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Antimicrobial activity and chemical screening of propolis extracts

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Abstract: Propolis is a resinous mixture that collects by honey bees from the plants. However, the physical character of propolis generally has been used by honey bees to protect their hive, but several beneficial properties of this compound could be considered for human being. In the present study, two propolis samples (P1&P2) were collected and subjected for extraction using different solvents. Then, their antimicrobial effects were evaluated against *Salmonella typhi* PTCC 1609*, Pseudomonas aeruginosa* PTCC 1047, *Staphylococcus aureus* PTCC 1112*, Escherichia coli* PTCC 1338*, Bacillus cereus* PTCC 1015*, Aspergillus niger* PLM 1140 *and Candida albicans* ATCC 1405 using Well Diffusion Agar. In addition, the bioactive compounds and functional groups of the extracts were determined by paper chromatography and Spray methods. The results obtained indicated that ethanol and methanol extracts of the propolis showed relatively more antimicrobial effect and both extracts exhibited similar responses against the antagonistic microorganisms. *Bacillus cereus*, *Salmonella typhi* , *Escherichia coli* and *Staphylococcus aureus* were sensitive whereas, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* were resistant to the extracts. *E.coli* was sensitive to methanol and resistant to ethanol extracts. Our finding concerning to the chemical analysis of the propolis exhibited the presence of flavonoid, tannin, steroid, alcohol and alkaloid in extracts. Overall, propolis has antimicrobial effect with different spectrum and therefore, it might consider a potent candidate for treatment of several clinical scenarios.

Keywords: Propolis, Compositions, Antimicrobial, Effect

1. Introduction

Propolis is a Greek word and means a substance in defense of the hive.

Propolis is sticky and soft resinous material with white, green, yellow and red colors that collects by worker bees from the leaf buds of numerous tree species such as birch, poplar, pine, alder, willow, palm, *Baccharis dracunculifolia*, and *Dalbergia ecastaphyllum* [17,18,4]. In fact, color of propolis depends on the plant resin source that is available for honey bees [8]. Composition of propolis is varied mainly due to season of collection and the variability of plant species growing around the hive [12].

The main chemical classes present in propolis are silver, mercury, copper, manganese, iron, calcium, vanadium, silis ,flavonoids, phenolics, and aromatic compounds. However, propolis contains some volatile oils, terpenes, and bee wax, but they could not be related to its antimicrobial effects [13]. Although several reports have been published on anti-inflammatory, antitumor, anti allergic, anti cancer, stimulation of Humoral and Cell Mediated Immunities[14]and anti blood pressure properties [18, 16], few information is available on the antimicrobial property of propolis. Hence, the present study was conducted to investigate the antimicrobial property of Iranian propolis on some pathogenic microorganisms.

2. Material and Method

2.1. Extraction and Sample Preparation

Two samples of Propolis were collected from different beehives in kazeroun city, Iran. The collected samples (P1&P2) were crushed after freezing and five gram of each dissolved in different solvents viz., methanol, ethanol, xylin, choloform and water to make 1:10, w/v concentration. The suspensions were kept in a shaker incubator at 150rpm, 30˚C for 24hr. Then the liquid phase withdrew and incubated at 45˚C for 24hr [19].

2.2. Antimicrobial Assay of the Propolis Extracts

To perform the experiment several microorganisms viz., *Salmonella typhi* PTCC 1609, *Pseudomonas aeruginosa* PTCC 1047, *Staphylococcus aureus* PTCC 1112, *Escherichia coli* PTCC 1338, *Bacillus cereus* PTCC 1015, *Aspergillus niger* PLM 1140 *and Candida albicans* ATCC 1405 separately were streaked onto Muller Hinton Agar, then wells were made in the agar using sterile borer. Afterward, 0.1 ml of Propolis extract was added into each well and the plates were incubated at 35˚C for bacteria and 28˚C for fungi for 24 to 72 hrs. To assess the antimicrobial effect of the Propolis extract, inhibiting growth zone for each microorganism was evaluated and recorded [3].

