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STUDY OF CHEMICAL ANALYSIS OF IRAQI PROPOLIS AND ACTIVE COMPONENT OF PROPLIS, IRAQ

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ABSTRACT

Propolis is a resinous substance collected by worker bees (*Apismellifera*) from the bark of trees and leaves of plants. The aim of this study is to elucidate the chemical nature of the bioactive compounds isolated from Iraqi propolis using different methods of analysis and Identification of propolis components by TLC. Propolis samples were collected during spring and summer seasons (2014). Phytochemical screening of propolis revealed presence of flavonoids, tannin, alkaloids, mucilage, saponin, fat, terpenoids, volatile oils phenolic and coumarins compounds as a major constituents and absence of glycosides with steroids. The major constituents of propolis revealed that proteins were predominant components followed by carbohydrates, fat, saponin, moisture and ash.The types of amino acids in proteins of Al-Museiabpropolis were proline, hydroxyl proline, tyrosine, tryptophan, arginine, cysteine, cystine, methionine, whereas casein was not present in Al-Museiabpropolis. Chemical screening of Al-Museiabpropolis revealed presence of six fatty acids represented by mgristic acid, palmtic acid, arachidic acid, olic acid, linoleic acid, and linolinic acid. The chemical analysis of mineral elements in Al-Museiabpropolis was also determined; K was predominant in Al-Museiabpropolis followed by Na, Fe, Mg and Ca, while Zn Cu, Co and Ni were presented in low concentration. Also we were isolated some compounds by TLC pates. The result of alcoholic and aquatic extracts of propolis analysis indicated that the contained 4 compounds for each extract. Thus it was concluded that Al-Museiabpropolis have active components as a major constituents and Al-Museiabpropolis were rich with proteins, carbohydrates, fat, and saponin. It has fatty acid and mineral elements.

Keywords: Propolis, Phytochemical analysis, Iraq.

INTRODUCTION

Propolis is a resinous substance collected by worker bees (*Apismellifera*) from the bark of trees and leaves of plants. This salivary and enzymatic secretions-enriched material is used by bees to cover hive walls to ensure a hospital-clean environment. As a natural honeybee hive product, propolis extracts have been used both internally and externally for thousands of years as a healing agent in traditional medicine. Propolis shows a complex chemical composition. Its biological properties-such as antibacterial, antiviral, antifungal, among other activities, have attracted the researchers' interest [1].Its biological properties may vary according to different plant sources. Many authors have demonstrated propolis antibacterial activity against *Enterococcus* spp, *Escherichia coli*, and *Staphylococcus aureus*. Reports have pointed out propolis efficient activity against Gram-positive bacteria and limited action against Gram-negative bacteria [2]. Different researchers [3, 4] have reported that propolis antibacterial activity is attributed to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters. Some prenylatedcoumaric acids were isolated from propolis in several countries [5]. Propolis and some of its cinnamic acid derivatives and flavonoids were responsible for uncoupling the energy transducing cytoplasmic membrane inhibiting bacterial motility, which might contribute to the antibacterial action [6]. Although numerous researchers have been reported the biological activities of propolis collected worldwide, information about Iraqi propolis are still absent. The aim of this study is to elucidate the chemical nature of the bioactive compounds isolated from Iraqi propolis using

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different methods of analysis and Identification of propolis components by TLC.

MATERIALS AND METHODS

Propolis samples

Propolis samples were collected from hives of honey bees of Al-Museiab, Iraqi during spring and summer seasons of 2014. Propolis samples were cleaned, free of wax, paint, wood, cut into small pieces, and placed in clean container.

Preliminary chemical determinations of active propolis components

Measurement of pH of propolis component

The measurement of pH in the concentration used in bioactivity was evaluated. 10 gram of propoliswere dissolved in 50 ml of distill water and shaked for half hr. at 37 ^{o}C. Then the solution filtered was and measured by pH-meter [7].

Resins

The procedure of Shihata [7] was followed by adding 50 ml of ethanol to 5 gmof propolis are in water bath boiling for 20 min. After cooling the mixture wasfiltered, and 10 ml of D.W. containing 4% HCI was added to the filtered solution. Turbidity of the mixture refers to resins existence.

