Physical Properties and Chemical Analysis of Iraqi Propolis

Ibrahim H. Y. Ali[*]; Awwad Sh. Daoud [1]; Adeeba Y. Shareef [2]

[1] *Department of Biology, College of Science , Tikrit University , Tikrit, Iraq.*

[2] *Department of Biology , College of Science , Mosul University, Mosul, Iraq.*

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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 shield 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], antiulcer [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 and 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, caffeic acids and their esters [13]. The flavonoids and polyphenolic compounds are the major 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: tschimgin (bornyl *p*-hydrox-ybenzoate), tschimganin (bornyl vanillate), ferutinin (ferutinol *p*-hydroxybenzoate) and tefernin (ferutinol vanillate). All of them were identified for the first time in propolis [15]. The results of the GC-MS analysis of Mediter-ranean 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 (Ranyia region, E 44º 52`, N 36º 15`).
2) Erbil Governorate (city center, E 43º 59`, N 36º 11`).
3) Dohuk Governorate (Aqraa region, E 43º 52`, N 36º 46`).
4) Nineveh Governorate (Sinjar region, E 41º 50`, N 39º 20`).
5) Kirkuk Governorate (city center, E 44º 23`, N 39º 20`).
6) Salah Al-Din Governorate (Al-Alam region, E 43º 43`, N 34º 39`).
7) Diyala Governorate (Ba'qubah region, E 44º 40`, N 43º 47`).
8) Al-Anbar Governorate (Al-Ramadi region, E 43º 17`, N 33º 26`).

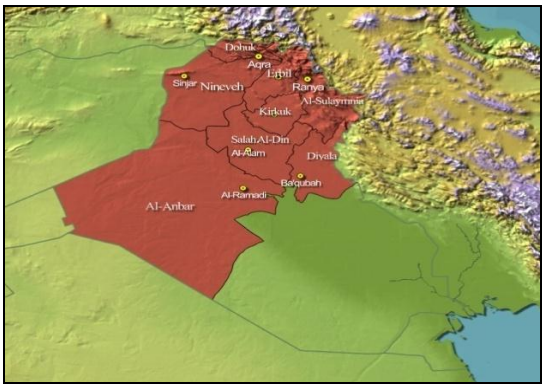

Figure (1): Distribution and geographical origin of Iraqi propolis samples.

Geographical positions of regions were measured from Curious World Maps v7.2b,(computer program)[26]. Physical prope-rties were recorded immediately and kept in refrigerator at 4–8ºC, until used for prepar-ation and fractionation.

Detection the Physical Properties of Iraqi Propolis

According to Lopez and co-workers [17], many physical parameters of Iraqi prop - olis were investigated by direct observation, including appearance, odor and color. Enviro - nmental variables depended on where the geo-graphical position of honeybees hives and the predominant flora in the apiaries.

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 20×20 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

Chromatogr-aphic system No.	Solvent system	Volume ratio
1	Toluene: ethyl acetate: formic acid	36:12:5
2	Toluene: ethyl acetate: acetic acid	30:15:5
3	n-hexane: ethyl acetate: acetic acid	31:14:5
4	Petroleum ether: ethyl acetate: formic acid	30:15:5
5	n-hexane: ethyl acetate: formic acid	31:14:5

Ten micro liters of propolis solution (containing 300mg of propolis extract, dissolved in 1ml of 96% ethanol) from Ranyia 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 approx-imately 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 Spectro-scopic – BRUKER device was used [20]. According to characteristic infrared absorp-tion frequencies (vibration mode and frequ-ency- 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 prop-olis

Origin place of samples (governorate, region)	Geograph-ical position	Color	Texture	Odor
Al-Sulaymania, Ranyia	E 44º 52`, N 36º 15`	Light brown	Rigid waxy	Very aromatic resinous
Erbil, city center	E 43º 59`, N 36º 11`	Brown	Rigid	Aromatic resinous
Duhok, Aqraa	E 43º 52`, N 36º 46`	Dark greenish brown	Waxy	Aromatic resinous
Nineveh, Sinjar	E 41º 50`, N 39º 20`	Reddish brown	Rigid waxy	Aromatic resinous
Kirkuk, city center	E 44º 23`, N 39º 20`	Yellow-ish brown	Rigid	Aromatic resinous
Salah Al-din, Al-Alam	E 43º 43`, N 34º 39`	Reddish brown	Rigid waxy	Aromatic resinous
Diyala, Ba'qubah	E 44º 40`, N 43º 47`	Browni-sh yellow	Rigid	Very aromatic resinous
Al-Anbar, Al-Ramadi	E 43º 17`, N 33º 26`	Dark brown	Waxy	Midly aromatic

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 posi-tion, 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 prop-olis 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, Ranyia region propolis. Figure(2) elucidate R_f of separated chemical comp-ounds in solvent system 1. Table (3) elucidate R_f values of standard and separated chemical compounds in 5 different solvent systems. Separated compounds were from flavonone, flavonol and phenolic acid groups.