2.3. Determination of Arbitrary Unit (AU)

The experiment was carried out follow by preparation of various concentrations of Propolis extracts (1/2, 1/4, 1/8, 1/16, ….1/512), then 100 µl from each dilution was added into the wells that made in cultivated Mueller Hinton Agar with sensitive microorganisms. The plates were incubated at 35 °C and AU for each extract was determined after 24 hrs. Arbitrary Unit of propolis extract for each sensitive bacterium was determined by the reciprocal of the highest dilution showing antimicrobial effect.

2.4. Chemical Screening of Propolis Extracts

To perform the experiment the crude propolis extracts was subjected for paper chromatography using ethyl acetate, acetic acid, water, with 10:50:40 proportions.

The experiment was carried out by spotting the extracts on the filter paper (whatman No. 1). The filter paper dipped into the solvents in chamber and the developing chamber was covered by watch glass to stop evaporation of eluent. When the solvent reaches the top, the filter paper pulled out, dried and placed on the cultivated Mueller Hinton Agar with sensitive bacteria. The plates were kept at 35˚C for 24 hr. afterward, the bioactive compound was recognized by exhibiting zone of inhibition interface of the filter paper.

To continue the study the bioactive compound fraction of each filter paper cut out and subjected for determination of chemical composition of the bioactive compound by spraying method.

2.5. Spraying Method

To perform the experiment, the filter papers were stained with solution of 50 drops nitric acid (65%) in 100 ml ethanol and dried by heating in an oven for thirty minutes at 110 °C. The appearance of pink and yellow color zones considered positive result for detection of primary amines and alkaloids groups [2].

To evaluate the presence of alcohols, phenols and steroids groups in structure of the bioactive compound, one gram vanillin was mixed with 100ml concentrated sulphuric acid, then the filter paper was stained by the solution and dried in an oven at 110˚C till appear maximal colored zones. Colored zones produced on a pale background indicated positive result for detection of alcohols, phenols, and steroids compounds [7].

For detection of sugars, the filter paper was stained by a reagent prepared by 5gram urea, 20ml hydrochloric acid and 100ml ethanol. The stained filter paper heated at 110˚C till maximum coloration. The appearance of blue color indicates positive results for the presence of ketoses and oligosaccharides [11].

To determine the presence of flavonoid and tannin in the bioactive compound of propolis , the filter paper piece contain the bioactive compound was pulled in 10ml ethyl acetate and kept in water bath 40˚C for 5 minutes. The filter paper pulled out and 1ml ammonia added into the solution. Observation of yellow color considered as positive result for the presence of flavonoeid. To continue the study the filter paper piece was pulled into 5ml water and boiled for 5 minutes. Afterward, the filter paper pulled out and 3 drops of ferric chloride were added to the solution. The appearance of dark brown color considered as positive results for the presence of tannin. It must be noted that the experiment was done on plain filter paper as control group [20].

3. Results

3.1. Antimicrobial Effect of Propolis Extracts

As expected, ethanol and methanol extracts of the propolis showed relatively more antimicrobial effect. In addition, both collected samples of propolis (P1&P2) showed similar responses on pathogenic microorganisms. In this regard, *Bacillus cereus*, *Salmonella typhi* , *Escherichia coli* and *Staphylococcus aureus* were sensitive whereas, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* were resistant to the extracts. Of all antagonistic bacteria *Escherichia coli* showed different response to the extracts, it was sensitive to methanol and resistant and ethanol extracts (Table1).

3.2. Arbitrary Units (AUs) of the Propolis Extracts

Our finding on determination of AUs of methanol and ethanol extracts of the P1 and P2 illustrated that lowest AU was found for methanol extract of P1 and P2 whereas, the highest AU was found for methanol extract of P2. In addition, *Salmonella typhi* and *Bacillus cereus* had relatively lowest and highest AUs respectively (Table2).

