

## دراسة فاعلية خلاصة العكبر في تثبيط النمو الجرثومي وتأثيره على بعض مضادات الاكسدة في دم الارانب البيض

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### الخلاصة

هدفت هذه الدراسة الى معرفة فاعلية الخلاصة الايثانولية للبروبولس (العكبر) في تثبيط نمو بعض الاحياء المجهرية الممرضة التي تتضمن :- البكتريا موجبه الغرام , والبكتريا سالبه الغرام *E. coli* , *Pseudomonas aeruginosa* . بلغت نسبة الخلاصة الايثانولية للبروبولس 1:3 عكبر:ايثانول. وكان قوامه لزجاً بلون أخضر داكن وله رائحة مميزة كما أظهرت نتائج الكشف الكيميائي التمهيدي الذي أجري في هذه الدراسة إحتواء مادة البروبولس على الفلافونويدات والرواتين والتربينات والفينولات وخلوها من القلوانيات والجليكوزيدات والكومارينات . تم تحضير (10) تراكيز متدرجة من الخلاصة الايثانولية للبروبولس وفحصت فاعليتها ضد نمو الاحياء المجهرية بطريقة الانتشار بالحفر فكانت جرثومة العنقودييه الذهبية الأكثر حساسية لتراكيز الخلاصة المختلفة تلتها الجرثومه التودية . فيما كانت جرثومتي الاشريكية القولونية والزائفة الزنجارية الأقل تأثراً هذه النتائج أكدت أن للمستخلص الايثانولي للبروبولس تأثيراً مضاداً لنمو الجراثيم وخصوصاً الموجبة غرام وأقل تأثيراً في نمو الجراثيم السالبة غرام . كما تناولت الدراسة معرفة التغيرات في بعض مضادات الاكسدة لمعرفة الاجهاد التاكسدي الناتج من نمو الجراثيم . استنتج من هذه الدراسة ان هنالك انخفاضاً في مستوى مضادات الاكسدة مما يدل على ان للجراثيم تأثيراً اجهادياً على الانسجة الحية ولخلاصة العكبر تأثيراً ايجابياً من خلال عمله كمضاد للاكسدة .

الكلمات المفتاحية : خلاصة العكبر , تثبيط النمو الجرثومي , مضادات الاكسدة , الارانب

## STUDYING THE EFFECTIVENESS OF PROPOLIS EXTRACT ON INHIBITING BACTERIAL GROWTH AND ITS EFFECT ON SOME ANTIOXIDANTS IN BLOOD OF WHITE RABBITS

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### ABSTRACT

This study aimed to investigate the antimicrobial effectiveness of ethanolic extract of propolis (EEP) in inhibiting the growth of some pathogenic microorganisms which include:- *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Corynebacterium*. Ethanol content of propolis was 1:3 propolis: ethanol. its constancy is sticky with a dark green color and has a distinctive smell. The results of the preliminary chemical analysis in this study revealed the propolis contain flavonoids, carbohydrates, trapezoides and phenols, and its absence from alkaloids, glycosides and comarins. (10) gradient concentrations of the ethanolic extract of Propolis were prepared and tested against the growth of microorganisms by diffusion method. The *Staphylococcus aureus* was the most sensitive to the concentrations of the different digestion followed by the *Corynebacterium*. While The *Escherichia coli* and *Pseudomonas aeruginosa* were less effective. These results showed that the ethanolic extract of Propolis had an antimicrobial effect, especially Gram positive, and less effective in the growth of Gram negative bacteria. The study also examined the changes in some antioxidants to determine the oxidative stress resulting from germ growth. The study concluded that there is a decrease in the level of antioxidants, which indicates that the bacteria have an effect on the living tissue and the extract of the propolis have positive effect due to its role as antioxidant.

