Antibacterial activity of Syrian Propolis extract against several strains of bacteria in vitro

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Abstract:

We aimed in this study to evaluate the effectiveness of Syrian propolis on some bacterial strains isolated from Al-Assad hospital in Latakia, to estimate its antimicrobial activity. Thus, we studied two propolis samples from two regions in the Syrian coast, and prepared ethanolic extracts of propolis (EEP) with different concentrations (0.5%, 1%, 5%, 10%, and 20%). Then we tested these extracts on four strains of bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*), using pore method on Muller Hinton Agar. As we observed, the bacteria showed diverse susceptibility to the extracts and the most susceptible bacterium was *Staphylococcus aureus* which was sensitive to all concentrations and especially for EEP 20%. However, the least affected bacteria were *E. coli* and Pseudomonas, as the 0.5% and 1% EEP had no efficacy. On the other hand, there were little differences in antimicrobial activity between the two samples of propolis.

Key words: Syrian Propolis, Ethanolic extract of Propolis, Antimicrobial activity.

Introduction:

Propolis is a mixture of beeswax and resins collected by the honeybee from different plant buds, leaves and exudates. Bees use propolis not only as a building material but also as a means of maintaining low levels of bacterial and fungal concentration in the hive. [1,2]

More than 150 components such as polyphenols, phenolic aldehydes, quinones, coumarins, amino acids, steroids and inorganic components have been identified in propolis samples. [1] Among the compounds reported to occur in these samples, phenolic acids and flavonoids are particularly important since many of propolis' alleged biological activities are attributed to these substances. [3]

Considering the complex structure of propolis, it cannot be used directly: The wax and organic debris are removed during processing, creating propolis tincture.[4] Then Propolis is extracted with suitable solvent. The most common solvents used for extraction are water, methanol, ethanol, chloroform, dichloromethane, ether, and

acetone.[2] It was observed that some extracts (ethanol, and 40% methanol extracts) found to be active against all the microbes while two extract was inactive that is, Petroleum ether and chloroform extracts.[4]

Propolis has long been used in oriental folk and in European ethno-pharmacology medicine for curing infections and as an antiseptic and anti-inflammatory agent for healing wounds and burns.[1,5] A number of investigations have shown that propolis possesses antibacterial, antiviral and antifungal properties. Moreover, it has been shown that there were variations in the antimicrobial activity according to the propolis origin. [5] Propolis is used to treat a variety of diseases, such as eczema, ulcers and eye, throat and urinary tract infections [6].

Preliminary scientific studies show that propolis has in vitro antibacterial and antifungal activity with active constituents including flavonoids like galangin and hydroxycinnamic acids and caffeic acid. [7] Thus propolis was found to have antibacterial activity against a range of commonly encountered cocci and Gram(+) rods, including the human tubercle bacillus, but only limited activity against Gram(-) bacilli. Thus propolis is more active on Gram (+) than on Gram (-) bacteria. On the other hand, it has been suggested that the killing of staphylococci is the result of the combined action of several components, none of which alone are effective. [8]

The objective of this work was to investigate antimicrobial properties of two propolis samples obtained from different regions of Syria against four strains of microorganisms.

Materials and methods:

Preparation of extracts:

The first sample was obtained from Latakia, Syria in September 2015 and the second was from Tartous, Syria in March 2016.

To prepare an ethanolic extract, the propolis sample was ground into a fine powder, and thereafter 2 g of the propolis powder was mixed with 10 ml of 95% ethanol to obtain 20% (w/v) propolis extract. Extraction was carried out at room temperature in the dark for 7 days, with periodical hand shaking. [5] After extraction, the mixture was centrifuged and supernatants were designated as an ethanolic extract of propolis (EEP). The extracts were of different concentration (0.5%, 1%, 5%, 10%, 20%). Then each sample was filtered by a 0.45 micrometer filter and collected into a sterile tube and freezed in -10 °C.

Preparation of Mueller-Hinton medium:

We suspended 38 grams in 1000 ml distilled water, heated until boiling to dissolve the medium completely. Then, we sterilized by autoclaving at 15 lbs pressure (121°C) for

15 minutes. Suspensions of microorganisms containing 10^6 cells/ ml were inoculated onto plate surfaces with a sterile cotton swab. Test plates (diameter 10 cm) were prepared with 20 ml of Mueller-Hinton agar (himedia laboratories pvt.ltd), and holes of 6mm in diameter were punched in the agar plates using cork borer. Each hole was filled with 50 µl of EEP. The diameters of the growth inhibition zones around the holes were measured after incubation for 48 h at 35°C. [5]

Results:

When the inhibition zone of propolis extract against both bacterial groups was greater than 6 mm is considered active [7].

Table (1): Diameters of inhibition zone of EEP from Lattakia city on bacterial strains

Bacterial strains	**Concentrations of EEP from Lattakia city**				
	0.5%	**%١**	**%٥**	**%١٠**	**%٢٠**
Staphylococcus aureus	٨ mm	١١mm	١٤mm	١٧mm	١٩ mm
Pseudomonas aeruginosa	٨ mm	٨ mm	٩ mm	١٢mm	----
Escherichia coli	----	----	----	٢١ mm	١٧ mm
Acinetobacter baumannii	٩ mm	١2 mm	١٧ mm	١٩ mm	١٥ mm

Table (2): Diameters of inhibition zone of EEP from Tartous city on bacterial strains

Bacteral strains	**EEP from Tartous city**				
	0.5%	**%١**	**%٥**	**%١٠**	**%٢٠**
Staphylococcus aureus	٩ mm	١١mm	١٤mm	١٦ mm	٢٢ mm
Pseudomonas aeruginosa	----	----	----	٧ mm	١٣ mm
Escherichia coli	١٤mm	٦ mm	١٠mm	١١ mm	١٣ mm
Acinetobacter baumannii	٧ mm	٨ mm	٩ mm	١٠ mm	١٢ mm

---: no inhibition zone

Discussion:

Due to our study we have been able to determine the therapeutic value of propolis based on the results that we got from our experiments. Firstly, we observed that the EEP is more effective on gram positive bacteria than the gram negative ones, which is in agreement with Grange et al. who found that dilution of 1: 20 in nutrient agar, the ethanolic extract of propolis completely inhibited the growth of Staphylococcus aureus (including the MRSA strains), Staph. epidermidis, and partially inhibited growth of Pseudomonas aeruginosa and Escherichia coli but had no effect on Klebsiella pneumoniae. (9)

The most susceptible species of studied bacteria was Staphylococcus aureus of inhibition diameters 22 mm for Tartous sample and 19 mm for Latakia sample, and the least sensitive bacteria against this extract were E.coli (Latakia EEP) and Pseudomonas (Tartous EEP).

The important point we noticed that all prepared concentrations (0.5% + 1% + 5% + 10% + 20%) were effective on Staphylococcus aureus, the most efficacy was referred to 20% concentration. Despite all these encouraging results we had some differences between the 2 propolis samples which can be explained by the difference of composition. Finally, 0.5% and 1% concentration were ineffective on E.coli.

We found that the two propolis samples had antibacterial activity against all four strains of bacteria with little variations in inhibition zones due to the difference in composition, which is in agreement with Abd EFK who found that all propolis samples showed an inhibition in the growth of all examined bacteria but the inhibition varied according to the propolis origin (10).

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