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## Chemical analysis and antioxidant content of various propolis samples collected from different regions and their impact on antimicrobial activities

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### ABSTRACT

**Objective:** To assess the antioxidant content, antimicrobial and antioxidant activities of various propolis samples. **Methods:** Seven propolis samples were collected from different locations in Morocco, which are characterized by different plant predominant vegetations. The resin, wax and balsam of hydroalcoholic extract of propolis content were identified, and the antioxidant content was analyzed with the use of HPLC and colorimetric methods. The antioxidant activity was assessed by DPPH, ABTS<sup>+</sup> and ferric reducing power assays. The antimicrobial activity was assessed against bacterial species, including methicillin resistant *Staphylococcus aureus* and *Candida albicans*, and expressed as the minimal inhibitory concentration. **Results:** The propolis samples showed significant variations in the chemical composition and in the antioxidant or antimicrobial activities even when the samples were collected from the same location. Propolis with high resin and low wax content had high level of antioxidant compounds, and strong antioxidant and antimicrobial activities. Gram-positive bacteria, especially, methicillin resistant *Staphylococcus aureus* were more sensitive to all propolis samples than Gram-negative bacteria and *Candida albicans*. **Conclusions:** The chemical composition and the antioxidant and antimicrobial activities of various propolis samples are different and rely on the geographic and plant origin of propolis collection. Propolis samples with low wax and high resin content might be more suitable to be used in future preclinical or clinical investigations.

## 1. Introduction

Propolis is assembled by the *Apis mellifera* bee from the bud and exudates of plants that are modulated by their enzymes. The main composition of propolis includes resin, wax, pollen, diterpenoid and flavonoids, which have anti-inflammatory, antimicrobial, and antioxidant activities[1-6].

Propolis has been utilized to treat human illness due to its

antimicrobial, wound healing, antitumor and anti-inflammatory properties[7-12]. However, few reports regarding propolis composition and activity from Morocco have been published. One study focused on the evaluation of *in vitro* and *in vivo* anticancer properties of Moroccan propolis[13]. The anti-inflammatory, anti-acetylcholinesterase, antioxidant and anticancer activities of

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propolis from different regions of Morocco have been reported[14,15].

Recent studies showed that Moroccan propolis has a strong antioxidant activity, and it has a marked protective activity against ethylene glycol induced hepatorenal toxicity[16,17]. Furthermore, propolis reduces proteinuria and has the capability to be used in the management of urinary tract stone formation[17]. A recent study from our laboratory showed that different samples collected from Morocco can inhibit glucosidase and amylase enzymes and prevent lipid peroxidation[14]. Another study showed that propolis collected from Morocco incorporated with magnetic nanoparticles can prevent methicillin resistant *Staphylococcus aureus* (MRSA) adherence to a catheter, which was ascribed to the propolis content of benzyl caffeate, pinocembrin, galangin, and isocupressic acid[18].

As propolis is collected by bees from different plants, its properties and characteristics could be related to seasonal time and geographical location. It was found that the chemical composition of propolis could be affected by plant origin and the collection region[19-22].

The aim of the present study was to investigate the chemical composition, antioxidant and antimicrobial activities of various propolis samples collected from different regions of Morocco, which are characterized by their different plant cultivation. This study will explore the effect of geographical locations and regional plant diversity on the chemical and functional characteristics of propolis, and it will help discover propolis that provides the most effective biological activities.