**Keywords: Propolis, Inhibiting Bacterial Growth, Antioxidants, Rabbits**

### INTRODUCTION

The word propolis originates from Greek: «pro» = in front, «polis» = city. The meaning, in front of the city,, suits well the protecting role of propolis for the bee colony. The Greek word propolis

means also to glue and describes also the role of propolis to cement openings of the bee hive. Propolis was already known in ancient Egypt, where it was probably used as an adhesive. It was mentioned by the Greek philosopher Aristoteles (1). Propolis

is the odorous, sticky, resinous, pale-yellow to dark-brown material which bees use to strengthen and join the hive cells, and to seal their hives from penetration by water and cold. It is furthermore thought to provide protection to the bee hives from microbial infection. Propolis is made of a complex mixture of bees wax, together with small amounts of sugars and plant exudates collected by honey bees from buds of some trees notably conifers and aromatic plants. The chemical composition of propolis depends on the season of collection and on the vegetation of the area and this is reflected in variations of its color and odor (2). Characteristically, it is a lipophilic material, hard and brittle when cold but soft, pliable, and very sticky when warm, hence the name bee glue (3). The chemical composition of propolis was: Flavonoids, phenolic acids and esters in ratio(5-45%), Bees wax and plant origin (25-35%), Proteins(16 free amino acids >1%), arginine and praline together 46% of total (5%), trace minerals, iron and zinc most common: ketones, lactones, quinines, steroids, benzoic acid, vitamins, sugars (5%) (2).

Depending upon its composition, propolis may show powerful local antibiotic, antiviral, antifungal, anti-inflammatory,

antioxidant, local anesthetic, hepatoprotective, immunostimulating, and cytostatic properties. Many authors have demonstrated propolis antibacterial activity against Gram-positive bacteria and Gram negative bacteria . However, Propolis used for the treatment of various diseases such as, diabetes, heart disease and keratitis ( 3) .

Many bacterial spp causing wound infection which include: *Pseudomonas* , *Staphylococcus* , *Streptococcus*, *E.coli* (4).

The aims of this study were:

- Prepare of propolis ethanolic extract .
- Study of chemical composition of propolis extract .
- Study the effectiveness of propolis ethanolic extract on bacterial growth *in vitro*
- Study treatment activity of propolis extract in rabbit.
- Study the effects of propolis on some antioxidants.

## **MATERIALS AND METHODS**

- Preparation of Propolis Ethanolic Extract (PEE): 70% ethanol was used for extraction in ratio 1:3 propolis: ethanol and kept on shaker 150 rpm for 5 days. The extract were filtrated by using whatman (no.1), supernatant were

collected and evaporated at room temperature (25°C) for 3 days. The remainder resin were collected to use in subsequent test (4).

- Chemical analysis (Qualitative method) (5):

a- Test for Flavonoids : (5) ml of the ethanolic extract (50%) was taken in the test tube and potassium hydroxide solution (0.5%) was added. Appearance of yellow color indicates the positive test for flavonoids.

b- Test for Phenols (Ferric chloride test): (0.5) ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates the presence of the phenolic compounds.

c- Test for Steroids (Liebermann - Burchards test):(2) ml of acetic anhydride was added to 0.5 ml of the extract and then added (2) ml of conc. Sulphuric acid slowly along the sides of the test tube. Change of colour from violet to blue or green indicates the presence of steroids.

d- Test for Tannins: One ml of the extract was added with 5ml of distilled water and kept for boiling in

hot water bath. After boiling, the samples were cooled down and 0.1% ferric chloride solution was added to each sample. Appearance of brownish green or blue black coloration confirms the presence of tannins .

e- Test for Terpenoids (Salkowski test): Five ml of extract was taken in a test tube then (2) ml of chloroform was added to it followed by the addition of 3ml of conc. Sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

f- Test for Glycosides (benedict test):One ml of extract was added to 2 ml of benedict reagent, followed by the transferred to water bath for a few minutes then cooled solution. Formation of red precipitate confirms the presence of glycosides.

- Bacterial isolates : provide from lab microbiology/ Vet.Med.Tikrit university which include: (*Staph.aureus* , *C.pyogenes*, *P.aeruginosa* , *E.coli*, *Streptococcus*).

- Determined MIC and MBC :-

a. Prepared of propolis ointment: the propolis extracted was mixed with

vasaline in ratio 1:1 ( two ointment concentration , the first 0.02 Mac\MI we used for treatment of rabbit infected experimentally with *E.coli* and *Pseudomonas* and 2 Mac \ MI concentration was used in the rabbit infected experimentally with *Corynebacteriam* , *Streptococcus* and *Staphylococcus*

- b. Experimental study : We used in this study eighteen rabbits . divided into six groups , each group contain three rabbits and one group consider as a control group . Each studied group exposed to one bacterial Spp., Which used *in vitro* study .

