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Research article

Microbiological studies on propolis extracts against some animal pathogens

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Abstract

Propolis means a gum that is gathered by bees from various plants having antibacterial, antifungal and healing properties. One of the common problems in the medical world, spreading of bacterial resistance against antibiotics, so one of the most important steps in microbiological researches is to find a new antimicrobial compound with minimal side effects. So the aim of this study is to investigate the antimicrobial activity of *propolis* aqueous and solvent extracts on some medically important bacteria and fungi. Hot and cold aqueous extracts in addition to successive solvent extracts (acetone, ethanol and chloroform extracts of *propolis)* were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* by agar well diffusion method. The chloroform extract showed significant antibacterial activity against *Escherichia coli* followed by acetone then ethanol extract finally the hot aqueous extract. Where the cold aqueous extract showed no significance against *Escherichia coli*. The most effective extract showed high significant antifungal activity was the cold aqueous extract against *Candida albicans* then the hot aqueous extract, finally ethanol extract. While the acetone and chloroform extracts did not show any significant antifungal activity against *Candida albicans*. Also the hot aqueous extract showed high significant antibacterial activity against *staphylococcus aureus* then ethanol extract. While the other extracts did not show any significant antibacterial activity against it. So it could be concluded that the *propolis* extract exhibited remarkable antimicrobial activity against microbial pathogens and can be introduced as an alternative to chemical antimicrobial drugs, but required wider investigation.

Introduction

The bee glue, is a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds and exudates, it is commonly named propolis [1]. This resin is masticated, salivary enzymes are added, and then it is mixed with beeswax and probably with other compounds of bee metabolism [2]. Etymologically the word *propolis* derives from the Greek *pro* (for 'in front of', 'at the entrance to') and *polis* (for 'community' or 'city'), meaning that this natural product contributes to hive defence. Due to its waxy nature as a protective barrier against external invaders or against weathering threats like wind and rain. And to embalm the carcasses of dead intruders, thus avoiding their decomposition and eliminating a potential source of microbial infections. Propolis is a complex mixture composed of beeswax, resins and plant balsams, essential oils, pollen and some organic and mineral compounds [1, 2]. Since ancient times, it has been extensively employed by man especially in folk medicine to treat several maladies. Egyptians used bee glue to embalm their cadavers due to its anti-putrefactive properties. Also Greek and Roman physicians used it as an anti-pyretic agent, mouth disinfectant and as an antiseptic and for wound healing [2]. In the Middle Age these therapeutic applications were perpetuated and among Arab physicians. Listed in the London pharmacopoeias of the 17th century as an official drug, and became very popular between the 17th and 20th centuries in Europe due to its antibacterial activity. Used in Italy, Stradivari as a violin varnish [4]. Also used in the Second Global War in several Soviet clinics as treatment for tuberculosis [6]. The first publishing scientific work with propolis, reported in 1908 [7]. The first patent was in 1968, [8] Propolis became the focus of great scientific interest during the last 30 years due to its medicinal and

biological properties mainly envisaging its application in human and veterinary medicine, pharmacology and cosmetics.

So the aim of this study is to investigate the antimicrobial activity of *propolis* aqueous and solvent extracts on some medically important bacteria and fungi.

Experimental

Collection of material

Propolis was purchased from Riyadh market.

Aqueous extraction

About 10 ml of each hot and cold distilled water was added to 5g of the propolis in sterile test tubes. The tubes were kept for 1 week at room temperature until use.

Solvent extraction

About 5 g of propolis was extracted with 10 mL of each solvent (Acetone, Chloroform and Ethanol) kept for 24 h. Then, it was filtered using Whattman filter paper. The solvent was evaporated to make the final volume as 1/2 of the original volume [9].

Preparation of inoculums

The inoculums (bacterial strains and fungi) were isolated from large animals and poultry farms on the outskirts of Cairo. The strains of bacteria (*Staphylococcus aureus*, *Escherichia coli)* & fungi (*C. albicans)* were inoculated Sabaroud dextrose agar (SAB) (Purchased from Witan – Bio-life Company produced by Jalil Medicals Company) & nutrient broth (Purchased from Witan–Bio-life Company produced by Jalil Medicals Company).for overnight at 37°C for bacteria & 25°C for fungi.