## 2. Materials and methods

### 2.1. Propolis samples

Seven different propolis samples (A, B, C, D, F and G) were collected from several areas of Morocco. These areas are different in their plants and climate. Propolis A was collected from the city of Boulmane, whose predominant vegetation consists of *Populus*, *Prunus*, *Ceratonia*, *Rosmarinus* and *Quercus*. Propolis B and C were collected from the city of Outat El Haj, whose predominant vegetation consists of *Olea*, *Pinus*, *Ceratonia*, *Eucalyptus*, *Rosmarinus* and *Quercus*. Propolis D was collected from the city of Sale, whose predominant vegetation consists of *Eucalyptus*, *Euphorbia*, *Juniperus* and *Quercus*. Propolis E was collected from the city of Sefrou, whose predominant vegetation consists of *Olea*, *Pinus*, *Quercus*, *Juniperus*, *Rosmarinus*, *Cistus*, *Lavandula* and *Pistacia*. Propolis F was collected from the city of Bhalil, whose predominant vegetation consists of *Olea*, *Pinus*, *Quercus*, *Juniperus*, *Rosmarinus*, *Cistus*, *Lavandula* and *Pistacia*. Propolis G was collected from the city of Serghina, whose predominant vegetation consists of *Populus*, *Prunus*, *Pinus* and *Quercus*.

### 2.2. Preparation of the ethanol extract of propolis

Propolis (10 g) was extracted with 100 mL 70% ethanol at ambient temperature by maceration under agitation for a total of seven

days. The hydroalcoholic extract solution was then filtered with the use of a Whatman filter paper to eliminate the residual mass and centrifuged for 10 min at 4 000 rpm.

### 2.3. Wax, balsam and resin extraction and quantification

The content of wax in different propolis samples was estimated according to methods described by Giulia Papotti *et al.* with some modifications[23]. The wax content was expressed as % w/w. The content of balsams was estimated according to Giulia Papotti *et al.*[23]. The 70% ethanolic filtrate obtained during the wax extraction was concentrated under reduced pressure at 60 °C. The results were expressed as % w/w. The content of resins was estimated according to Giulia Papotti *et al.* with some modifications[23]. The residual propolis obtained after the wax extraction was treated with chloroform and ethanol 1:1 (v/v) by maceration under stirring for 48 h. The results were expressed as % w/w.

### 2.4. Determination of total phenol, flavone and flavonol content

The total phenol content in the propolis extracts was determined by the Folin ciocalteu colorimetric method according to Ahn *et al.*[24]. The total polyphenol content was expressed as mg/g of gallic acid equivalents. The content of flavone and flavonol was quantified as described by Miguel *et al.*[25]. Quercetin was used as a standard for the construction of calibration curve.

### 2.5. HPLC method for the alcoholic extract of propolis

Pinocembrin, chrysin, caffeic acid and galangin (2 mg each) were added into 20 mL measuring flask and filled with MeOH. The dilutions were kept in the refrigerator (4 °C) till use. Before injection, they were diluted 1/10. The injection volume was 10 µL. Before injection, the samples were put into the ultrasound bath for 10 min and filtered through 0.45 µm filters.

### 2.6. Total antioxidant capacity

The total antioxidant capacity of different propolis extracts was determined according to the ammonium molybdate colorimetric method[26]. Ascorbic acid was used as the standard calibration. The results were expressed as milligram of ascorbic acid equivalent per gram of sample.

### 2.7. Free radical scavenging activity on DPPH, ABTS<sup>+</sup> and ferric reducing power

The effect on DPPH radical was evaluated by the method of Kumazawa[20]. Absorbance measurements were read at 517 nm, and the percentage inhibition was plotted against phenol content to determine IC<sub>50</sub>. The ABTS<sup>+</sup> scavenging activity was measured[27]. Several concentrations were made and the percentage inhibition was

plotted against phenol content to determine IC<sub>50</sub>. The ferric reducing power was identified according to the method described by Moreira et al [28]. Ascorbic acid was used as positive control.

### 2.8. Microorganisms

Different clinical isolates of Gram-negative bacteria: *Proteus mirabilis* (*P. mirabilis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*); Gram-positive bacteria: *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*), MRSA, and *Streptococcus agalactiae* (*S. agalactiae*); fungus: *Candida albicans* (*C. albicans*) were obtained from the Institute for Microbiology and Immunology, Medical Faculty in Ljubljana. All the microbes were cultivated on Mueller Hinton agar at 37 °C for 48 h and transferred to Mueller Hinton broth until the concentration of 0.5 McFarland was obtained.