Tannins

The procedure of [8] was used for the detection of tannins. Ten gm of propolis were added to 50 ml D.W. then heated tillboiling and after cooling the mixture was filtered .Then each extract was equally divided into two conical flasks. For the first flask, few drops of 1%lead acetate solution were added, the appearance of white gelatinous pellet was considered as a positive indicator for tannins existence, while for the second flask, and few drops of 1% Ferric chloride solution were added. The appearance of blue-green color was an indicator for the presence of tannins.

Alkaloids

For detection of alkaloids, three methods were used as followed:

a- Dragnadroff reagents [9]

According to this procedure,10gm of propolis were dissolved in 50 ml of D.W.with (4%) of HClthen boiled for 10 minute and cooling. Then the solution was filtered and 0.5ml from this solution were placed in 4 petridishs and treated with 1ml of:

a-Dragnadroff reagents, the development of orange precipitate indicates for the presence of alkaloids

b- Mayer reagent,The development of white precipitate indicates for the presence of alkaloids.

c-Wankerreagent, The development of brown precipitate indicates for the presence of alkaloids.

d-Becrick acid, reagent, The development of yellow precipitate indicates for the presence of alkaloids.

Flavonides

The presence of flavonoids was detected according to Jaffer*et al* [10] .The procedure induced two solutions as follow. The first solution was prepared by dissolving 10 gm of ethanolic extract of propolis in 5ml of ethanol (95%) and then the solution was filtered.

The second solution was prepared by mixing 10 ml of 50% ethanol+10 ml of 50% potassium hydroxide. Both solution were mixed to gather. The development of yellow color is an indication for the presence of flavonoids.

Phenolic compounds

About 3ml of ethanolic extract of propolis were treated with 1% ferric chloride then exposure to ammonia. The development of blue color is an indication for presence of phenolic compounds [11].

Glycosides

For detection of Glycosides, three methods were used as followed:

A. Five ml of aquatic extract of propolis was mixed with 5ml of Fehling reagent and boiled in water bath for 10 minutes.

B. 1 ml of aquatic extract of propolis was mixed with 5ml of Benedict reagent.

C. 5 ml of aquatic extract of propolis were treated with few drops of HCl and boiled in water bath for 20 minute then the pH was adjusted with NaOH then Fehling reagent was added. The appearance of red precipitate indicating for presence of reducing sugars [11].

Saponins

For detection of Saponins, three methods were used as followed:

A-Five ml of alcoholic extract of propoliswereshaked vigorously intest tube. If the formation of permanent foamis an indicator the presence saponins [11].

B- Five ml of aquatic extract of propolis was mixed with 3ml of mercuric chloride. The appearance of white precipitate indicating for presence ofsaponin.

C-Five ml of aquatic extract of propolis was mixed with 5ml of Ag (NO_3) boiling in water bath with shaken appearance of mirror on the walls of tube after cooling indicating for presence ofsaponin [7].

Comarins

According to Jaffer*et al* [10], 3ml of ethanolic extract of propoliswere placed in test tube and covered by filter paper whatman No.1 saturated with NaOH thenthe test tube boiled in the water bath. The filter paper was exposed to UV-light. The appearance of yellow greenish coloris an indication for the presence of

comarins.

Steroids and Terpenes

The procedure being recommended by Al-Maisary [12] was employed for detection of these compounds by mixing one gram of propolis with 2 ml of chloroform and one drop of glacial acetic acid, and then one drop of concentrated H2SO4 were added. The appearance of brown color was an indicator of the presence of terpenes, and then if a blue color was appeared, this time is an indicator for the presence of steroids

Volatile oil

According to the Muhsan [13], a filter paper was saturated with 10 ml of the propolis and examined under UV light. The appearance of bright pink color is an indicator of the presence for volatile oils.

Determination of major propolis component

Carbohydrates

A drop of ethanolic extract was mixture with 0.5 ml of Benedict reagent in the test tube and heated for 10 minute. The appearance of green bluish color is an indicator for the presence ofCarbohydrate [14].

Proteins

Using total protein kits, Biuret reagent .20 µl of ethanolic extract+1ml of Biuret reagent. 20 µl of kit+1ml of protein standard. After 10 minute the OD was read by spectrophotometer at 550 nm. Then the following equivalence was applied:

Result=AB (assay)\Abs of standard × standard concentration (60 gm\L)

General tests for proteins

Ninhydrin test

Two drop of 0.1% Ninhydrin was added to 1 ml of protein solution and boiled for 1-2 minutes and then allowed to cool. Blue colored complex indicates a positive test. Proline and Hydroxyproline give a yellow color [15].

