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The Effect of Ethanolic of Propolis in Growth of Staphylococcus aureus and E.coli Isolated from Wounds Infections

By

Hassan Abdul Hussain Abdullah

Sajad Raid Abed

Hussain Kareem faraj

Supervised by

Asist .Dr .Miran Abdul Ameer Attieh Al-Ramahi

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بسم أللّه الرحمن الرحيم

((وقل رب زدني علما))

صدق أللّه
العلي
العظيم
سورة طه- آية (114)

SUPERVISOR CERTIFICATE

I certify that this research entitled (**The Effect of ethanolic of propolis in Growth of Staphylococcus and E.coli Isolated from Wounds Infections**), was prepared by **Hassan Abdul Hussain Abdullah , Hussain Kareem Faraj** and **Sajad Raid abed** under my supervision at the College of Biotechnology /University of Al-Qadisiyah as required for the degree of *Bachelor* of Science in *Medical Biotechnology Sciences*.

Miran Abdul Ameer Attieh Al-Ramahi.Dr. Assist

Apr / 2018

الإهداء

إلى نور الهدى

(محمد)

(صلى الله عليه وعلى أله وسلم)

إلى من قال بحقيهما تعالى

((وقضى ربك ألا تعبدوا إلا إياه وبالوالدين إحسانا))

والدي و والدتي

إلى سندي وأعواني

إخوتي وأخواتي

إلى كل من يسعده نجاحي

اهدي جهدي المتواضع

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God grants success

Hassan , Sajad and Hussain

Abstract

The objective of this study was to investigate the effectiveness of the effect of the alcohol extract of Propolis to determine its ability to inhibit the growth of some isolated air bacteria from wound suppression .

A sample of propolis was collected from one of the honey cells hives in Diwaniyah governorate and alcohol

The propolis is keep in the freezer for 2 days until the milling process is easy . The large particles of the propolis were grinded for a powder and put this powder in container and we added ethyl alcohol 70%.

And put for 7 days until the alcohol completely volatiles and then grind the propolis again and save in the container

We take 3 sizes of powder propolis is 25-50-100 and this material is culture on staphylococcus and E.coli and observe the results after two days form planting the propolis on dishes .

Where are find propolis inhibition growth staphylococcus and E.coli

Inhibition increases more where in the dish 100 mg/ml we see the largest diameter of inhibition .

Introduction and literature review

Drug resistance of pathogenic bacteria is life threatened (World Health Organization, 2014). Complications of nosocomial and community _acquired Infections, mainly in patients with weak immune system and patients with several diseases are due to the emergence of resistant microorganisms to commonly used antibiotics. Perhaps, even more important is the emergence of multi-resistant opportunistic infectious Agents (Klein and Laxminarayan2007). Among these opportunistic pathogens are the enterococci, the coagulase-negative staphylococci, methicillin-resistant Staphylococcus aurous and *Escherichia coli* (Cabrera et al2011), which may lead to serious and even fatal infections in otherwise healthy hosts.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prominent pathogens causing community and livestock-associated infections (Stefani et al 2012, Xiao et al 2013). They have been the subject of interest in the last two decades due to an increase in resistance to MRSA by most potent glycopeptide antibiotics (Fu and Yao 2013). MRSA has altered penicillin-binding proteins (PBPs) with reduced affinity to penicillin and other available-lactam antibiotics (Weese 2010).

Staphylococcal infections are of major importance in both human and veterinary medicine. *Staphylococcus aureus* is a major inhabitant of the human skin. It occasionally lives on domestic animals, although these are usually colonized by other species of staphylococci. Furthermore, it has been a frequent cause of subclinical mastitis in animals (Pantosti and Monaco 2007, Weese2010).The development of alternative antimicrobial methods has become one of the top priorities of medicine

And biotechnology, to combat these kinds of resistant organisms. In this regard, propolis has proved to be a plausible alternative for this purpose. Propolis is a bee product that contains phenolic substances including cinnamic acid derivatives and some flavonoids (Marcucci et al.2001, Borrelli et al.2002). Flavonoids and cinnamic acid derivatives have been considered the main biologically active components in propolis (Borrelli et al.2002). It has been extensively used in folk medicine and also, because of its antibacterial, antiseptic, anti-inflammatory and anesthetic activities, in alternative medicine(Krol et al.2013).Several studies have documented the biocidal functions of propolis, including antibacterial, antifungal, antiprotozoal, antiviral, anti-tumor, immune-modulation and anti-inflammatory activities(Mello et al. 2010,Al-Abbadi et al.2015). It has also been used as an alternative treatment for infections (Sanghani et al.2014).