#### - Antioxidants

- a. Superoxide Dismutase activity determination of it follows the method described by (6).

- b. Glutathione The determination of glutathione concentration follows the method described by (7).
- c. Catalase determination follows the method described by (8).
- d. Malondialdehyde measurement of it follows the method described by (9).

#### **STATISTICAL ANALYSIS**

The data were analyzed using the SPSS program for values representing the standard rate and error and analyzed the data using the ANOVA Analysis of variance one way. The differences between the groups were determined using the Duncan multiple range test. At a probability level ( $P \leq 0.05$ ).

#### **RESULTS**

Results of bacterial inhibition tests :- The concentration were integrated in  $10^5$  cell/ml and when it added on diluted tube ethanolic extraction , the results were :-

A- MIC : table (1) revealed that the MIC for E.coli and *Pseudomonas* were 0.02mac/ ml while the MIC for *Corynebacteriam*, *Streptococcus* and *Staphylococcus* were 2mag/ml

Dilution	Bacterial growth				
	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>C.pyogen</i>	<i>Steptococcus</i>	<i>Staph.aureus</i>
0.2 mg/ml	-	-	-	-	-
0.02 mg/ml	-	-	-	-	-
2 mac/ml	-	-	-	-	-
0.2 mac/ml	-	-	+	+	+
0.02 mac/ml	-	-	+	+	+
2 ng/ml	+	+	+	+	+

A- MBC: The table (2) shows that MBC of (*Staph.aureus* , *C.pyogenes*, *Srtreptococcus* ) were 0.2mg/ml and for *E.Coli* and *P.Aeruginosa* were 0.2mac/ml .

Dilution	Bacterial growth				
	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>C.pyogen</i>	<i>streptococcus</i>	<i>Staph.aureus</i>
0.2 mg/ml	-	-	-	-	-
0.02 mg/ml	-	-	-	-	-
2 mac/ml	-	-	+	+	+
0.2mac/ml	-	-	+	+	+
0.02mac/ml	+	+	+	+	+
2 ng/ml	+	+	+	+	+

**Table (3) comparative between antioxidants during treatment and after healing**

<b>Groups</b> <b>Parameters</b>	<b>During treatment</b> <b>Mean ± SD</b>	<b>After healing</b> <b>Mean ± SD</b>
<b>SOD U/ml</b>	<b>3.67 ± 0.93 b</b>	<b>6.87 ± 0.36 a</b>
<b>GSH μmol/L</b>	<b>5.23 ± 0.31 b</b>	<b>6.98 ± 0.12 a</b>
<b>CAT U/L</b>	<b>3.04 ± 0.37 b</b>	<b>6.25 ± 0.19 a</b>
<b>MDA μmol/L</b>	<b>2.08 ± 0.01 a</b>	<b>0.74 ± 0.02 b</b>

- Values represent the mean ± standard deviations

- Different characters in the same row mean significant difference at a significant level ( $P \leq 0.05$ )

## DISCUSSION

The incorrect curing methods was encouraged for cases of resistant germ to antibiotics in many disease, so that we must find alternative ways to get scaled this phenomenon. One of these methods is the use of bee products, which include honey, wax, royal jelly, venom, pollen, and propolis, and the last one was the most important in medicine, therapeutic side as it contains active chemical component like, flavonoids and antioxidant (10). The use of ethyl alcohol (70%) for extraction because it is a good solvent for most the active ingredients found in propolis as well as easy of evaporation compared to other solvents (2). Preliminary chemical results which done on propolis into out ignition conclusions indicate the presence of compounds (flavonoids) and that in

agreement with the sentiments of the researchers in their studies since have confirmed the presence of these compounds in a major (11). Flavonoids are one of the main chemical compounds aggregates in propolis and responsible for a large part of the vital process as antioxidants (12) and antimicrobial (13). The analysis also showed the presence of (resins) which agrees sentiments with (14). Also (15) Noted that the resins materials consist 50-70% from terpens, phenols, the result of chemical detection has shown its presence in the article has been reaction to the bacteria and fungi in collaboration with flavonoids material (16), it was chosen four types of germs. Gram positive (*Staph.aureus*, *C.pyogenes*) have been more sensitive to different concentrations extract. While Gram negative (*E.coli*,