Xanthoproteic test

One ml of concentrated nitric acid was added to 2 ml of protein solution. A white precipitate was obtained due to denaturation of proteins. The solution was heated for 1 minute and cooled under tap water. A yellow color was obtained. 5 ml or more of 40% sodium hydroxide solution was added. The yellow color deepens to an orange color [15].

Sakaguchi test

Two drops of 1% α-naphthol in alcohol (Molisch's reagent) were added to 2ml of sample solution,4% sodium hydroxide and 8-10 drops of bromine water. The appearance of red complex indicates a positive test [15].

Test for Sulphur-containing Amino acids

Two 2 ml of 40% sodium hydroxide was add to 2 ml of protein solution and boiled for 3 minutes, cooled and then 2-3 drops of lead acetate were added. The appearance of black or brown precipitate indicates a positive test [15].

Test for Carbohydrate group

Two 5 drops of Molisch's reagent were added to 2 ml of albumin solution, mixed, and carefully suspended to 2 ml of concentrated sulphuric acid, purple ring is formed at the interface [15].

Test for Organic Phosphorus

Perform the test with only casein (milk protein)

Half ml of 40% sodium hydroxide were added to 3 ml of casein and heated strongly, and cooled under tap water. Then 0.5 ml of concentrated nitric acid was added and the mixture was filtered. To the filtrate pinch of solid ammonium molybdate was added and warmed gently. Note the canary yellow color of the precipitate [15].

Moisture

Two of propoliswere put in oven at 130ºC for 1hr.then put the propolis in Discater which contain silica gel. After Weighted the propolis recovered into oven 130ºC then the propolis put in Discater and weighted the propolis to happen the stable weight and calculate the percentage [16].

Ash

The presence of ash in propolis was detected according to the standard method being recommended by Aldellaly and Alhekeem [17], since 2 grams of propolis put in moffle furnace at 550ºC; the sample converted into light gray color. The sample was weighted and the percentage determination.

Determination of minerals elements

The percentage and types of minerals elements present in propolis were detected according to [18], since 2 gm of propolis digested with 10 ml of acidic mixture (H_2NO_3, H_2SO_4 and Brecloric acid) at the following ratio 1:5:2, respectively. The mixture was heated at 40-60 ºC, filtering the solution and evaporation, the semisolid materiel diluted with deionized water into 25 ml, finally detection the elements by atomic absorption spectroscopy.

Determination of fats

Total fats

The total fats in propolis were determined by using soxhlet apparatus. 200 ml of petroleum ether in the round flask and put 10 gm of propolis into thumble for 8

hr. after extraction evaporation of the solvent at 50 ºC and calculate the fat weight (17).

Free fatty acids

In this procedure 0.3 gm of ethanolic extract of propolis was converted into methyl ester by adding 1 ml from reagent (25 ml of methanol in 0.1 acetyl chloride). The mixture was heated in water bath for 25 minute then cooled the solution and measurement by GC[19]

RESULTS AND DISCUSSION

Phytochemical screening of propolis

Phytochemical screening of propolis from different samples (Al-Museiabpropolis) revealed presence of flavonoids, tannin, alkaloids, mucilage, saponin, fat, terpenoids, volatile oils phenolic and coumarins compounds as a major constituents and absence of glycosides and steroids (Table-1). These results were in agreement with other studies conducted by several authors [20-22] who mentioned that propolis contained flavonoids, saponin, fat, volatile oils and phenolic compounds although they tannin, glycosides and alkaloids were absence. The differences in contents of propolis samples might be due to localities and geographical variation, different plants and different parts of plants.

The pH of propolis samples investigated in the present study ranged from 5.15 to 5.23. This acidity is due to presence of organic acids and phenolic acids of propolis (such as gallic acid, tannic acid and free fatty acids such as;mgristic acid, Palmitic acid, Arachidic acid, Oleic acid, Linoleic acid and Linoleic acid) [23].These acids are related to glycosides such as a glucoside ester of gallic acid and hydrolysable tannins (esters of phenolic acids),Pentagalloyl glucose was abundant in the hydrolysable tannin fraction [24] which may be responsible for low pH of propolis.