Antimicrobial screening

The preliminary study of antimicrobial activity of different extracts of propolis was performed by using agar well diffusion method [10]. The sensitivity of all extracts was tested against *E. coli, S. aureus* and *C. albicans*. The anti-microbial activity was measured by the inhibition zones produced in milliliter. All experiments were duplicated. Ciprofloxacin (10 µg) and penicillin (10 µg) used as positive control while distilled water (100 µg) used as negative control for antibacterial screening. Nystatin (10 µg) was used as positive control while distilled water (100 µg) used as negative control for antifungal screening.

Results and Discussion

Results

This investigation of antimicrobial activity was performed on five different extracts of *propolis* as shown in (Table 1). The screening step in the preliminary study for antimicrobial activity was done using the Agar well Diffusion Method. The diameter of the clear zone indicated the inhibition activity. The chloroform extract showed significant antibacterial activity against *E. coli* (2.4 mm) followed by acetone (2.3mm) followed by ethanol extract (1.4 mm) finally the hot aqueous extract (1.1mm). However, the cold aqueous extract showed no significance against *E. coli*. The most effective extract showed high significant antifungal activity was the cold aqueous extract against *C. albicans* (3.8 mm) *followed by* the hot aqueous extract (2.6 mm) followed by ethanol extract (2 mm). While the acetone and chloroform extracts did not show any significant antifungal activity against *C. albicans*. Also the hot aqueous extract showed high significant antibacterial activity against *Staphylococcus aureus* (2.2 mm) followed by ethanol extract(1.2mm) While the chloroform, acetone and cold aqueous extracts did not show any significant antibacterial activity against *Staphylococcus aureus* as shown in Table (2, 3, 4 and 5).

Table 1. List of propolis different extracts used to evaluate antimicrobial activity

No.	Name	Color of used extract
1	Ethanol	Green
2	Chloroform	Dark brown
3	Acetone	Milky
4	Hot aqueous extract	Brown
5	Cold aqueous extract	Light brown

Table 2. Antimicrobial activity of propolis different extracts against *E.coli* , *S. aures* and *C. albicans* isolates in (mm)

Type of extract → / Type of microorganism ↓	Acetone	Chloroform	Ethanol	Cold aqueous extract	Hot aqueous extract	Penicillin	Ciprofloxacin	Nystatin	Distilled water
E coli	2.3	2.4	1.4	0	1.1	1.1	2.8	0	0
S aures	0	0	1.2	0	2.2	0	0	0	0
C albicans	0	0	2	3.8	2.6	0	0	1.6	0

Table 3. Diameter (mm) of zone of inhibition produced by propolis against *E. coli* comparing with reference drug ciprofloxacin and penicillin

Type of extract → / Type of micro organism ↓	Acetone	Chloroform	Hot aqueous extract	Ethanol	Ciprofloxacin	Penicillin
E. coli	2.3	2.4	1.1	1.4	2.8	1.1

Table 4. Diameter (mm) of zone of inhibition produced by *propolis* against *S. aureus* comparing with reference drug ciprofloxacin and penicillin

Type of extract → / Type of micro-organism ↓	Hot aqueous extract	Ethanol	Ciprofloxacin	Penicillin
S. aureus	2.2	1.2	2.8	1.1

Table 5. Diameter (mm) of zone of inhibition produced by propolis against *C. albicans* comparing with reference drug Nystatin

Type of extract → / Type of micro-organism ↓	Ethanol	Hot aqueous extract	Cold aqueous extract	Nystatin
C. albicans	2	2.6	3.8	1.6

Discussion

Nowadays either in dairy or broiler farms veterinary apitherapy is documented rather than in immunomodulation performance [11,12,13]. The antimicrobial activity of propolis have been studied by several authors [14,15]. These is due to its several biological properties and its chemical composition especially though propolis composition which have been mentioned in literature and newly reported such as flavonoids, which may be responsible for fungicidal, anesthetic biological and antibacterial properties, etc. [16].