*P.aeruginosa*) less affected ,and these results agree with past studies by researchers on propolis that have been collected from different regions despite the variation in the degree of this effect, which may be attributable to the class basis of existing plants variation within the geographical area in which the bees exist and which are visited and combines them resin article(15). These results confirmed that the ethanolic extract of propolis influence to counter the growth of germs, especially Gram positive and less influential in the growth of Gram negative bacteria (17). This can be explained that the isolated Gram negative bacteria has alipopolysaccharide in its outer wall as a self\_barrier which common with complex proteins have the ability to prevent many antioxidants passage into the germ cell, compared with the Gram positive bacteria through chemical study results that could be installed by which the presence of flavonoids materials, terpens and phenols have attributed the antibacterial action to local propolis. The presence of these compounds as many studies have confirmed that these compounds influence to counter the growth of germs, especially Gram positive bacteria (16). The activities variation of ethanolic extract of propolis

depend on types of microbes, and propolis concentration in the media (18), They reported that the zones of inhibition of *S.aureus* were 16 mm at 50% concentration of propolis collected from Al-Kufa, Iraq. (19) pointed out that the phenolic compound was causing protein denaturation of microbes through the inhibition of enzymatic action of metabolic reactions and destroy the microorganism. On the other hand, the results of agar diffusion methods of tannins extracts revealed that tannins had highest activity against most Gram negative and positive bacteria. The inhibitory zones ranged from 12-30 mm against the bacterial isolates. Tannins are toxic for bacteria, fungi and yeast due to combine with the microbial cell wall which inhibits the bacterial growth. The use of tannins showed a high activity against isolated fungi and bacteria by inhibiting their growth by stopping one or more of physiological processes of microorganisms (20). The researcher (21) referred that the ability of tannins in disturbing the growth or changes the morphology of certain microorganisms. It was suggested that the main action of this extract would be primarily on the cell wall of fungi (21). other study (22) found that the ethanolic extract was effect on nucleic



acid DNA and RNA and some bioactivities of bacterial enzymes. In terms of biochemical parameters The cells of the skin, similarly to the cells of the heart or another organ, contain very large numbers of mitochondria that produce energy during the respiration process (23). The main physiological function of mitochondria is production of ATP but it includes also the control of cell survival and death (24). The activation of the respiration and energy production of mitochondria processes strengthens the protective function of the skin, decreases transepidermal moisture loss, and improves skin regeneration (25). The results were agreed with (26) who reported that Antioxidants in many phytomedicines, such as propolis, scavenge free radicals which are responsible for the oxidative damage of lipids, proteins and nucleic acids. The regenerative properties offered by propolis are also attributed to its superoxide and free radical inhibiting properties. (3) reported that The total polyphenol and flavonoid content account for much of the antioxidant activities in the ethanolic extracts of propolis. (27) explained that the Flavonoids in propolis may inhibit lipid peroxidation as well as may influence the lipoxygenase and cyclo-

oxygenase pathways.(28) Caffeic acid phenyl ester (CAPE) also plays a role in anti-oxidant properties of propolis.(29) revealed that the antioxidant properties actions involve scavenging of reactive oxygen species, metal ion chelation and synergistic action with other antioxidant compounds. the normal physiology of wound healing depends on low levels of ROS. High level of ROS leads to impaired wound healing. Antioxidants enhance the healing of infected and non-infected wounds by reducing the damage that is caused by oxygen radicals (30), and some studies refers to role of propolis as antioxidant properties and various biological activities (31 ). (32 ) reported that the propolis extract diminished the lipid peroxidation and lowering the level of Malondialdehyde (MDA) and increase the level of antioxidants enzyme like (SOD, GSH, CAT ) and we noticed these results in our study. There are other researchers like (33) who explained that oxidative stress was decreased with a treatment of propolis extract. Propolis proved to be beneficial in decreasing the levels of MDA. Therefore, propolis extract provided protection against free radicals and lipid peroxidation measured as MDA. Also, the present results showed that the

treatment with propolis caused reduction in (MDA) level and increased the activities of SOD, CAT and the level of GSH in the serum. These results are in agreement with the results obtained by (34) who reported that propolis caused reduction in the malondialdehyde (MDA) level and increased the activities of the antioxidant enzymes (SOD, GSH and CAT) and the reason of this role of propolis may be due to the flavonoids in it which can increase the activities of the antioxidant enzymes and reduce the level of ROS.

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