The bactericidal action of propolis extracts have been shown to be effective mainly against yeasts and gram-positive bacteria such as Staphylococcus aureus and Streptococcus spp.(Krol et al.1993). Recent studies demonstrated antimicrobial activity of propolis against methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant clinical isolates (Astani et al.2013, Saddiqa and Abouwarda 2016). On the other hand, a minor action against gram-negative bacteria has been demonstrated in previous studies (Silici and Kutluca2005, Rahman et al.2010). Nonetheless, the chemical composition of propolis will depend on the tree bark and leaf buds taken by the bees (Apis mellifera) (Stepanovic et al.2003). The number of methicillin-resistant *Staphylococcus aureus* infections and other multi drug resistant infections is increasing, and treatment with antibiotics is problematic (Shelburne et al.2004, Rosenberg et al.2012).

In the current study, we have investigated for the first time the antimicrobial effect of 5 propolis extracts taken from different regions of Colombia against isolates of Staphylococcus spp .methicillin-resistant Propolis is a rubbery, sticky, brown, thermoplastic resin collected by honey-bees from tree buds. Honey bees use propolis in their hives as a universal means of repairing crevices, as a surface cover, hardener and preservative. It is probably also used as a repellent since it is applied inside the beehive and around its entrance. The term propolis originates from the Greek words promeaning before/in front and polis– meaning town (Jankovic, 1968) and denotes the fact that bees use propolis to con-struct the entrance to the beehive – "town". It is also known as "bee glue" Propolis has bactericidal and fungicidal properties and it is used as an alternative treatment for infections. The wide range of action of propolis on various microorganisms is the result of the combined activities of flavonoids and aromatic compounds. In principle, active components of a natural preparation can be separated by solvents in the process of extraction. Solvents of different polarity may be used as extractants. For this purpose polar (water, glycerol, methanol), less polar (ethanol, propyl alcohol, acetone, and others) and nonpolar (dichloromethane, chloroform, carbon tetrachloride, diethyl ether, benzol) solvents are used. Today, ethanol is generally the solvent used in the process of extraction of propolis and all published data show the effects of propolis extracts dissolved in ethanol. Sin-ce ethanol belongs to the group of less polar sol-vents, when used as an extractant only less polar active substances are extracted, while flavonoids are extracted in minimal quantities. Using nonpolar solvents, nonpolar flavonoids can better be extracted from the basic substrate, leading to an increase of their concentration in the final preparation. Pharmacologically active constituents of pro-polis have been discovered in fractions that are soluble in solvents such as ethanol.

Several large classes of active substances have been identified in a variety of constituents of propolis. Among these are: flavones, flavonoles and flavanones (known under the collective name of flavonoids), various phenols and aromatics. Flavonoids represent a large group of natural pigments and active compounds of herbal origin. All flavonoids have two benzene rings linked by a triple bond. The most significant phenols are: cinnamyl alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid and caffeic and ferulic acids (which are derivatives of cinnamon acid with an extra hydroxy and/or methyloxy group). Propolis also contains rare compounds such as phenol triglycerides (Popravko et al., 1983), pterostilbene and eugenol and phenethyl ester of caffeic acid. The principal pharmacological constituents of propolis are natural antibiotics – flavonoids which exert most of the healing properties of propolis. The anti-microbial activity of propolis has been well investigated (Ghisalberti,1979; Grange and Davey,1990). Flavon pinocembrin affects numerous bacte-ria, fungi and molds and it is, together with galangin, 3-acetyl pinobanksin and caffeic and ferulic acid, probably responsible for many of the biological activities of propolis (Hegazi and Abd El Ha-dy, 2001;Hegazi and Abd El Hady, 2002a;Hegazi and Abd El Hady, 2002b; Popova et al.,2004). During the physiological processes in an organism the mechanism of homeostasis maintains the pH value of the internal environment around the neutral level. However, during pathophysiological processes, the chemical reactions that take place decrease the level of pH, making the affected environment slightly acidic. The principal hypothesis of this study was that the bactericidal and antimicrobial effects of propolis vary depending on: the type of solvent used during the extraction, bacterial species and acidity of the environment.