Percentage of major component of propolis

Figure (1) shows that the percentages of constituents of Al-Museiabpropolis. Results revealed that proteins were predominant components followed by carbohydrates with a percentage of 23% and 20% respectively. The percentages of fat, saponin, moisture and ash were 20%, 19%, 16% and 2.22% respectively. Carbohydrates were predominant components of most types of propolis due to highly nutrition properties and also contained saccharin (such as cellulose and pectin). The Saponin of propolis samples in the present study ranged from 19 to 18%. Saponin was tritrepenoid which has pharmacological activities for many diseases which affect the digestive tract [9]. Regarding, the percentages of proteins were 23-19 %. The presence of proteins in the most types of propolis may be attributed to the presence of inoculator's grains in highly amounts. Proteins were important in the making of plants tissues, hormones and enzymes [25].

Table (3) shows that the types of amino acids in proteins of Al-Museiabpropolis were proline and hydroxyl proline determined by Ninhydrin test. Principle of this test reacts with α amino group of proteins (propolis extract) and free amino acids to give a purple colored complex. This is answered by all proteins, peptones, peptide, amino acids and other primary amines.The α-amino acids react with Ninhydrin to form aldehyde and hydrindantin. Hydrindantin in turn reacts with Ninhydrin to form the blue colored complex while Proline and hydroxyl proline showed a yellow color. Tyrosine and tryptophan were determined by Xanthoproteic test and gave yellow color. Principle of this test benzene ring of tyrosine and tryptophan in (propolis extract) undergo nitration on treatment with strong nitric acid in higher temperature. Nitration of phenylalanine under this condition normally didn't take place. Arginine was determined by Sakaguchi test and gave red color. The principle of this test depends up on the Arginine or arginyl residue in the protein (propolis extract) which reacts with α-naphthol and alkali hypobromite to give a red complex. The reaction is specific for guanido group of Arginine. Cysteine and cystine in (propolis extract) were determined by test for sulphur containing amino acid and revealed black color. The principle of this test depends up on cysteine, cystine or proteinous containing these amino acids will convert to sulfide when boiled with strong alkali, organic sulphur, and addition of lead acetate to this solution causes the precipitation of insoluble lead sulfide (black in color). Methionine did not give this test as the sulphur group in this amino acid is not free and did not released by treatment with alkali. Glycoprotein in propolis extract was determined by Molisch's test for carbohydrate group and gave purple color, whereas the proteins of Al-Museiabpropolis had not casein determined by test for organic phsphrus (casein). Chemical analysis of Al-Museiabpropolis detected for types and percentages of free fatty acids by chemical solvents after conversion of propolis extract into methyl ester and determined by HPLC (Table 3).

Chemical screening of Al-Museiabpropolis revealed presence of six fatty acids. Three fatty acids represented by mgristic acid (7.35%), Palmtic acid (17.84%) and Arachidic acid (4.02%) contained only one chemical bond. One fatty acid had one double bond which represented byOlic acid (12.08%) and one fatty acid has two double bond -Linoleic acid (6.27%)- while Linolinic acid (3.16%) has three double chemical bond.

Fatty acids are very important in physiological activities in any life system. Linoleic acid is essential in different metabolic reactions and hypotension and treatment of ENT infections, asthma and stomach sore [26]. Decrease the level of fatty acid causes defect in RBCs, Eczema, skin dry, hair loss and men sterility [27]. Palmtic acid has importance in making beautifying conjured up due to it's antifungal, antibacterial and

antiviral activities. Pharmacological and biological activities of propolis attributed to its free fatty acids. The chemical analysis of mineral elements in Al-Museiabpropolis was also determined (Table 4). Potassium was predominant (0.0025 mg\ml) in Al-Museiabpropolis compared with other elements followed by Na, Fe, Mg and Ca which were 0.00162, 0.00130, 0.00123 and 0.0014 mg\ml respectively, while Zn Cu, Co and Ni elements were low since they accounted for 0.00114, 0.00107, 0.00026 and 0.00018 mg\ml respectively. Lead (Pb) was not detected in Al-Museiabpropolis. These results were in agreement with author [28] who mentioned that propolis contained K, Ca, Na, Co, Mg, V, Fe, Cu, and Ni. The mineral elements have physiological importance in human and animal bodies, especially Na and Ca which play important roles in bones building. K keeping on pH for body fluids while Zn plays an important role in wounds treatment. In general the mineral elements increase the pharmacological and biological activities of propolis [29].

