Physical Properties and Chemical Analysis of Iraqi Propolis

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Abstract

Propolis is a complex resinous substance manufactured by honeybees (Apis mellifera L.) to mainly protect the hive against pathogens. Physical properties of Iraqi propolis from eight regions (Al-Sulaymania, Erbil, Dohuk, Nineveh, Kirkuk, Salah Al-Din, Diyala and Al-Anbar) were investigated. Chemical analysis was achieved by thin layer chromatography (TLC) technique using five of different mobile phases including Toluene: ethyl acetate: formic acid; Toluene: ethyl acetate: acetic acid; n-hexane: ethyl acetate: acetic acid; Petroleum ether: ethyl acetate; formic acid and n-hexane: ethyl acetate: formic acid. Functional groups of separated chemical compounds were detected by IR spectroscopy. Results revealed variations in color and texture of Iraqi propolis, while odor was ranged between midly aromatic to high aromatic resinous according to geographical origin. Chemical analysis showed availability of ten important bioactive compounds in Iraqi propolis: Flavanone, 3-Hydroxyflavone, Chrysin, Quercetin, Galangin, Apigenin, Kaempferol, O-coumaric acid, Caffeic acid and Ferulic acid.

Key words: Propolis, thin layer chromatography, physical properties, IR spectroscopy.

Introduction

Propolis is a resinous honeybees prod-uct, its color varies from yellow-green to dark brown depending on its source and age. It is used to make the protective shielde at the entrance of beehive [1]. It manufacture by mixing Honeybees waxes with resinous sap that obtained from the bark and leaf-buds of certain trees, and other flowering plants, used as a sealant and sterilizer in Honeybees nests[2].Propolis has a wide range of biolo-gical activities, as antibacterial [3], anti-inflammatory [4], anti-ulcer [5], antioxidant [6], hepatoprotective [7], and tumoricidal [8] activi-ties, and high repellent index against ants [9]. It collected by honeybees from different types of plants, especially poplar and conifer trees. Bees use it along with beeswax to construct their hives [10,11]. Propolis contain approximately 50% resin and vegetable balsam, 30% wax, 10% essential aromatic oils, 5% pollen, and 5% other substances as minerals and vitamins [12]. It contains a large number of biologically active components including different flavo-noids, polyphenolic esters, terpenoids, ster-oids, amino acids, caffeine acids and their esters [13]. The flavonoids and polyphenolic compounds are the ma-jor constituents of propolis making 45-55% in most samples from different countries. In addition, propolis contains over 16 different vitamins [14]. Iranian propolis from Isfahan province contain five individual components: the prenylated coumarin suberosin, and four terpene esters: tshchin (bornyl p-hydroxy-ybenzoate), tschimangan (bornyl vanillate), ferutinin (ferutinpol p-hydroxybenzoate) and teferin (ferutinol vanillate). All of them were identified for the first time in propolis [15]. The results of the GC-MS analysis of Mediter-anean Propolis from Greece of samples collected from different locations, as groups of compounds were: aliphatic acids, fatty acids, flavonoids, diterpenes, triterpenes, sugars and sugar derivatives. More than 50 individual compounds were identified in the samples analyzed, among them sugars, flavor-noids, fatty acids, and 37 diterpenes. Twenty of the diterpenes were detected in propolis for the first time [16]. The aim of our study was to elucidate the more significant physical properties of Iraqi propolis and investigation about impor-tant bioactive compounds by chemical analy-sis using TLC technique.

Materials and Methods

Propolis Collection

A crude sample of Apis mellifera L. propolis was collected between September-2009 and January-2010 by scraping it from a honeybees hive, especially between the frames and in the internal wall of the hive. It was collected from eight regions in Iraq, where the vegetation was composed of native plants plus orange ; oak ; apple ; pines tree ; ... etc . Every geographic origin (G.O) of propolis was located within a different Iraqi province (figure 1), as follows:

