

Biological Activity of Anthraquinone Compounds of the Alcoholic Extract Propolis

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Abstract

Different products from a unique Propolis extract, Propolis (bee glue), an of resinous consistency produced by bees, has been used as indigenous medicine for the treatment of several diseases in some contrary. Safety assessment of Propolis extract with respect to antimicrobial activity against three bacterial isolates, *Proteus Mirabilis*, *Klebsiella Pneumonia* and *Staphylococcus Qureus*. The result showed that the main composition of the extract was Anthraquinone, the last one was responsible for biological activity of the extract. FT-IR, UV results showed more than eight peaks for represent the main fine composition of the extract. The present study also showed that the extract has antibacterial activity against three bacterial isolates

Key words: Propolis, Soxhlet, Anthraquinone, antibacterial activity, *Proteus Mirabilis*, *Klebsiella Pneumonia*, *Staphylococcus Qureus*.

Introduction

Propolis(bee glue) is known as complex sticky resin that honeybees[1] collect from plant blooms and then treat it with salivary secretions rich in enzymes to form a compound called Propolis[2,3].It is used by bees as an insect repellent and lays the cell from the inside to preserve it and the eggs from insects and diseases. This substance resists the action of some types of bacteria and fungi responsible for the process of decomposition and decomposition through its biological activity [4], Propolis has a distinctive and strong smell because it contains volatile oils, either in terms of consistency and is variable according to temperature, it is solid and easy to break at a temperature (10 ° C) and then softens and becomes adhesive At a temperature of (25-45 °C) and at a temperature of (70-60° C), its strength becomes viscous liquid [5,6,7]. Propolis is a substance soluble in water and organic solvents such as alcohol and ether. The chemical analyzes of Propolis and its components contain phenols, esters, acids, fats, etc. It also contains some mineral elements other than that and it has effectiveness against bacteria, fungi, and viruses [8,9,10]. The phenolic acids and gallic acid are the compounds found in higher concentration. However, other Gallic acid derivatives as hydrolysable tannins are present in higher concentrations as compared with other compounds [11,12,13]. Flavonoids as Kaempferol, quercetin are also found in Propolis. Concentrations up to 20% of essential oil can be found in the Propolis [14,15],many reports confirmed the antibacterial, antifungal, antiviral and ant carcinogenic properties of this Propolis [16,17]. Therefore, the purpose of this study was to investigate the chemical composition the extract of Propolis solution by identification of active group of Propolis extract, (FT-IR) and UV- analysis.

Materials and methods

1- Preparation of Propolis ethanol extract

The followed method [18] in the work of extracting Propolis, it's called continuous extraction, where grams of Propolis was placed in a thimble and put in Soxhlet the extracted was done by EtOH for 12 hours. The goal of this is to separate the fat from the extract and then filter the extract from the fat and stored for tests.

2- Isolation of Propolis compounds [19]

TLC technology was used to separate [5] Propolis compounds and use silica gel sheets (20×20 cm and 2 mm thickness [Merck company] , mobile phase (BEA) Butanol –Ethanol-Acetic acid and the ratio (5: 4: 1) was used . After Confirm the position of the compounds as in Figure 1, and determine the relative flow rate $R_f=2$

3- Identification of active group of Propolis extract

Active groups were revealed by TLC technology, The separated Propolis spots were scraped, collected, and dissolved in ethanol. The chemical effective groups were identified in inorganic chemistry laboratory in the Chemistry Department - College of Science - Kufa and the following was [20, 21].

-Sodium Fusible: To small piece of sodium were added drops of Propolis extract in a clean and dried test tube because the sodium is very effective with water, then that heated to be red. 5 drops of ethenol were added and cooled mixture, then heated again to clean sodium. 4-5 mL of distilled water was added to cooled mixture after that boiled again and filtrated. The Propolis Extract Sodium Fusible filtrated solution (PESF_{fs}) was used for the following experiment:

-Nitrogen testing: 2 mL (PESF_{fs}) was put in a test tube containing 0.1-0.2 gm of FeSO₄. The mixture was boiled to convert the Fe II to Fe III then, diluted H₂SO₄ was added to produce the blue complex.