Number of the compounds in the active components of Al-Museiabpropolis

The result of TLC analysis (Table 5) indicate that the alcoholic and aquatic extracts of propolis contained four compounds for each extract when used the mobile phase Chloroform: acetic acid: ethyl acetate: ethanol. The relative rat (R_f) for alcoholic extract were I93, 86.6, 76.6, 63], while R_f for aquatic extract were [88, 84.6, 57.6, 42]. When compare these results with standard tables were found similar compounds for some flavonoids were kaempferal, quercetin, and quercetin 5-methylether [9]. The result of TLC analysis (Table 5) revealed that the phenolic compounds of propolis contained three compounds when mobile phase (N-butanol: acetic acid: water) used. R_f for these phenolic compounds were [95, 90, 28.5].The candidate compounds were ρ-coumaric acid, gallic acid, isoferulic acid or caffic acid. The result of TLC analysis (Table 5) revealed that the flavonoids compound of propolis contained one compounds when mobile phase Chloroform**:** acetic acid: water used. R_f for flavonoids compounds were [91.6]. The candidate compounds were kaempferal. On the other hand, the result of TLC analysis (Table 5) was revealed that the alkaloids compounds of propolis contained three compounds when mobile phase Methanol: ammonia used. R_f for alkaloids compounds were [96, 92.5, 14.8].
Regarding, the result of TLC analysis (Table 5) revealed that the tannin compounds of propolis contained two compounds when mobile phaseIsobutanol**:** acetic acid: water used. R_f for tannin compounds were [95, 40].

Moreover, the result of TLC analysis (Table 5) revealed that the Coumarins compounds of propolis contained four compounds when mobile phase N-butanol: acetic acid: water used. R_f for Coumarins compounds were [84.6, 50, 23, 15]. The candidate compounds were scopoletin and aesculin.

The result of TLC analysis in (Table 5) revealed that thesaponin compounds of propolis contained two compounds when mobile phase Chloroform: acetic acid: ethyl acetate: ethanol used. R_f for saponin compounds were [96, 92]. The result of TLC analysis (Table 5) revealed that the mucilage compounds of propolis contained eleven compounds when mobile phase Chloroform: acetic acid: ethyl acetate: ethanol used. R_f for mucilage compounds were [92, 88, 84.6, 76.9, 65, 50, 15, 79, 83, 81.5, 91], while, the result of TLC analysis (Table 6) revealed that the tritrepenoid compounds of propolis were contains three compounds when Mobile phase hexane: ethyl acetate used. R_f for tritrepenoid compounds were [92.8, 85.7, 71].The results of TLC analysis (Table 6) revealed that the diterpenoids compounds of propolis contained five compounds when mobile phase n- hexane-ethyl acetate (n- hexane- EtOAc) used. R_f for diterpenoids compounds were [96, 88, 80.7, 69, 61.5].

The result of TLC analysis (Table 6) revealed that the monoterpenoids compounds of propolis contained five compounds when mobile phase hexane: ammonia used. R_f formonoterpenoids compounds were [95, 90, 86, 81.8, 22.7].The result of TLC analysis (Table 6) revealed that the sesquterpenoid compounds of propolis contained nine compounds when mobile phase benzene-Chloroform (benzene-CHCls) used. R_f forsesquterpenoid compounds were [90, 20, 85, 75, 65, 60, 52, 42, 57]. These results of TLC were in agreement with other study that isolated many different compounds especially alcoholic, aquatic, phenolic and flavonoids compounds (16). From the results of TLC analysis, the related percentages of mucilage compounds of all active component in propolis were predominant, followed by sesquterpenoid and diterpenoids compounds whereas the lowest number of active component of propolis were flavonoids compounds.

Results also revealed that the mobile phase of Chloroform: acetic acid: ethyl acetate: ethanol [50:5:35:10] was the best in separation of alcoholic, aquatic, mucilage and saponin compounds of propolis. This difference may be due to differences in nature of compounds especially type, degree of polarity, and functional group of the solvent [30]. The Mobile phase of benzene-Chloroform (benzene-CHCls) and N-butanol: acetic acid: water was good mobile phases in separation of sesquterpenoid compounds, phenolic, andcoumarins compounds respectively.