So the aim of this study is to investigate the antimicrobial activity of propolis aqueous and solvent extracts on some medically important bacteria and fungi.

While some authors found propolis samples active only against Gram-positive bacteria and some fungi [17,18,19], others found also weak activity against Gram-negative bacteria [20, 21]. In addition to Fernandes *et al.* [22], who observed a marked action of propolis against Gram-positive bacteria and limited activity against Gram-negative ones. The antimicrobial activity of propolis have been investigated most extensively, and its inhibitory capacity may be influenced by some factors such as (tested microorganisms, extract preparation, propolis origin, bee species, etc) [23, 24]. Some authors attribute the reason for its antimicrobial activity to its complex composition, and its mechanisms of action [25, 26].

On the other hand, the mechanism of propolis antimicrobial activity is more complex and might be attributed to the synergistic activity between its various potent biological ingredients that more than 300 compounds mainly phenolics and flavonoids [27]. It was found that propolis affects bacterial cytoplasmic membrane, and it inhibits motility, enzyme activity, cell division, and protein synthesis through inhibition of RNA-polymerase, which can explain partially the synergism of propolis with drugs [28]. Moreover, galagin and caffeic acid derived from propolis are enzymatic inhibitor agents for bacteria [29].

In this study, investigation of antimicrobial activity was performed on five different extracts of propolis as shown in (Table 1). The screening step in the preliminary study for antimicrobial activity was done using the Agar well Diffusion Method. The diameter of the clear zone indicated the inhibition activity. The chloroform extract showed significant antibacterial activity against *E. coli* (2.4 mm) followed by acetone (2.3 mm) followed by ethanol extract (1.4 mm) finally the hot aqueous extract (1.1 mm). However the cold aqueous extract showed no significance against *E. coli*. The most effective extract showed high significant antifungal activity was the cold aqueous extract against *C. albicans* (3.8 mm) followed by the hot aqueous extract (2.6 mm) followed by ethanol extract (2 mm). While the acetone and chloroform extracts did not show any significant antifungal activity against *C. albicans*. Also the hot aqueous extract showed high significant antibacterial activity against *Staphylococcus aureus* (2.2mm) followed by ethanol extract (1.2 mm) While the chloroform, acetone and cold aqueous extracts did not show any significant antibacterial activity against Staphylococcus aureus as shown in Table (2, 3 , 4 and 5).

Investigating results of the antibacterial activity of propolis was on *Escherichia coli* generally confirmed a very

significant inhibitory activity of propolis on the growth of *Escherichia coli* which were in agreement with the results mentioned by Brumfitt *et al.*, Simuth *et al.*, Qiao Z *et al.,* Simuth *et al.* [30,31,32,33].

Results of this investigation have shown that the antibacterial effect of propolis extract against bacteria (Staphylococcus aureus). Very similar to results that obtained by Brumfitt *et al.*, Simuth *et al.*, Qiao Z *et al.* [30, 31and 33].

In addition to the antimicrobial screening, which clearly indicated that cold aqueous extract of propolis, had much more powerful antifungal activity comparing with the standard antifungal Nystatin.

Based on these results propolis could be applied in an adequate solvent in cases of infection by the investigated bacteria with positive outcome especially in cases of hypersensitivity or resistance to certain antibiotics, propolis could be used as replacement therapy. The extraction of propolis by solvents, such as acetone, ether and chloroform, are toxic substances with harmful effects on animals even if applied in minimal quantities.

Due to that it have to be evaporated from extraction by a vacuum evaporator and be in a solid state, i.e. powder, which contains all the active substances of propolis extracted by the solvent. Then the propolis powder may be dissolved in drinking water of diseased animals or used in production of ointments for external application.

Conclusion

So it could be concluded that all tested samples were sensitive to the antimicrobial activity of propolis extracts, where *C.albicans* was the most sensitive one among the tested *samples* taking into account the results of this research encourage the utilization of propolis extract for treatment of animal bacterial and fungal infections which could be improved by administrating with an adequate antibiotic, depending on the seriousness of the disease. Also those results have been encourage to conduct additional tests to confirm the specific use of the studied solutions to treat the bacterial infections caused by bacteria found to be sensitive to our extracts.

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