-Sulfur testing: The few of PSF_{fs} with CH₃COOH and mixed with few drops of Pb(CH₃COO)₂ to produce black precipitate of PbS.

-Halogens testing: Few drops of HNO₃ with 3 ml of the (PESF_{fs}) then mixed with AgNO₃ to produce yellow precipitate.

-Phenol testing: Few mL of (PESF_{fs}) was mixed with few mL of FeCl₃ solution to produce purple solution.

- Aldehydes and Ketones testing: Few mL of the (PESF_{fs}) was mixed with 3 drops of Brady's reagent the black precipitate indication of positive results of presence of carbonyl group. Also mixed the same amount of volume of (PESF_{fs}) with 3 drops of Fehling's reagent then boiled the mixture for 5 minutes, red precipitate hint of aroma aldehydes. The testing of ketones was done by mixing 1 ml of the (PESF_{fs}) with few drops of sodium nitroprusside to produce red solution [19].

- Anthraquinones testing: Few drops of the (PESF_{fs}) were mixed with 2 mL of diluted NaOH, the color of solution should be changed from brown to black because the oxidation effect in case of positive result [22].

4-FT-IR Spectroscopy and UV-Spectrum Analysis

FT-IR (FT-IR-Prestige 21-Shimadzu Spectrophotometer) and UV- spectrum (shimadzu visible 165-UV) analysis were done the biochemical laboratory at the Faculty of Pharmacy, University of Kufa, Najaf, Iraq.

5-Study the anti-bacterial activity

Three types of Isolates of bacteria (Proteus Mirabilis, Klebsiella Pneumonia and Staphylococcus Qureus) were selected to the antibacterial activity .Propolis extract was studied in the laboratories of the Department of Biology - College of Science - University of Kufa ,depending on the following process:

-Preparing the agricultural medium: The media was prepared by Muller_ Hinton Agar according to method [23].

- Anti-bacterial efficacy testing: The diffusion method was followed by using the disc diffusion method, as the paper discs were prepared using a (Whatman no .1) filter paper. Filter papers were cut to circles disc (0.5 cm Di) then sterile by autoclave device at a temperature of 121 °C and pressed 15 pounds / 2 inches for 15 minutes .The disc of papers were dried and submerged in different concentration of the Propolis extract (1, 2, 3,V/V%). After 24 hour, the papers were dried by electric oven at temperature 37 °C for 30 min. Each bacterial isolate was spread onto Muller Hinton agar and left upright for 2 h. The prepared papers were put onto plates and incubated at 37 °C for 18-24h. The results were observed by clear zone around the discs [24].

Results and discussion

Extraction, separation and purification of compounds of alcoholic extract Propolis (Bee glue)

In this study, the extraction process was worked by the Soxhlet device with ethanol, the method was eliminated[18]. After filtrated the extract was without oil fats. separation process TLC Chromatography technique was used . One spot was observed then it was clear then the chemical components were extracted by dissolved using the ethanol. [25] Alcoholic extraction of Propolis was used in further experiments .

Identification of alcoholic extract of Propolis (Bee glue)

The identification tests groups of the extract has given good and important results, where the testing of the sulfur, nitrogen and halide groups gave a negative results , this is concordant with 1. However, testing of phenols, aldehydes and ketones has given a positive results , Anthraquinones new testing is a good because of all researcher were didn't used this test (anthraquinones) in biological activity [26], it was a positive revelation as the solution became black due to the acute oxidation process [27,28,29]

FT-IR analysis

Figure 1 showed FT-IR analysis figure ,there is a evident and high potent band (3200-3404/cm) which indicate to OH groups of phenol extract. Another band (1600-1650/cm indicated to ester group C-O or aromatic aldehyde and ketone groups C=O which bunch then ring. The band (1643/cm) which is also the manners match the frequency of the aromatic carbonyl group pertinence to quinine [30,31]. The bands at 1410 /cm illustrated the frequency of manner of aromatic group C=C, and other strong band (908 or 979 /cm) which illustrated frequency of manner of group C-O. Another moderate bands at (651 /cm) illustrated the frequency of manner of groups OH [32].

