. de O., Barreto, G. de A., Cerqueira, J. C., Anjos, J. P. dos, Andrade, L. N., Padilha, F. F., Druzian, J. I., & Machado, B. A. S. (2019). Evaluation of the antioxidant profile and cytotoxic activity of red propolis extracts from different regions of northeastern Brazil obtained by conventional and ultrasound-assisted extraction. *PLOS ONE, 14*(7), 1–27. <https://doi.org/10.1371/journal.pone.0219063>.

Rosli, N. L., Roslan, H., Omar, E. A., Mokhtar, N., Hapit, N. H. A., & Asem, N. (2016). Phytochemical analysis and antioxidant activities of Trigona Apicalis propolis extract. *AIP Conference Proceedings 1791*, 020018. <https://doi.org/10.1063/1.4968873>.

Salatino, A., Fernandes-Silva, C. C., Righi, A. A., & Salatino, M. L. F. (2011). Propolis research and the chemistry of plant products. *Natural Product Reports, 28*(5), 925–936. <https://doi.org/10.1039/C0NP00072H>.

Savickas, A., Majiene, D., Ramanauskiene, K., Pavilionis, A., Muselik, J., Masteikova, R., & Chalupova, Z. (2005). Chemical composition and antimicrobial activity of Lithuanian and Czech propolis. *BIOLOGIJA, 4*, 59–63.

Shi, H., Yang, H., Zhang, X., & Yu, L. (2012). Identification and Quantification of Phytochemical Composition and Anti-inflammatory and Radical Scavenging Properties of Methanolic Extracts of Chinese Propolis. *Journal of Agricultural and Food Chemistry, 60*(50), 12403–12410. <https://doi.org/10.1021/jf3042775>.

da Silva, C. C. F., Salatino, A., Motta, L. B. da, Negri, G., & Salatino, M. L. F. (2019). Chemical characterization, antioxidant and anti-HIV activities of a Brazilian propolis from Ceará state. *Revista Brasileira de Farmacognosia, 29*(3), 309–318. <https://doi.org/10.1016/j.bjp.2019.04.001>.

Silva, V., Genta, G., Möller, M. N., Masner, M., Thomson, L., Romero, N., Radi, R., Fernandes, D. C., Laurindo, F. R. M., Heinzen, H., Fierro, W., & Denicola, A. (2011). Antioxidant activity of uruguayan propolis. In vitro and cellular assays. *Journal of Agricultural and Food Chemistry, 59*(12), 6430–6437. <https://doi.org/10.1021/jf201032y>.

Singh, S., & Singh, R. P. (2008). In Vitro Methods of Assay of Antioxidants: An Overview. *Food Reviews International, 24*(4), 392–415. <https://doi.org/10.1080/87559120802304269>.

- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (Vol. 299, pp. 152–178). Academic Press. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- Soltani, E.-K., Cerezuela, R., Charef, N., Mezaache-Aichour, S., Esteban, M. A., & Zerroug, M. M. (2017). Algerian propolis extracts: Chemical composition, bactericidal activity and in vitro effects on gilthead seabream innate immune responses. *Fish & Shellfish Immunology*, *62*, 57–67. <https://doi.org/10.1016/j.fsi.2017.01.009>.
- Tiloke, C., Anand, K., Gengan, R. M., & Chuturgoon, A. A. (2018). Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer. *Biomedicine & Pharmacotherapy*, *108*, 457–466. <https://doi.org/10.1016/j.biopha.2018.09.060>.
- Tomazzoli, M. M., Neto, R. D. P., Moresco, R., Westphal, L., Zeggio, A. R. S., Specht, L., Costa, C., Rocha, M., & Maraschin, M. (2015). Discrimination of Brazilian propolis according to the seasoning using chemometrics and machine learning based on UV-Vis scanning data. *Journal of Integrative Bioinformatics*, *12*(4), 15–26. <https://doi.org/10.1515/jib-2015-279>.
- Touzani, S., Al-Waili, N., Menyiy, N. E., Filipic, B., Pereyra, A., Arabi, I. E., Al-Waili, W., & Lyoussi, B. (2018). Chemical analysis and antioxidant content of various propolis samples collected from different regions and their impact on antimicrobial activities. *Asian Pacific Journal of Tropical Medicine*, *11*(7), 436. <https://doi.org/10.4103/1995-7645.237188>.
- Verzelloni, E., Tagliacruzchi, D., & Conte, A. (2007). Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsamic vinegar. *Food Chemistry*, *105*(2), 564–571. <https://doi.org/10.1016/j.foodchem.2007.04.014>.
- Wang, X., Sankarapandian, K., Cheng, Y., Woo, S. O., Kwon, H. W., Perumalsamy, H., & Ahn, Y.-J. (2016). Relationship between total phenolic contents and biological properties of propolis from 20 different regions in South Korea. *BMC Complementary and Alternative Medicine*, *16*(1), 65. <https://doi.org/10.1186/s12906-016-1043-y>.
- Yadav, R. & Agarwala, M. (2011) Phytochemical analysis of some medicinal plants. *Journal of Phytology*, *3*(12), 10-14. Zeitoun, R., Najjar, F., Wehbi, B., Khalil, A., Fayyad-Kazan, M., Dagher-Hamalian, C., Faour, W. H., & El-Makhour, Y. (2019). Chemical Composition, Antioxidant and Anti-inflammatory Activity Evaluation of the Lebanese Propolis Extract.

Current Pharmaceutical Biotechnology, 20(1), 84–96.
<https://doi.org/10.2174/1389201020666190206201241>.