### 2.9. Statistical analysis

The tests were performed in triplicate, and the results were expressed as Mean ± standard deviation. Statistical comparisons were made with one-way ANOVA followed by *post hoc* Tukey's Multiple Comparison Test using Graph Pad Prism 5 software.

## 3. Results

The wax, resin and balsam content of the various propolis samples was demonstrated in the Table 1. Propolis samples with high wax content had low resin content. Samples A, E and F contain higher amount of wax as compared to other samples, while samples B, C and G contained higher amount of resin. Regarding balsam content, there were significant differences between propolis samples except between propolis A and G, propolis C and G, and propolis B and E. Propolis B showed the highest amount as compared to the other samples except propolis E.

**Table 1**

Wax, balsam, and resin contents of propolis samples (%).

Samples	Waxes	Resins	Balsams
A	75.34±0.02	19.69±0.11	1.87±0.02
B	19.89±0.12*	70.30±0.10*	3.01±0.09*
C	20.12±0.11*	59.51±0.09 <sup>#</sup>	2.09±0.12 <sup>#</sup>
D	29.33±0.01 <sup>##</sup>	47.33±0.08 <sup>##</sup>	2.67±0.08 <sup>##</sup>
E	74.32±0.08 <sup>##+β</sup>	18.89±0.12 <sup>##+β</sup>	2.89±0.10 <sup>+</sup>
F	60.86±0.09 <sup>##+αβ</sup>	32.43±0.12 <sup>##+αβ</sup>	2.32±0.02 <sup>##βα</sup>
G	20.16±0.07 <sup>*αβπ</sup>	60.21±0.09 <sup>##+αβπ</sup>	2.08±0.03 <sup>#αβπ</sup>

\*P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column; <sup>π</sup>P<0.05 as compared to propolis F in the same column.

Regarding phenol, flavone and flavonol content, it was found that propolis B, C and G had significant higher content as compared to the other propolis samples (Table 2). Furthermore, propolis B, C and G had a higher total antioxidant activity as compared to other samples. Therefore, the total antioxidant activity increased in the propolis samples with high content of phenols, flavone and flavonol. However, propolis samples with high amount of wax showed lower antioxidant content as well as lower total antioxidant activity. With the use of DPPH, ABTS<sup>+</sup> and FRAP, it was found that propolis samples B, C and G showed higher antioxidant activity as compared to the other propolis samples. Propolis B demonstrated similar activity to ascorbic acid (Table 3).

**Table 2**

Total phenolic content, flavone and flavonol and total antioxidant capacity of hydro-alcoholic extract of propolis.

Samples	Phenols (mg GAE/g)	Flavone and flavonol (mg QE/g)	Total antioxidant activity (mg AAE/g)
A	12.02±1.02	9.98±1.32	6.56±0.82
B	168.43±1.09*	160.56±0.59*	90.87±2.91*
C	135.15±1.42 <sup>#</sup>	130.19±0.41 <sup>#</sup>	67.82±3.46 <sup>#</sup>
D	73.75±0.79 <sup>##</sup>	56.97±2.44 <sup>##</sup>	48.01±0.51 <sup>##</sup>
E	31.45±2.01 <sup>##+β</sup>	26.31±1.29 <sup>##+β</sup>	29.03±1.67 <sup>##+β</sup>
F	40.46±1.31 <sup>##+βα</sup>	26.52±1.31 <sup>##+β</sup>	30.98±2.01 <sup>##+β</sup>
G	134.04±1.37 <sup>#βα</sup>	128.09±1.98 <sup>#βα</sup>	87.67±1.92 <sup>*+βα</sup>

\*P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column.

**Table 3**

DPPH, ABTS<sup>+</sup> and ferric reducing antioxidant power of hydro-alcoholic extract of propolis (IC<sub>50</sub>, mg/mL).