3.3. Chemical Composition of the Bioactive Compound of Propolis

As shown in Figure 1, paper chromatography detected bioactive compound of the propolis extracts. Furthermore, the results obtained from determination of the chemical composition of the bioactive compound illustrated the presence of alcohol, phenols, steroid and alkaloid groups in the bioactive compound of Propolis extracts. In addition, observation of yellow and dark brown color after

performing the spray method resulted the presence of flavonoid and tannin in the bioactive compounds.

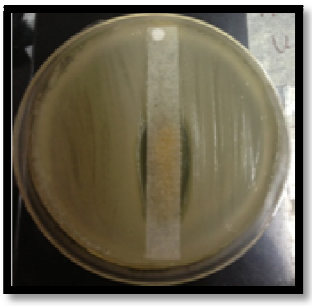
Table 1. *Antimicrobial assay of propolis against pathogenic microorganisms*

Pathogenic microorganisms	Propolis extracts of			
	P1		P2	
	Methanol	Ethanol	Methanol	Ethanol
Salmonella typhi	+	+	+	+
Staphylococcu aureus	+	+	+	+
Escherichia coli	+	-	+	-
Bacillus cereus	+	+	+	+
Pseudomonas aeruginosa	-	-	-	-
Candida albicans	-	-	-	-
Aspergillus niger	-	-	-	-

Table 2. *Determination of Arbitrary Units (AUs) of Propolis extracts*

Pathogenic microorganisms	AUs of extracts			
	P1		P2	
	Methanol	Ethanol	Methanol	Ethanol
Salmonella typhi	*16*	*32*	*16*	*32*
Staphylococcus aureus	32	*128*	*32*	*128*
Bacillus cereus	*32*	*256*	*512*	*256*

a



b

Figure 1. *a: Active compound of ethanol extract of propolis against Bacillus cereus, b: Active compound of methanol extract of propolis against Bacillus cereus*

4. Conclusion

Propolis is a resinous substance collected by honey bees (*Apis mellifera*) from various tree species. This compound usually used by bees to coat hives, seal cracks and protect the hive against different contaminations [1]. As mentioned above, composition of propolis and its properties depended on the kind of the plants and geographical area. [5, 1,9]. Therefore, the present study was conducted to evaluate the antimicrobial property of Iranian propolis against the pathogenic microorganisms. Our finding illustrated that the propolis samples (P1&P2) were extracted relatively more by methanol and ethanol. Likewise, these extracts showed potent activity against *Bacillus cereus*, *Salmonella typhi* , *Escherichia coli* and *Staphylococcus aureus* however, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* were resistant to them. Although, both type of propolis have shown similar responses against pathogenic microorganisms, antimicrobial property of their extracts against *Escherichia coli* was differ. It means this bacterium was sensitive to methanol and resistant to ethanol extracts of P1&P2. Therefore, it can be concluded that both propolis extracts have similar antimicrobial compounds. In addition, chemical analysis of the propolis samples was carried out by TLC and spray methods. The results obtained exhibited the presence of flavonoid, tanin, steroid, alcohol and alkaloid in the extracts. On the other hand probably the bioactive property of propolis probably is related to flavonoid followed by tanin, and steroid. In this regard, several papers supported our finding concerning to antimicrobial property of flavonoid, tanin, and steroid [9, 15,13]. These scientists believe that the antimicrobial property of the propolis is related to the geographical areas. Hence, it might be interpreted that the antimicrobial property of different propolis is not identical. However, our finding suggests that the antimicrobial action of the propolis as an adjuvant to therapy and it might be considered a potent candidate for treatment of several clinical scenarios. However, further study should be done to achieve a superior dose to kill the target microorganisms.

Therefore, our study believes that the propolis promotes healing. On the other hand, the propolis can be used as a natural alternative to antiobiotics. Recently, pharmaceutical industries conducted to introduce the new antimicrobial components with potent activity against pathogenic bacteria. Therefore, the new sources of remedy such as propolis might be considered valuable component for investigation.

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