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Aim

The study aim to determine which preparation of propolis has the most antimicrobial activity which thereby could improve the way propolis applications.

The bactericidal effect of propolis extracted by solvents (ethanol) aged for 2 days on cultures of 2 different species of bacteria {Escherichia coli, Staphylococcus aureus}.

Materials and Methods

1- Extraction of Propolis

(100 g) of raw propolis sample was cut into small pieces, then it was grounded and macerated in 300 mL of 95% ethanol (w/v) at room temperature for 3 days. The suspension was filtered to remove rough particles under What man filter paper No.1. The previous steps were repeated three times as figure 1(a,b,c,d,e) .The filtrate was evaporated to dry under reduced pressure using a rotary evaporator to remove the solvent and obtain the Ethanol Extract of Propolis (EEP).The dry extracts were then weighted to calculae the yields of extracts before they were kept in the refrigerator at 10 ^{o}C for future antibacterial activity and chemical determination experiments.

Images show the method of collecting samples and how they work

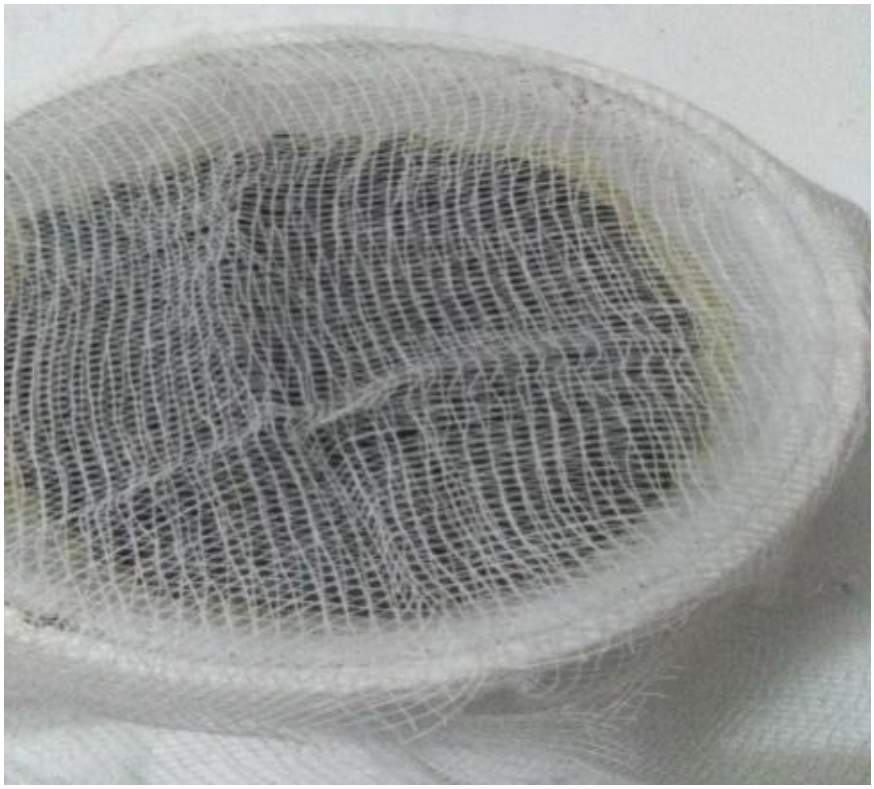
A

B

C

D

E



2-Antibacterial activity

Bacterial cultures

Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria were tested for the antibacterial activity of EEP. Both bacteria were obtained from the Department of zoonotic unit veterinary medicine .Glycerol stocks of S. aureus and E. coli were streaked on nutrient agar. All cultures were incubated aerobically at 37 °C for 24 then, 5 mL of nutrient broth was inoculated with a randomly selected single colony of each bacterial isolate. The bacterial suspensions were then incubated aerobically, 37 °C for 12 h. Each