1) Al-Sulaymania Governorate (Ranya region, E 44° 52’, N 36° 15’).
2) Erbil Governorate (city center, E 43° 59’, N 36° 11’).
3) Dohuk Governorate (Aqraa region, E 43° 52’, N 36° 46’).
4) Nineveh Governorate (Sinjar region, E 41° 50’, N 39° 20’).
5) Kirkuk Governorate (city center, E 44° 23’, N 39° 20’).
6) Salah Al-Din Governorate (Al-Alam region, E 43° 43’, N 34° 39’).
7) Diyala Governorate (Ba’qubah region, E 44° 40’, N 43° 47’).
8) Al-Anbar Governorate (Al-Ramadi region, E 43° 17’, N 33° 26’).
Chemical Analysis of Iraqi Propolis

The analysis of Iraqi propolis and separation of its compounds was performed by Thin Layer Chromatography (TLC) technique, using different mobile phases. Analysis was performed on precoated alumi-nium plates 20x20 cm TLC plates F$_{254}$ silica gel 60 – MERCK, Germany. The plates were dried in air and activated in oven at 110ºC for 30minutes.$^{18,19}$ Five different mobile phases (table 1) were selected to establish the RF values for every sample (all solvents were of analytical grade).$^{19}$

Table (1): Solvent systems of TLC technique

<table>
<thead>
<tr>
<th>Chromatographic system No.</th>
<th>Solvent system</th>
<th>Volume ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene: ethyl acetate: formic acid</td>
<td>36:12:5</td>
</tr>
<tr>
<td>2</td>
<td>Toluene: ethyl acetate: acetic acid</td>
<td>30:15:5</td>
</tr>
<tr>
<td>3</td>
<td>n-hexane: ethyl acetate: acetic acid</td>
<td>31:14:5</td>
</tr>
<tr>
<td>4</td>
<td>Petroleum ether: ethyl acetate: formic acid</td>
<td>30:15:5</td>
</tr>
<tr>
<td>5</td>
<td>n-hexane: ethyl acetate: formic acid</td>
<td>31:14:5</td>
</tr>
</tbody>
</table>

Ten micro liters of propolis solution (containing 300mg of propolis extract, dissolved in 1ml of 96% ethanol) from Ranya region were placed at a distance of 1.5 cm at the lower edge of the plate, and RF values of standards were used according to Medić-Šarić and co-workers.$^{19}$ The plates were developed at room temperature in a vertical separating chamber to the height of approximately 25 cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). After drying, visualization was performed in two methods:

i) Exposure to ammonia vapor, then direct eye-visualization.

ii) In short UV light (254nm).$^{18}$

For functional groups identification of separated chemical compounds, IR Spectroscopic – BRUKER device was used.$^{20}$ According to characteristic infrared absorb- tion frequencies (vibration mode and frequency- cm$^{-1}$) of common classes of organic compounds, important functional groups of Iraqi propolis compounds were detected.

Results and discussion

Physical Properties of Iraqi Propolis

Results of physical properties of Iraqi propolis showed wide differences especially in color between different samples of Iraqi propolis depending on geographical origin and flora vegetation in that area. Table (2) summarizes the important physical properties of Iraqi propolis from different localities:

Table (2): Physical properties of Iraqi propolis

<table>
<thead>
<tr>
<th>Origin place of samples (governorate, region)</th>
<th>Geographical position</th>
<th>Color</th>
<th>Texture</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Sulaymania, Ranya</td>
<td>E 44º 52´, N 36º 15´</td>
<td>Light brown</td>
<td>Rigid wax</td>
<td>Very aromatic resins</td>
</tr>
<tr>
<td>Erbil, city center</td>
<td>E 43º 59´, N 36º 11´</td>
<td>Brown</td>
<td>Rigid</td>
<td>Aromatic resins</td>
</tr>
<tr>
<td>Duhok, Aqraa</td>
<td>E 43º 52´, N 36º 46´</td>
<td>Dark greenish brown</td>
<td>Waxy</td>
<td>Aromatic resins</td>
</tr>
<tr>
<td>Nineveh, Sinjar</td>
<td>E 41º 50´, N 39º 20´</td>
<td>Reddish brown</td>
<td>Rigid wax</td>
<td>Aromatic resins</td>
</tr>
<tr>
<td>Kirkuk, city center</td>
<td>E 44º 23´, N 39º 20´</td>
<td>Yellowish brown</td>
<td>Rigid</td>
<td>Aromatic resins</td>
</tr>
<tr>
<td>Salah Al-din, Al-Alam</td>
<td>E 43º 43´, N 34º 39´</td>
<td>Reddish brown</td>
<td>Rigid wax</td>
<td>Aromatic resins</td>
</tr>
<tr>
<td>Diyala, Ba'qubah</td>
<td>E 44º 40´, N 43º 47´</td>
<td>Brownish yellow</td>
<td>Rigid</td>
<td>Very aromatic resins</td>
</tr>
<tr>
<td>Al-Anbar, Al-Ramadi</td>
<td>E 43º 17´, N 33º 26´</td>
<td>Dark brown</td>
<td>Waxy</td>
<td>Mildly aromatic</td>
</tr>
</tbody>
</table>