Samples	DPPH	ABTS <sup>+</sup>	FRAP
A	1.190±0.030	0.983±0.020	1.080±0.130
B	0.019±0.020*	0.021±0.110*	0.039±0.120*
C	0.023±0.010*	0.026±0.090*	0.048±0.060*
D	0.029±0.020 <sup>##</sup>	0.043±0.120*	0.042±0.030*
E	0.529±0.010 <sup>##+β</sup>	0.631±0.020 <sup>*+##β</sup>	0.401±0.110 <sup>##+β</sup>
F	0.351±0.120 <sup>##+β</sup>	0.421±0.150 <sup>*+##βα</sup>	0.268±0.140 <sup>##+βα</sup>
G	0.024±0.110 <sup>*απ</sup>	0.027±0.120 <sup>*#απ</sup>	0.062±0.020 <sup>*+βαπ</sup>
Ascorbic acid	-	-	0.030±0.070 <sup>*+βαπφ</sup>

\*P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column; <sup>π</sup>P<0.05 as compared to propolis F in the same column; <sup>φ</sup>P<0.05 as compared to propolis G in the same column.

Different flavonoids content is shown in the Table 4. The result demonstrated that the propolis samples B, C and G showed higher content of caffeine total, caffeine, caffeine 1, chrysin, pinocembrin and galangin with the use of HPLC. Propolis sample B contains the highest amount of all the flavonoids except caffeine, which was highest in the propolis sample G.

**Table 4**

Different flavonoids content in various Moroccan propolis samples (mg/mL).

Samples	Caffeine-total	Caffeine	Caffeine1	Chrysin	Pinocebrin	Galangin
A	0.73±0.06	0.28±0.02	0.44±0.03	6.23±0.56	1.70±0.15	10.96±0.98
B	5.46±0.49 <sup>*</sup>	1.69±0.15 <sup>*</sup>	3.38±0.29 <sup>*</sup>	258.90±23.30 <sup>*</sup>	28.64±2.57 <sup>*</sup>	482.50±43.42 <sup>*</sup>
C	5.24±0.42 <sup>*</sup>	0.28±0.02 <sup>#</sup>	2.77±0.24 <sup>*</sup>	76.71±6.90 <sup>*#</sup>	2.68±0.24 <sup>#</sup>	52.63±4.73 <sup>*#</sup>
D	3.53±0.31 <sup>*#+</sup>	0.84±0.07 <sup>*#+</sup>	0.73±0.06 <sup>*#+</sup>	26.80±2.41 <sup>*#+</sup>	10.02±0.92 <sup>*#+</sup>	94.75±8.52 <sup>*#+</sup>
E	3.51±0.31 <sup>*#+</sup>	0.62±0.05 <sup>*#β</sup>	1.84±0.16 <sup>*#β</sup>	17.26±1.55 <sup>*#β</sup>	6.80±0.61 <sup>*#β</sup>	50.88±4.57 <sup>*#β</sup>
F	4.08±0.36 <sup>*#</sup>	1.42±0.12 <sup>*#β α</sup>	2.95±0.26 <sup>*#β α</sup>	40.27±3.62 <sup>*#β α</sup>	15.39±1.38 <sup>*#β α</sup>	105.27±9.47 <sup>*#β α</sup>
G	4.76±0.42 <sup>*β</sup>	27.02±2.43 <sup>*#β α π</sup>	1.84±0.16 <sup>*#β π</sup>	141.90±12.77 <sup>*#β α π</sup>	15.22±1.36 <sup>*#β α</sup>	236.87±21.30 <sup>*#β α π</sup>
STD	7.20±0.64 <sup>*#β α π ϕ</sup>	3.47±0.31 <sup>*#β α π ϕ</sup>	3.69±0.33 <sup>*#β α π ϕ</sup>	21.09±1.89 <sup>*#β α π ϕ</sup>	21.48±1.93 <sup>*#β α π ϕ</sup>	21.93±1.97 <sup>*#β α π ϕ</sup>

<sup>\*</sup>P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column; <sup>π</sup>P<0.05 as compared to propolis F in the same column; <sup>ϕ</sup>P<0.05 as compared to propolis G in the same column.