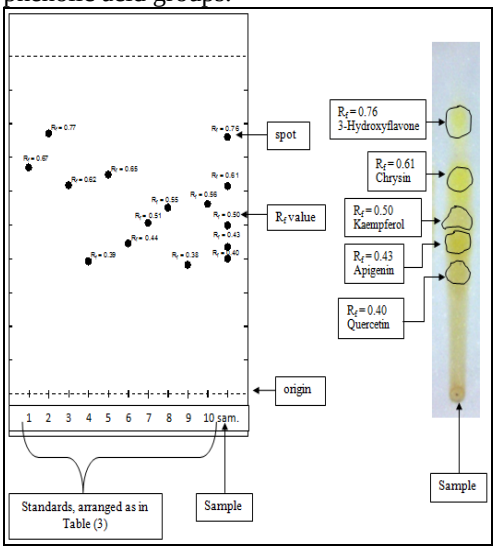

Figure (2): TLC plate showed spots of separated compounds by solvent system 1.

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

TLC Systems No. / compounds		1		2		3		4		5	
No.		$R_{f(s)}$	$R_{f(c)}$	$R_{f(s)}$	$R_{f(c)}$	$R_{f(s)}$	$R_{f(c)}$	$R_{f(s)}$	$R_{f(c)}$	$R_{f(s)}$	$R_{f(c)}$
1	Flavanone	0.67	--*	0.62	--	0.75	--	0.76	0.76	0.38	0.40
2	3-Hydroxyflavone	0.77	0.76	0.80	0.82	0.82	0.82	0.51	--	0.56	0.57
3	Chrysin	0.62	0.61	0.60	--	0.68	--	0.38	--	0.36	--
4	Quercetin	0.39	0.40	0.27	--	0.30	--	0.27	--	0.22	--
5	Galangin	0.65	--	0.64	0.65	0.72	--	0.44	--	0.37	--
6	Apigenin	0.44	0.43	0.47	--	0.39	--	0.33	--	0.21	--
7	Kaempferol	0.51	0.50	0.50	--	0.47	--	0.37	--	0.23	--
8	*o*-Coumaric acid	0.55	--	0.51	--	0.73	0.73	0.36	--	0.37	--
9	Caffeic acid	0.38	--	0.30	--	0.43	--	0.62	0.63	0.22	--
10	Ferulic acid	0.56	--	0.49	--	0.63	0.62	0.32	--	0.28	0.30

$R_{f(s)}$: R_f values of standards; $R_{f(c)}$: R_f values of separated compounds; * 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] invest-igated Jordanian propolis from Amman city by column chromatography and identified three pure phenolic compounds: pinobanksin-3-*O*-acetate, pinocemberin and chrysin.

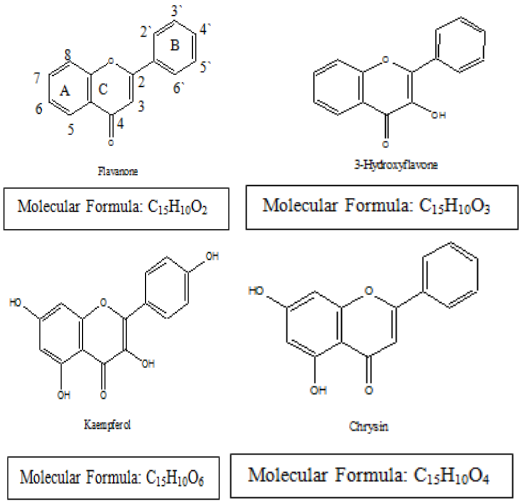

Figure (3): chemical structure and molecular formula of separated compounds.