Mobile phase n-hexane-ethyl acetate (17:3) and hexane: ammonia (1 : 1) was good mobile phases in separation ofditerpenoids compounds andmonoterpenoids compounds respectively. Mobile phase from Methanol: ammonia (200:3) was good mobile phases in separation of alkaloids compounds.

Mobile phase from Chloroform**:** acetic acid: water (5:45:50) and mobile phase Isobutanol**:** acetic acid: water was lower than other mobile phases in separation of flavonoids compound and tannin compounds respectively.

The isolated active compounds of propolis (detected by visible and UV light) were different in $R_f$values and different groups according to degree of polarity, function groups of each compound, and type of mobile phase. Large values of $R_f$indicated high dissolvability of compound in mobile phase, therefore the compounds readily move to up, whereas small $R_f$values indicated low dissolvability of compound in mobile phase. Therefore the compounds move slowly to up [31].

Most compounds were colored using visible light that is due to the phenol, flavonoids and tannins (yellow), alkaloids (yellow, brown and purple), coumarins (yellow, brown), saponin and mucilage (Brown, dark red, purple and gray),terpenoids (Brown, yellow, olive, and gray). Brown, yellow, and gray were due to carotenes and xanthen. Purple and red colors were due to enthocyanin. Olive color due to chlorophyll. Chlorophyll could not be removed from the original substance because the solvents for chlorophyll such as alcohol, petroleum ether dissolve large quantities of mixture [32].

Table 1. Phytochemical analysis of propolis

Constituents of propolis	Al-MuseiabPropolis
Alkaloids	+*
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+
Coumarins	+
Resins	+
Terpenoids	+
Volatile oils	+
Glycosides	-**
Steroids	-
pH	5.15

*: present, **: absent

Table 2. Types of amino acids in propolis from Al-Museiab district

Test	Result	Color	Types of amino acids
Ninhydrin	+	Yellow	Proline and hydroxyl proline
Xanthoproteic	+	Yellow	Tyrosin and tryptophan
Sakaguchi	+	Red	Arginine
Test for sulphur containing amino acid	+	Black	Cysteine and cystine
Molisch's test for carbohydrate group	+	Purple	Glycoprotiens
Test for organic phsphrus(casein)	-	-	Have not casein

Table 3. Free fatty acids of Al-Museiabpropolis detected by HPLC

No.	Free fatty acids	%	No. of carbon atoms	No. and type of bond
1	mgristic acid	7.35	C 14=0	One bond
2	Palmtic acid	17.84	C 16=0	One bond
3	Olic acid	12.08	C 18=1	One double bond
4	Linoleic acid	6.27	C 18=2	Two double bond
5	Linolinic acid	3.16	C 18=3	three double bond
6	Arachidic acid	4.02	C 20=0	One bond

Table 4. Quantities of mineral elements in Al-Museiabpropolis

No.	Mineral element	Quantity (mg\ml)
1	Fe	*0.00130
2	Zn	* 0.00114
3	Mg	*0.00123
4	Ni	*0.00018