Regarding the antimicrobial activity, none of the Gram-positive or Gram-negative microorganisms showed a resistance to any of the propolis samples. As compared to ethanol, propolis samples showed better antimicrobial activity. Interestingly, MRSA was the most sensitive to the propolis samples A, B and C, while *S. aureus* was more sensitive to propolis samples B, D, E and F (Table 5). *S. pyogenes*, *S. agalactiae* and *Micrococcus luteus* were much more sensitive to propolis B. Therefore, propolis B and C, which have a high resin content, showed the highest antimicrobial activity against most of the Gram-positive pathogens.

Regarding Gram-negative microorganisms, the results showed that *E. coli* was sensitive to all propolis samples except F (Table 6). *P. aeruginosa* was more sensitive to propolis C than the other samples, while *P. mirabilis* was more sensitive to samples B and G. Propolis

C is the most effective against *P. aeruginosa* and *A. baumannii*. Furthermore, the most effective propolis samples against *C. albicans* are B, C and G, which contained high amount of antioxidant content and resin.

Propolis B and C were collected from the same area and showed both high resin, phenols, flavones and flavonol content and high total antioxidant and antimicrobial activity in comparison to the other samples. However, propolis B contained significantly more resin than propolis C and also it contained higher phenols, flavones and flavonol content, and higher total antioxidant and antimicrobial activity than propolis C. With the use of DPPH, ABTS<sup>+</sup> and FRAP, propolis samples B and C showed higher activity as compared to other samples, and propolis B has higher activity as compared to propolis C.

**Table 5**

Activity of propolis samples against Gram-positive bacteria (MIC, mg/mL).

Samples	MRSA	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>Streptococcus mutans</i>	<i>Micrococcus luteus</i>
A	0.007±0.001	0.015±0.004	0.031±0.002	0.062±0.003	0.003±0.001	0.031±0.001
B	0.007±0.001	0.007±0.001 <sup>*</sup>	0.015±0.001 <sup>*</sup>	0.031±0.003 <sup>*</sup>	0.007±0.001 <sup>*</sup>	0.015±0.002 <sup>*</sup>
C	0.003±0.002 <sup>*#</sup>	0.015±0.002 <sup>#</sup>	0.015±0.004 <sup>*</sup>	0.062±0.002 <sup>#</sup>	0.007±0.002 <sup>*</sup>	0.062±0.011 <sup>*#</sup>
D	0.015±0.002 <sup>*#+</sup>	0.007±0.001 <sup>*#</sup>	0.031±0.001 <sup>*#+</sup>	0.062±0.001 <sup>#</sup>	0.003±0.001 <sup>*#+</sup>	0.062±0.012 <sup>*#</sup>
E	0.031±0.012 <sup>*#β</sup>	0.007±0.002 <sup>*#</sup>	0.031±0.002 <sup>*#</sup>	0.062±0.001 <sup>#</sup>	0.062±0.004 <sup>*#β</sup>	0.125±0.002 <sup>*#β</sup>
F	0.015±0.002 <sup>*#α</sup>	0.003±0.001 <sup>β+*# α</sup>	0.031±0.001 <sup>*#</sup>	0.062±0.002 <sup>#</sup>	0.031±0.005 <sup>*#β α</sup>	0.125±0.002 <sup>*#β</sup>
G	0.015±0.002 <sup>*#α</sup>	0.007±0.001 <sup>π+*</sup>	0.015±0.002 <sup>*π β α</sup>	0.031±0.004 <sup>*β+π α π</sup>	0.062±0.010 <sup>*#π β</sup>	0.125±0.003 <sup>*#β</sup>
EtOH	0.062±0.012 <sup>α π *#β ϕ</sup>	0.062±0.008 <sup>π β+*# ϕ α</sup>	0.250±0.011 <sup>*#π+ϕ β α</sup>	0.250±0.011 <sup>*#π+ϕ β α</sup>	0.500±0.011 <sup>*#π+ϕ β α</sup>	0.500±0.012 <sup>*#π+ϕ β</sup>