bacterial suspension was adjusted with fresh medium to obtain a 0.5 McFarland standard turbidity using a spectrophotometer (Gene Quant 1300) at 625 nm. For each bacterial culture, The agar well diffusion was prepared by adding 0.1 ml of 1×10^{8}cfu from each bacteria in to the mauler Hinton agar in polemic flask and homogenized slowly for a few seconds . The mixture was then poured in to a Petri dish and allowed to solidify prior to the preparation of 6 mm diameter wells made by using a sterilized pasture pipette after that 0.1ml of propels ethanoic extract solution in three different concentrations (25,50,100 mg/ml) were transferred in to each well allowed to set , while use ethanol solution (25%)as a negative control for antimicrobial activity. All the plates were incubated at 37° c and the

diameter of inhibition zone surrounding each well were calculated by mm

The MIC of propolis against Staphylococcus isolates and E.coli was determined by the tube dilution method according to the procedures recommended by the National Committee for Clinical Laboratory Standards(C.L.S.I.2013).

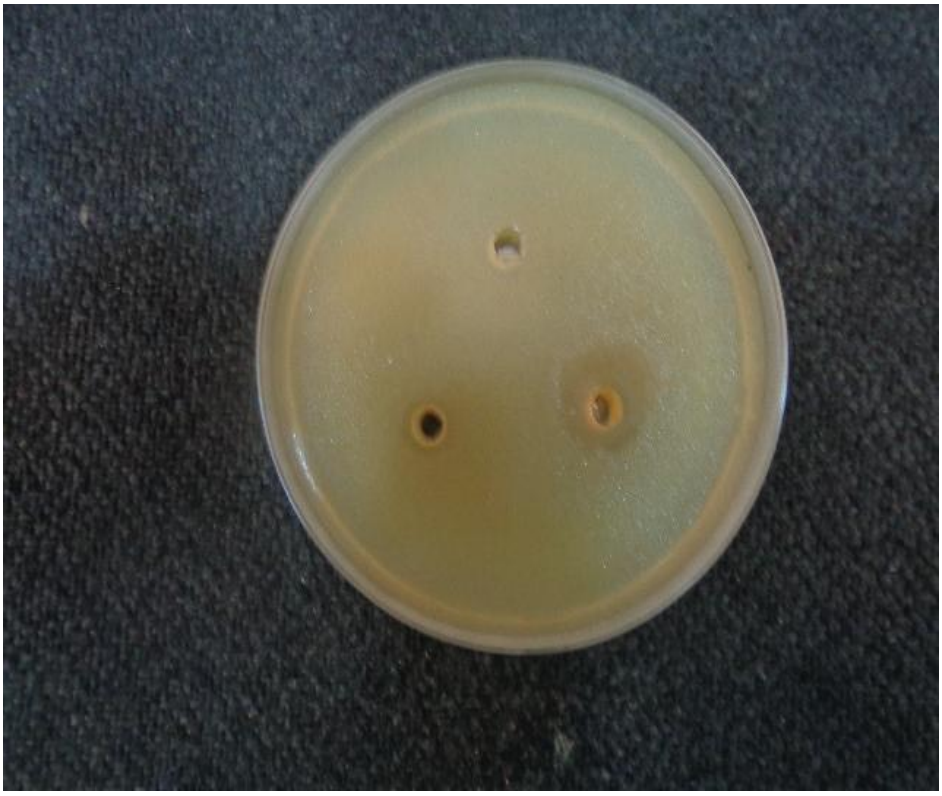
Results

The finding revealed that the inhibitory effect of all concentrations propolis' extract on *S. aureus* and E coli growth was distinct, EEP has a statistically significant ($p<0.001$), inhibitory effect ,but greater inhibitory effect was in cultures with concentrations 100 as in table1 and figure 2,3

Table (1):The effect of propolis extract in three different concentrations against growth of some pathogenic bacteria .

propolis concentration (mg/ml)	zone of inhibition measure in (mm)	
	S. aureus	E.coli
25	7± 0.88	9.12± 0.18
50	11.33± 0.70	10.66± 0.28
100	17.33± 0.95	16.14±1.08

❖ Values for 6 isolates for each bacteria with SE.