Colors of different samples of Iraqi propolis had a broad range of varieties, it ranges between brownish yellow in Ba'qubah propolis sample and dark brown in Al-Ramadi propolis sample depending on flora vegetation that was mixed with different plants, and according to geographical position, as mentioned in the table (2). Flavonoids and polyphenolic compounds form about 45-50% of propolis composition in general and they had an important role in propolis color.$^{1,14}$ Whereas Silva and co-workers$^{21}$ reported chemical composition and botanical origin of red propolis as a new type of Brazilian propolis, which depended on secretions of plant species that were often mentioned as its probable botanical source. Texture of Iraqi propolis mainly was rigid or rigid waxy depending on the amount of beeswax, and this extrusive proportionate with beeswax that increased the softness of propolis from rigid to rigid waxy or waxy, and in general, propolis
contains about 30% of wax, which affects the texture 
[12]. These results approximating to Mot and co-
workers [22], their study was conducted on Romanian 
propolis, and flora was complex or Meadow, high or 
low content of mixture of deciduous forests. 
Concerning Iraqi propolis odor, most of the samples 
were aromatic resinous depends on flora vegetation, 
and types of chemical compounds were essential and 
aromatic oils, which form 10% of propolis composition 
[12]. Iraqi propolis odor was very 
aromatic resinous in Ranyia region, while Al-Ramadi 
region it was midly aromatic, other samples from 
other regions were aromatic resinous. The chemical 
analysis of Iraqi propolis was achieved using a TLC 
technique, five different solvent systems were used 
(table 1) according to Medić-Šarić and co-
workers [19] and results revealed separation ten of the important 
bioactive compounds from Al-Sulaymania, 
Ranya region propolis. Figure(2) elucidate Rf of separated 
chemical compounds in solvent system 1. Table (3) 
elucidate Rf values of standard and separated 
chemical compounds in 5 different solvent systems.

Table (3): Rf values of standard and separated compounds of Iraqi propolis.

<table>
<thead>
<tr>
<th>TLC Systems No.</th>
<th>compounds</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Flavanone</td>
<td>0.67</td>
<td>--</td>
<td>0.62</td>
<td>--</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>3-Hydroxyflavone</td>
<td>0.77</td>
<td>0.76</td>
<td>0.80</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>Chrysin</td>
<td>0.62</td>
<td>0.61</td>
<td>0.60</td>
<td>--</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>Quercetin</td>
<td>0.39</td>
<td>0.40</td>
<td>0.27</td>
<td>--</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>Galangin</td>
<td>0.65</td>
<td>--</td>
<td>0.64</td>
<td>0.65</td>
<td>0.72</td>
</tr>
<tr>
<td>6</td>
<td>Apigenin</td>
<td>0.44</td>
<td>0.43</td>
<td>0.47</td>
<td>--</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td>Kaempferol</td>
<td>0.51</td>
<td>0.50</td>
<td>0.50</td>
<td>--</td>
<td>0.47</td>
</tr>
<tr>
<td>8</td>
<td>o-Coumaric acid</td>
<td>0.55</td>
<td>--</td>
<td>0.51</td>
<td>--</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td>Caffeic acid</td>
<td>0.38</td>
<td>--</td>
<td>0.30</td>
<td>--</td>
<td>0.43</td>
</tr>
<tr>
<td>10</td>
<td>Ferulic acid</td>
<td>0.56</td>
<td>--</td>
<td>0.49</td>
<td>--</td>
<td>0.63</td>
</tr>
</tbody>
</table>

\[R_{f_{std}}: R_{f} \text{ values of standards; } R_{f_{sep}}: R_{f} \text{ values of separated compounds; } ^*\text{ the chemical compound was not detected at this } R_{f}\]