<sup>\*</sup>P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column; <sup>π</sup>P<0.05 as compared to propolis F in the same column; <sup>ϕ</sup>P<0.05 as compared to propolis G in the same column.

**Table 6**

Activity of propolis samples against Gram-negative bacteria and *C. albicans* (MIC, mg/mL).

Samples	<i>C. albicans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>P. mirabilis</i>
A	0.250±0.021	0.062±0.010	0.031±0.002	0.250±0.012	0.062±0.003
B	0.062±0.010 <sup>*</sup>	0.031±0.012 <sup>*</sup>	0.031±0.003	0.062±0.007 <sup>*</sup>	0.015±0.002 <sup>*</sup>
C	0.062±0.012 <sup>*</sup>	0.031±0.040	0.015±0.002 <sup>*#</sup>	0.015±0.009 <sup>*#</sup>	0.125±0.009 <sup>#</sup>
D	0.125±0.012 <sup>*#+</sup>	0.031±0.031	0.031±0.006	0.125±0.012 <sup>*#+</sup>	0.062±0.010 <sup>*#</sup>
E	0.125±0.022 <sup>*#+</sup>	0.031±0.032	0.031±0.005	0.125±0.052 <sup>*#+</sup>	0.125±0.012 <sup>*#β</sup>
F	0.125±0.009 <sup>*#+</sup>	0.250±0.022 <sup>*#β α</sup>	0.031±0.012	0.125±0.030 <sup>*#+</sup>	0.062±0.030 <sup>*#α</sup>
G	0.062±0.002 <sup>*π</sup>	0.031±0.018 <sup>π</sup>	0.031±0.002	0.125±0.012 <sup>*#+</sup>	0.031±0.002 <sup>*#β</sup>

<sup>\*</sup>P<0.05 as compared to propolis A in the same column; <sup>#</sup>P<0.05 as compared to propolis B in the same column; <sup>+</sup>P<0.05 as compared to propolis C in the same column; <sup>β</sup>P<0.05 as compared to propolis D in the same column; <sup>α</sup>P<0.05 as compared to propolis E in the same column; <sup>π</sup>P<0.05 as compared to propolis F in the same column.

#### 4. Discussion

The results showed that various propolis samples brought from different areas in Morocco exhibited significant variations in their chemical composition and in their antioxidant or antimicrobial activity. Propolis with high resin and a low wax content has a high level of antioxidant compounds and high antioxidant and antimicrobial activity. The propolis samples collected from areas, where *Olea*, *Pinus*, *Ceratonia*, *Eucalyptus*, *Rosmarinus*, *Populus*, *Prunus*, and *Quercus* are predominant, have a high resin content and high antimicrobial and antioxidant activity. Propolis samples B, C and G contain high level of caffeine, chrysin, pinocembrin and galangin. Therefore, propolis samples with high flavonoid content exhibits high antimicrobial and antioxidant activity; these samples have a high resin content. Therefore, other biological ingredients rather than flavonoids might play the major role as antimicrobial agents. Further studies are needed to identify such ingredients. The variation in the propolis samples composition is dependent on the type of trees predominant in the region of the sample collection. However, samples B and C were collected from the same area at the same time, but showed variations in their composition as well as their antimicrobial and antioxidant activity. With the use of HPLC, it was found that propolis B contains higher amount of caffeine total, caffeine1, chrysin, pinocembrin, galangin than other samples, including sample C, which was collected from the same area. Some of the samples contain high amount of one or two of the ingredients, but did not show high antioxidant or antimicrobial activity. Therefore, the effect is not related to one ingredient, but related to the multiple ingredients with possible synergismes, or to unidentified ingredients. It was proposed that the ratio between wax and plant resin might depend on the resins availability and the use of propolis by bees. With a low amount of resins, honey bees use more wax in propolis[29].