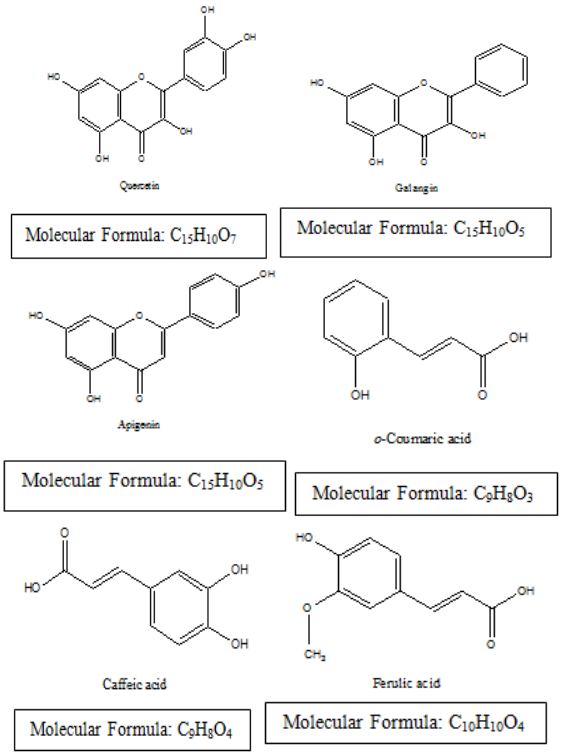

Figure (3): *continue…*

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 3300–2700cm^{-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.

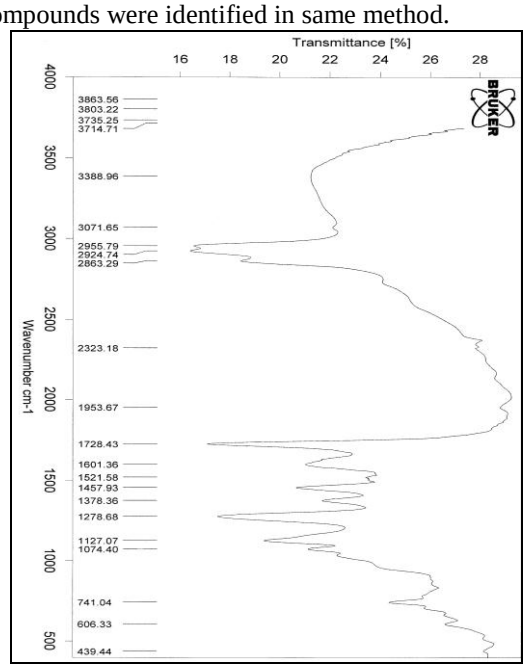

Figure (4): IR spectra of separated kaempferol.

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 avail-ability ten of important bioactive compo-unds, 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 .

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الخصائص الفيزيائية والتحليل الكيميائي للبروبوليس العراقي

إبراهيم حسن ياسين علي، عواد شعبان داود[١]، أديبة يونس شريف[٢]

[١] *جامعة تكريت، كلية العلوم، قسم علوم الحياة، تكريت، العراق*

[٢] *جامعة الموصل، كلية العلوم، قسم علوم الحياة، الموصل، العراق*

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الملخص

البروبوليس هو مادة راتنجية معقدة، تُصنع بواسطة نحل العسل لحماية خلية النحل من المُمْرضات بشكلٍ رَئيسي. تم جمع البروبوليس العراقي من ثمانية مناطق (السليمانية، اربيل، دهوك، نينوى، كركوك، صلاح الدين، ديالى، الانبار) لدراسة الخصائص الفيزيائية و إجراء التحليل الكيميائي. أنجز التحليل الكيميائي بواسطة تقنية كروماتوغرافيا الطبقة الرقيقة و 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 and n-hexane: ethyl acetate: formic acid. كما تم التحري عن المجاميع الفعالة للمركبات المفصولة باستخدام طيف الاشعة تحت الحمراء (IR spectroscopy). كشفت النتائج عن وجود اختلافات باللون و البنية للبروبوليس العراقي، بينما كانت الرائحة العطرية متفاوتة بين متوسط العطرية الى راتنجي عالي العطرية و ذلك حسب الموقع الجغرافي لعينة البروبوليس. أظهر التحليل الكيميائي وجود عشرة من المركبات الفعالة في البروبوليس العراقي و هي: Flavanone, 3-Hydroxyflavone, Chrysin, Quercetin, Galangin, Apigenin, Kaempferol, *O*-coumaric acid, Caffeic acid and Ferulic acid.