Results revealed ten of important bioactive-natural 
chemical compounds in Iraqi propolis and these were: 
flavanone, 3-hydroxyflavone, chrysin, quercetin, 
galangin, apigenin, kaempferol, o-coumaric acid, 
caffeic acid and ferulic acid. Chemical structures 
and formula of these compounds were found out from 
ChemDraw® Ultra v8.0 (computer program)[23] 
indicated in figure (3). Identification of separated 
chemical compo-unds was indeed done by IR 
Spectroscopic device. Some of these compounds 
were similar to results of Naama and co-workers [24], 
who identified six chemical compounds from Iraqi 
propolis for different regions of Baghdad governorate 
by TLC and HPLC, these were: chrysin, galangin and 
caffeic acid, naringenin, p-coumaric acid and 
pinocembrin. While Darwish and co-workers [25] 
investigated Jordanian propolis from Amman city by 
column chromatography and identified three pure 
phenolic compounds: pinobanksin-3-O-acetate, 
pinocembrin and chrysin.
Figure (3): chemical structure and molecular formula of separated compounds.

**Figure (3): continue…**

IR spectroscopy was used to ascertain the identification [20]. Figure (4) showed the IR spectra of kaempferol. The C–H bands of aromatic compounds appear in the 3300–2700 cm⁻¹ range, carbonyl group (C=O) gave rise to a strong absorption in the region 1820–1660 cm⁻¹, the peak was often the strongest in the spectrum. Also O–H group found at 3300–2500 cm⁻¹, and C=C band was a weak absorption near 1650 cm⁻¹. Aromatic ring as general gave rise weak absorption in the region 1650–1450 cm⁻¹. All other compounds were identified in same method.

**Figure (4): IR spectra of separated kaempferol.**

As conclusion, our findings have shown that Iraqi propolis samples had a wide variations in color and approximately constancy in texture and odor, and that regard as an important characteristic for arriving to primary step of Iraqi propolis standardization. Also, chemical analysis demonstrated availability ten of important bioactive compounds, TLC technique was used successfully as an inexpensive method and for its specificity, as well a possibility to use it for qualitative and quantitative analysis of Iraqi propolis.

References
26. Curious World Maps v7.2b, (computer program), Copyright © 2002-2006 Curious Software Company Ltd. US.
الخصائص الفيزيائية والتحليل الكيميائي للبروبوليس العراقي

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الملخص
البروبوليس هو مادة راتنجية معقدة، تصنع بواسطة نحل العسل لحماية نحل من المفترضات بشكل رئيسي. تم جمع البروبوليس العراقي من ثمانية مناطق (السليمانية، اربيل، دهوك، نينوى، كركوك، صلاح الدين، ديالى، الانبار) لدراسة الخصائص الفيزيائية وإجراء التحليل الكيميائي.

أنجز التحليل الكيميائي بواسطة تقنية كروماتوبغرافيا الطبقية الوقيقة، باستخدام خمسة أطوار متحركة تضمنت Toluene: ethyl acetate: formic acid; Toluene: ethyl acetate: acetic acid; n-hexane: ethyl acetate: acetic acid; Petroleum ether: ethyl acetate: formic acid; Petroleum ether: n-hexane: ethyl acetate: formic acid. كما تم التحري عن المجامع الفعالة للمركبات المفصولة باستخدام طيف الأشعة تحت الحمراء (IR spectroscopy). كشفت النتائج عن وجود اختلافات باللون والبنية للبروبوليس العراقي، بينما كانت المركبات (Flavanone, 3-Hydroxyflavone, Chrysin, Quercetin, Galangin, Apigenin, Kaempferol, O-coumaric acid, Caffeic acid and Ferulic acid) موجودة بشكل متعدد في البذور والعشة من المركبات الفعالة في البروبوليس العراقي.