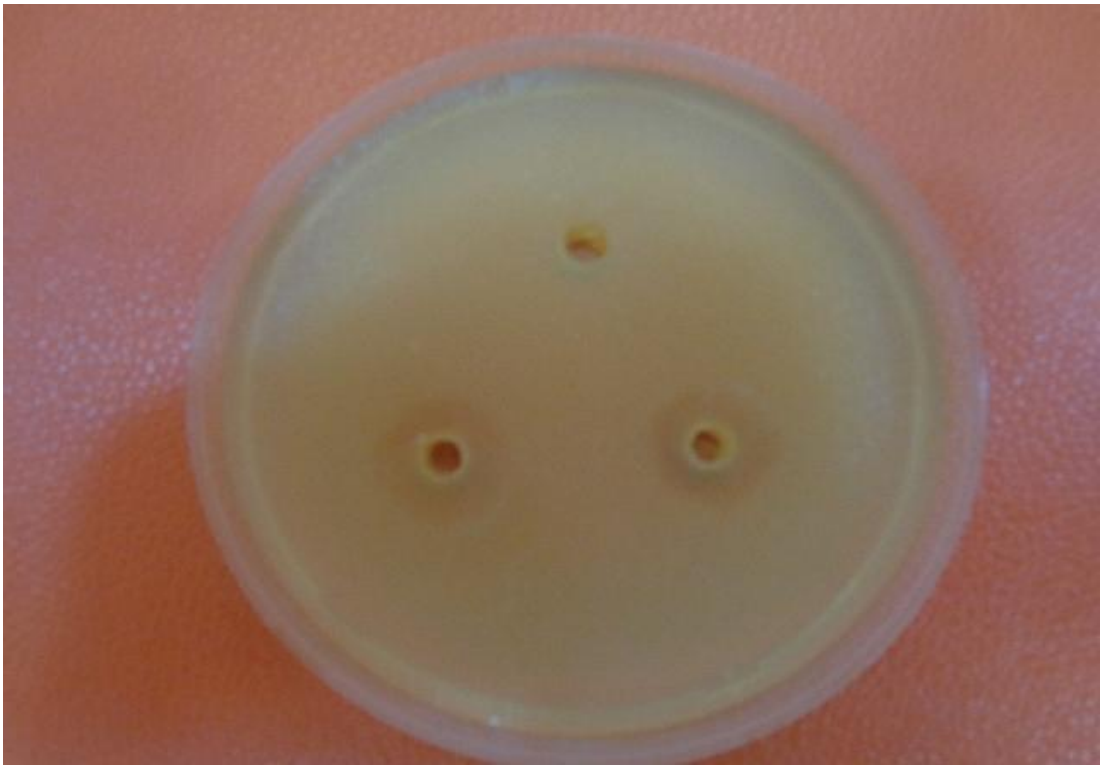


Figure 3 Inhibition Zone *E coli* Against Propolis with ethanol in Different Concentration of Propolis from 25 ,50,100%

The presented study aimed at the investigation of antimicrobial properties of Iraq propolis. The different concentrations (25, 50, and 100%) on the growth of bacteria was also determined. Effective inhibition of the growth of the selected Gram negative and Gram positive strains was obtained, particularly after the incubation with ethanolic propolis.

DISCUSSION

Antibacterial activity

The results of the inhibitory effect of ethanolic extract of
propolis on *S. aureus* and *E. coli* were shown in Table 1. All ethanolic extracts of propolis at 25% concentration were unable to
induce clear zone on both bacteria. When the concentration of EEP samples was increased, all tested propolis demonstrated only a weak activity on both (positive control) was the highest on both *S. aureus* and E.coli

Recommendations

1- Encouraging bee keeping throughout the country and confirmation the need to benefit from secondary products for bees from medical purposes .

2- Conduct further studies to isolate the active medical substances in the propolis and study their effect on the growth on inhibition of other types of pathogenic bacteria even in vivo .

3- Study on the mixing of propolis with the black bean should be done on other microorganisms such as fungi .

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