In a previous study, it was found that the chemical composition of three different propolis extracts collected from three regions of Morocco (Fez, Rabat and Gharb) were different[14]. The propolis samples from Fez and Rabat have poplar flavonoids. Propolis from Rabat also contained high percentage of flavonoids, but it had a significantly lower amount of phenolic acid esters, and it did not contain pinobanksin-3-O-acetate. Propolis from Bhalil in Fez contained high level of diterpenes (74.3%). All the three samples inhibited glucosidase and amylase enzymes, prevented lipid peroxidation, and scavenged free radicals[14].

In another study from Morocco, the chemical analyse of propolis from the region of Fez-Boulmane, showed that the total flavonoids content was 31.9%, total diterpenes content was 21.5%, and total phenolic acid esters content was 16.5%. The percentage of the other main compounds are 8.1 for diterpene isocupressic acid, 7.4 for the pinocembrin, 5.9 for the pinocembrin chalcone, and 5.3 for the galangin[18]. However, studies have found that propolis samples collected in Asian, African, and European regions contain predominantly naringenin, galangin, pinocembrin, cinnamylidene acetic acid, caffeic acid, *p*-coumaric acid, apigenin, pinobanksin,

quercetin, cinnamic acid, kaempferol, chrysin, cinnamyl caffeate, and aromatic acids[30,31]. Furthermore, diterpene was found to be the main compounds present in the propolis collected from tropical zones such as Brazil[32].

In Europe, the analysis of propolis from Ireland, Germany, and the Czech Republic showed that the main compounds are aromatic alcohols, aromatic acids, cinnamic acid, fatty acids, and chrysin[33]. Furthermore, propolis from Czech contains the highest phenolic content [(129.83±5.90) mg CAE/g] followed by Irish propolis and German propolis; however, the Irish propolis contains the highest content of total flavonoid [(2.86±0.20) mg QE/g][33]. In the present study, it was found that the seven propolis collected from the different regions in Morocco showed high phenols and flavones content and all the samples contain different amounts of caffeine total, caffeine, caffeine 1, chrysin, pinocembrin and galangin.

It is well known that propolis has a strong antioxidant activity[16,34–35]. In the present study, all the propolis samples showed considerable total antioxidant activity ranging from 6.56 mg AAR/g to 90.87 mg AAR/g. The antioxidant activity of the propolis was evaluated with the use of DPPH, ABTS<sup>+</sup>, and ferric reducing power methods with ascorbic acid as a control. All the propolis extracts showed a strong free radical scavenging activity with IC<sub>50</sub> ranging between 0.19 mg/mL and 1.19 mg/mL with the use of DPPH, ranging between 0.021 mg/mL and 0.983 mg/mL with the use of ABTS<sup>+</sup>, and ranging between 0.03 mg/mL and 1.08 mg/mL with the use of ferric reducing power. It was found that ethanol propolis extracts from Ireland and Czech Republic demonstrate a high free radical scavenging activity with IC<sub>50</sub> (26.45±3.80) µg/mL and (27.72±5.20) µg/mL respectively with the use of DPPH method, while the aqueous extract of propolis showed a moderate antioxidant activity with IC<sub>50</sub> (36.40±3.20) µg/mL[33]. Irish propolis exhibited the highest antioxidant activity (IC<sub>50</sub>=26.45 µg/mL)[33]. The antioxidant activity of the seven propolis samples from different regions in Morocco is evident in the present study. However, large differences were found in their antioxidant activity, which are related to their chemical composition, especially, the total phenolic and flavonoid content.