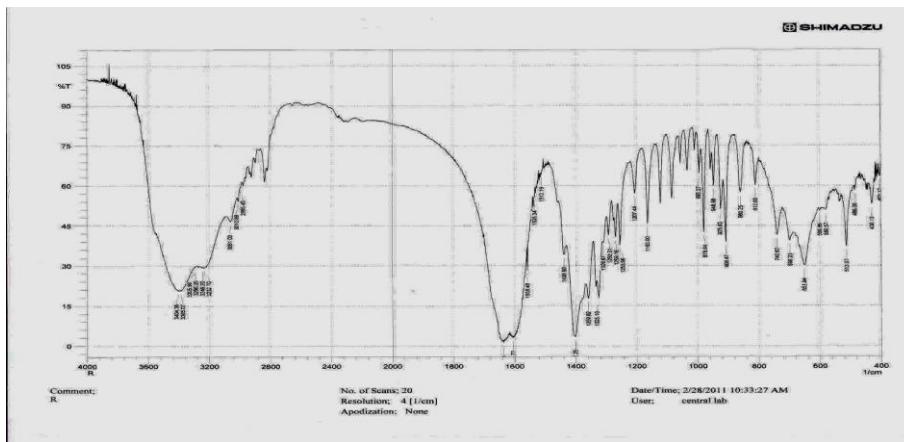


Fig.1 FT-IR analysis of alcoholic extract of PropoliS

UV- Spectrum Analysis

The results showed of UV- analysis in figure 2 for extract of Propolis. The absorption at (219nm) is due to the electronic transfer of type $n-\pi^*$ to the phenol group associated with the aromatic ring and absorption from (281 nm) due to the electronic transmission of type $n-\pi^*$ of carbonyl bind with the aromatic ring of the Quinone and absorption of (342 nm) is due to the $\pi-\pi^*$ electronic transmission of the ketone group bind with the aromatic ring [33,34].

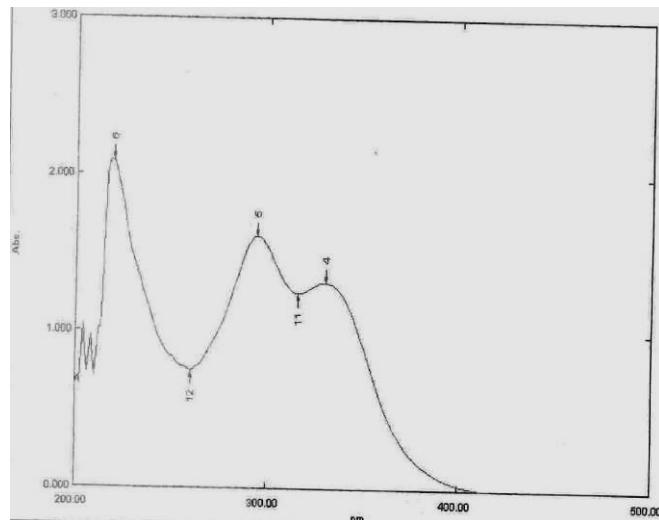


Fig.2 Uv- Spectrum analysis of Propolis extract .

Antibacterial activity of alcoholic extract Propolis

The results of antibacterial activity of alcoholic extract of Propolis showed by Table 1 against three bacterial isolates, Concentrations of alcoholic extracts were effective in reducing of inhibition present , because of Quinone carbonyl groups were very active of process ,why? because of atoms oxygen that the presence of the duple of electronic that react with DNA of Bacteria host, which lead of inhibits the action of effective proteins for bacteria[34,35], also the ketones group has the same work .

Table.1 Inhibition zone that produced by extract of Propolis

Bacteria	Cons.% of alcoholic extract Propolis V/V			Control
	1	2	3	
K.pneumonia	+	+++	+++++	0
ST.areus	++	++++	+++++	0
P. mirabilis	+	+++	+++++	0

+ = 0-6mm, ++ = 6-11mm, +++ = 11-17mm, +++++ = 17-25mm

Conclusion

This study demonstrated that propolis contains the Quinone group, which inhibited the effectiveness of the bacteria selected for a study with chemical compound groups, where the vital activity of three bacteria was measured after extracting, separating and diagnosing some effective chemical compounds using the alcoholic extract of propolis,

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