5	Co	*0.00026
6	Cu	*0.00107
7	Ca	**0.00121
8	Na	**0.00162
9	K	**0.0025

Atomic Absorption Spectroscopy* **Flame photometer

Table 5.R_f values (×100) and colors of extract of propolis

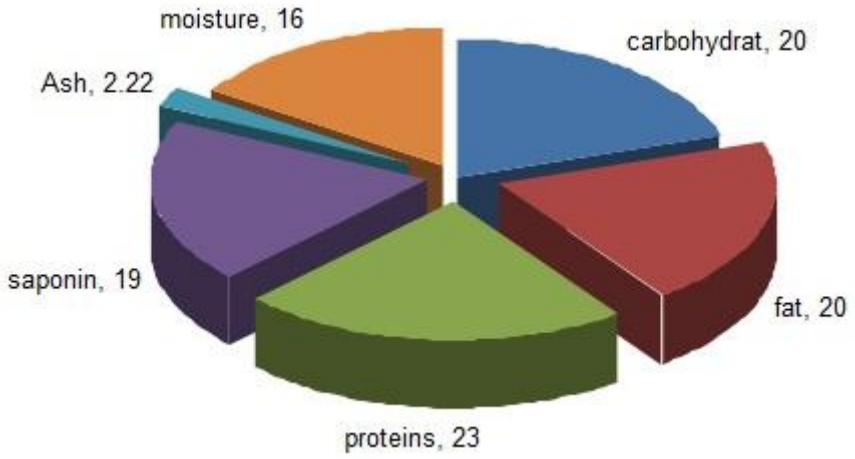
Type of extracts	Compound	R_F	Color	
			visible	UV
alcoholic extract of propolis	1	93	Brown	Brown
	2	86.6	Brown	Olive
	3	76.6	Blue	Purple
	4	63	Yellow	Brown
aquatic extract of propolis	1	88	Yellow	Brown
	2	84.6	Brown	Olive
	3	57.6	Yellow	Purple
	4	42	Yellow	Purple
phenolic extract of propolis	1	95	Yellow	Yellow
	2	90	Yellow	Yellow
	3	28.5	Yellow	Yellow
flavonoids extract of propolis	1	91.6	Yellow	Yellow
alkaloids extract of propolis	1	96	Yellow	Brown
	2	92.5	Brown	Purple
	3	14.8	Yellow	Olive
tannin extract of propolis	1	95	Yellow	Yellow
	2	40	Invisible	White
Comarins extract of propolis	1	84.6	Brown	Brown
	2	50	Yellow	Yellow
	3	23	Brown	Brown
	4	15	Yellow	Yellow
saponin extract of propolis	1	96	Brown	Dark red
	2	92	Dark red	Purple
mucilage extract of propolis	1	92	Brown	Dark red
	2	88	Dark red	Red
	3	84.6	Brown	Purple
	4	76.9	Purple	Purple
	5	65	Gray	Purple
	6	50	Purple	Purple
	7	15	Brown	Brown
	8	79	Purple	Purple
	9	83	Brown	Brown
	10	81.5	Olive	Olive
	11	91	Pink	Pink

Table 6. R_f values (×100) and colors of trepenoid extract of propolis

Type of extract	Compound	R_F	Color	
			visible	UV
tritrepenoid extract of propolis	1	92.8	Yellow	Purple
	2	85.7	Olive	Dark red
	3	71	Yellow	Brown
ditrepenoid extract of propolis	1	96	Yellow	Olive
	2	88	Yellow	Purple
	3	80.7	Gray	Dark Purple

	4	69	Olive	Dark red
	5	61.5	Yellow	Brown
monotrepenoid extract of propolis	1	95	Brown	Dark brown
	2	90	Brown	Red
	3	86	Yellow	Brown
	4	81.8	Brown	Olive
	5	22.7	invisible	White
sesquterpenoid of propolis	1	90	Yellow	Brown
	2	20	Brown	Brown
	3	85	Yellow	Brown
	4	75	Yellow	Pink
	5	65	Yellow	Dark red
	6	60	invisible	White
	7	52	Olive	Brown
	8	42	Gray	Green
	9	57	Pink	Dark red

Fig 1. Major component of Al-Museiabpropolis



CONCLUSIONS

Thus it was concluded that Al-Museiabpropolis have active components as a major constituents and Al-Museiabpropolis were rich with proteins, carbohydrates, fat, and saponin, but they have trace ash. It has fatty acid and mineral elements.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Simone-Finstrom M and Spivak M. Propolis and bee health: the natural history and significance of resin use by honey bees. *BehavEcolSociobiol*, 64, 2010, 1-609-1617.
2. Park YK, Alencar SM, Aguiar CL. Botanical Origin and Chemical Composition of Brazilian Propolis. *J. Agric. Food Chem*, 50, 2005, 2502-2506.
3. Trusheva B, Popova M, Bankova V, Simova S, Marcucci MC, Miorin PL, Pasin FR, Tsvetkova I. Bioactive constituents of Brazilian red propolis. *Evid-Based Compl. Alt*., 3, 2006, 294-254.
4. Yaghoubi, SM, Ghorbani GR, Soleimanian, ZS and Satari R. Antimicrobial activity of Iranian propolis and its chemical composition. *DARU*, 15, 2007, 45-48.