Propolis has a well-known antimicrobial activity[36–40]. It was demonstrated that poplar propolis has an antibacterial effect against both Gram-positive and Gram-negative bacteria, including MRSA[36]. Another study showed that Turkish propolis has activity against different mycobacteria[41]. We have found that propolis collected from Arabic peninsula is more potent as an antimicrobial agent than propolis collected from Egypt, and it demonstrates a synergistic activity with honey against Gram-positive and Gram-negative bacteria[42].

In the present study, all the propolis samples showed antimicrobial activity against Gram-positive and Gram-negative bacteria as well as *C. albicans*. Gram-positive bacteria showed higher sensitivity to propolis than Gram-negative bacteria with MIC ranging between 0.003 mg/mL to 0.125 mg/mL for Gram-positive and between 0.030 mg/mL to 0.250 mg/mL for Gram-negative bacteria. *C. albicans* showed less sensitivity to propolis samples and the MIC

ranged between 0.062 mg/mL to 0.250 mg/mL. MRSA was much more sensitive to the propolis samples than other bacteria and the MIC ranged from 0.030 mg/mL to 0.062 mg/mL. However, propolis samples from Ireland, Germany, and Czech Republic showed a moderate antibacterial effect against Gram-positive bacteria with MIC ranging from 0.08 mg/mL to 2.50 mg/mL. Moreover, ethanol extract of propolis exhibited moderate activity against Gram-negative bacteria with MIC between 0.6 mg/mL to 5.0 mg/mL. Regarding fungal growth, it was found that ethanol extract of propolis showed a moderate antifungal activity with MIC values between 0.6 mg/mL and 2.5 mg/mL[33]. Therefore, propolis samples collected from Morocco exhibited more activities against Gram-positive or Gram-negative bacteria than samples collected from Europe. This is most likely due to variation in their chemical compositions.

Data on propolis from many countries showed that propolis are mainly active against Gram-positive bacteria; for example, propolis from North American, South American and European countries had MIC ranging from 0.125 mg/mL to >0.500 mg/mL, while propolis collected from Africa and Asia had MIC ranging from 0.08 mg/mL to >0.50 mg/mL[43]. Furthermore, it was found that Gram-positive bacteria are sensitive to low propolis concentration while Gram negative bacteria were sensitive to high propolis concentration[44]. These results are in agreement with the present findings.

It is well-known that propolis causes inhibition of cell division, protein synthesis and bacterial motility, disruption of cell walls, enzyme inactivation, and bacteriolysis[45,46]. The polyphenols of propolis affect microbial proteins by forming hydrogen and ionic bonds[47,48]. In addition, the inhibition of MRSA by propolis has been ascribed to phenolic esters and flavonoids (pinocembrin, galangin and their derivatives)[49]. In the present study, MRSA was found to be very sensitive to Moroccan propolis samples, which might be due to the high content of pinocembrin and galangin. Furthermore, it was also found that propolis with high resin and low wax shows higher activity against Gram-positive or Gram-negative bacteria as well as *C. albicans*. This might be due to the high level of phenols and flavonoids or due to synergestims between different substances available in propolis with a low wax content.

The results showed that the compositions of various propolis samples are not identical and might depend on area of collection and its predominant plants. Propolis with a high wax has low resin and low antioxidant content and activity, and has lower antimicrobial activity as compared to propolis with high resin content. Gram-positive bacteria, in particular, MRSA are more sensitive to propolis than Gram-negative bacteria or *C. albicans*. Interestingly, the data showed that various propolis samples collected from different regions, or even from the same region, have different compositions, and they are different in their antimicrobial activity. This study supports previous finding by Dr Noori Al-Waili, which showed that mixing two different propolis samples together yielded new propolis with higher biological activity than individual propolis (Al-Waili, submitted). Furthermore, testing propolis with high resin content in preclinical and clinical settings might lead to identify certain types of propolis with the highest biological activity.

## Conflict of interest statement

The authors of this article confirm that they have no conflict of interest.

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