5. Kosalec I, Bakmaz M, Pepeejnjak S, Knezevi SV. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *ActaPharmaceut*, 54, 2004, 65-72.
6. Bankova VS, Castro DSL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. *Apidologie*, 31, 2000, 3-15.
7. Shihata IM. Apharmcological study of (Analagisarvensis). AID,Vct.thesisCario university, 1951.
8. Jawad AA. Ethological studies and assessing the anti-aggressive effects of some Iraq medical plants in laboratory mice. Ph.D. Thesis, College of Education. Univ. of Basrah, 1996.
9. Harbone JB. Phytochemical methods. Aguide to modern techniques of plants analysis. London, New York, Chapman and Hall, 1984.
10. Jaffer HJ, Mahmod MJ, Jawad AM, Najis A and AL-Naib A. Phytochemical and Fitoterapia, LIX, 229, 1983.
11. Al-Khazraji SM. Biopharmacological study of Artmisiaherbaalba. M.Sc. thesis, College of Pharmacy, Baghdad Univ, 1991.
12. AL-Maisary M. Effect of oil and alcoholic extract of Azdirachtaindica on some pathogenic, fungi of plant M.Sc. Thesis .Science College AL-Mustansria University, 1999.
13. Muhsa SM. Physiological and histological effects of Origanumvulgare L extract (aqueous and alcoholic) on the spp. reproductive system in male mice treated with methotrexate. Degree of Doctor of philosophy of Science in Biology College, University of Baghdad, 2010.
14. Saadalla RA. Biochemistry practical manual. Basrah University Press, Basrah, Iraq, 1980.
15. Anchary VP and Mohanty PK. A complete work book on clinical biochemistry. first edition. Taypeebrothers medical publishers (P) LTD. India, 2010.
16. Al-Ammar MHM. Effects activity of propolis components on some spp.of pathogenic bacteria.M.sc. Thesis. University of Kufa, 2001.
17. Al-dellaly BK and Al-hekeem SH, Food analysis. Al-mosul university Republic of Iraqi Ministry of Higher Education, 1987.
18. Wisconsin Drumm. Variability in the Saccharide, Protein, phenolic acid and Saponin contents of four market classes of edible dry bean s. *J.Sci. Food Agric*, 51, 1990, 285-297.
19. Al-Kaisey MT. Some Chemical and Nutrianal Properties of Soybean Seeds, Basrah. *J.Agric -Res,Scl*, 1992, 21-28.
20. Greenaway W, Scaysbrook T and Whatley JFR. The composition and plant origins of propolis: a report of work at Oxford. *Bee World*, 71, 1990 b, 107-118.
21. Greenaway W, May J, Scaysbrook T and Whatley FR. Identification by gas chromatography - mass spectrometry of 150 compounds in proplis. *Zeitschrift fur Naturforschung*, 46, 1990a, 111-121.
22. Bankova V, Dyulgerov A, Popov S, Evstatieva, L, Kuleva L, Purb O, and Zamjansan Z. Propolis produced in Bulgaria and Mongolia: Phenolic compounds and plant origin. *Apidologie*, 23, 1992, 79-85.
23. Sariözlu NY and Kivac M. Isolation of gallic acid-producing microorganisms and their use in the production of gallic acid from gall nuts and sumac. *African J. Biotechnol*., 8(6), 2009, 1110-1115.
24. Kosar M, Bozan B, Temelli F and Baser K. The dose and time of the treatment. *J. Biochem. Mol. Biol.,* 34, 2007, 250-258.
25. Clydesdale FA. Food, Nutrition and Health.AVI Publishing -Companyjnc; Westport Connecticut, 1985.
26. Aurand LW, Woods AE and Wells MR. Food Composition and analysis AVI Book Van Nostr and Reinhold Concrete, New York, 1987.
27. WilsonCC, Gisvold O, and Doerge RF. Textbook of organic medicinal and pharmacutical chemistry, L .B. Lippn.Co.H.company, 1971.
28. Melliou E and Chinou I. Chemical analysis and anti microbial activity of Greek propolis. *Planta Med*, 70, 2004, 515-519.
29. Lima B, Tapia A, Luna L and Fabani M. Main flavonoids, DPPH activity, and metal content allow determination of the geographical origin of propolis from the province of san Juan (Argentina). *J. Agric. food Chem*, 57, 2009, 2691-2698.
30. Grange JM and Davey RW. Antibacterial properties of propolis (bee glue). *J. Roy. Soc. Medicine*, 83(3), 1990, 159-160.
31. Keroynz N and Anthrykin J. Identification organic compounds. Translated by Yasinshandallah and Nazar AL-Jubory.AL-MouselUniv, 1986.
32. Roth L and Rupp G. Roth collection of natural products. Concise descriptions and spectra.VerlagsgesellschaftmbH, Germany, 1995, 512.