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Antibacterial and Antifungal Activity of Iranian Propolis Against *Staphylococcus aureus* and *Candida albicans*

[1]Yousef-Beigi Ghasem, [1]Ownagh, Abdolghaffar and [2]M. Hasanloei
[1]Department of Microbiology, School of Veterinary Medicine, University of Urmia, Iran
[2]Graduated of the Urmia Veterinary School, University of Urmia, Iran

Abstract: Propolis samples from West North region of Iran were studied for their antibacterial (against *Staphylococcus aureus*) and antifungal (against *Candida albicans*) activities. In this article, yield of extracts and their pH values were measured. Antibacterial and antifungal activities of Ethanol- Extracted Propolis (EEP) were investigated by Petri dish bioassay method. Dilutions of EPP in agar with serial concentrations ranging from 0/04 to 10% (W/V) were prepared and antimicrobial activities were determined as Minimal Inhibitory Concentrations (MIC). All samples were active against the fungal and bacterial test strains. MIC values for different propolis samples against *Staphylococcus aureus* were, respectively 4, 3 and 1.5% (W/V) and against *Candida albicans* were, respectively 2, 4 and 3% (W/V).

Key words: Propolis, antibacterial, antifungal, *Staphylococcus aureus*, *Candida albicans*

INTRODUCTION

Propolis (bee glue) is a sticky dark colored substance collected by honeybees of various climates, mainly from poplar (polulus) genus. Bees mix the original propolis with beeswax and β-glucosidase they secrete during the propolis collection. The resulting material is used by bees to seal holes in the hives, exclude draught, protect against invaders and mummify their carcasses (Garedew *et al*., 2004; Kujumgiev *et al*., 1999; Marcucci *et al*., 2001; Pietta *et al*., 2002; Popova *et al*., 2005).

Propolis has been used by man since early times, for various purposes, especially as a medicine because of its antimicrobial properties.

The medical use of propolis was nearly for gotten in modern era due to the discovery and effective use of antibiotics. Nowadays, however, since several pathogens are developing resistance to potent antibiotics and antibiotics' side effects in humans, the need to search and screen for new antimicrobial agents is increasing (Garedew *et al*., 2004). The present letter reports the results of a study on the antibacterial and antifungal activities of ethanol extracts of propolis samples in Iran (temperate zone).

Numerous reports describe the antimicrobial properties of bee glue and many active components have been identified (Bankova *et al*., 2000).

MATERIALS AND METHODS

Preparation of ethanol extract: Propolis samples by the *Apis mellifera* bees were obtained from Khoy, Urmia and Maragheh in North West Iran.

All samples were collected by scraping from frames and walls of the hives. Extraction in 70% ethanol (1:10 W/V) for 24 h was performed on solid samples gathered.

pH values of extracts were measures by using a pH meter at 25°C. The extracts were filtered and then were dried at 40°C while their weights were monitored at an of 24 h intervals starting from the 10th incubation day. Complete dryness of the samples was ascertained by the absence of weight loss during the last 3 continuous weightings and was achieved in about 15 days. The yield of extraction of each propolis sample was determined from the proportion of dry weight of extracted sample to that of fresh sample (Garedew *et al*., 2004; Kujumgiev *et al*., 1999; Popova *et al*., 2005).

The pH values of different propolis samples and yields of extraction are summarized in Table 1.

The dried propolis samples were dissolved in 60% ethanol to prepare 10% stock solutions, which were diluted further with the same solvent to achieve varying experimental concentrations (Although extraction of propolis was done with 70% ethanol, treatments were done with 60% ethanol to minimize the effect of a highly concentrated solvent).

Corresponding Author: Yousef-Beigi Ghasem, Department of Microbiology, School of Veterinary Medicine, University of Urmia, Iran

Table 1: Physical properties and yield of extractions of the propolis samples, yields (%W/W) of EEP (ethanol-extracted propolis) and pH value and description of the raw propolis quality

	EEP		
Samples	pH	Yields (W/W)	Remarks
1 (Khoy)	5.12	30.40	Pure propolis, very sticky
2 (Urmia)	5.48	37.06	Pure propolis, slightly sticky
3 (Maragheh)	5.42	36.20	Pure propolis, very sticky

Biological material: Bioassays of antimicrobial activities of samples were performed on *Candida albicans* and the *Staphylococcus aureus*.

Petri dish bioassay: Agar dilution in plates: MIC values for different propolis samples against bacterial and fungal species were determined by agar dilution method, according to recommendations of the National committee for Clinical Laboratory Standards Guidelines. Fifty microliter of propolis solution or of varying concentrations were added to the sterile agar solutions at a temperature of 48°C, to achieve final concentrations of 0.04, 0.06, 0.08, 0.1, 0.2, 0.5, 1, 1.5, 2, 3, 4 and 5% W/V propolis in the saboraud dextrose agar and Muller Hinton agar. The contents were mixed thoroughly and then 7.5 mL of each growth media culture were poured into sterile Petri dishes and allowed to cool. Standard (final) inocula containing about 3×107 CFU bacteria and yeasts in 1 mL suspension were prepared using McFarland nephelometer standards. Inoculations were done by uniformly distributing 50 μL of each standard inoculum on the surface of the propolis-containing agar layers using sterile 2-shaped glass rods. The plates were then incubated at a temperature of 37°C for 24 h for bacteria and at 25°C for 48 h for fungi.

The Minimal Inhibitory Concentration (MIC) was the lowest concentration of propolis that inhibited any visible growth of bacteria or yeasts. Control experiments were performed similarly with solvent to measure the antimicrobial activity of solvent (Garedew *et al*., 2004; Popova *et al*., 2005).

RESULTS

Differences in the yield of extraction and physical properties of propolis samples: The pH values of extracted propolis samples were acidic between 5.12 and 5.48, showing slight differences among samples of different geographic sources. The yield of extractions for sample 1 (Khoy), 2 (Urmia) and Maraghe were, respectively 30.40%, 37.06% and 36% W/W. The yields showed dependency on the quality of raw propolis. Those raw samples that were pure and stiky had higher yields.

Table 2: Minimal Inhibitory Concentrations (MIC) of propolis against bacteria and yeasts (%W/V)

Samples	Geographic origin	*Staphylococcus aureus*	*Candida albicans*
1	Khoy	4.0	2
2	Urmia	3.0	4
3	Maragheh	1.5	3

Antimicrobial activities of ethanol extract of different propolis samples: Comparisons of the antimicrobial activities of the various propolis samples against the different test organisms were done at several levels of propolis concentrations by using the Minimal Inhibitory Concentration (MIC). The activities were increased with increasing concentrations above the MIC Values for each. Table 2.

DISCUSSION

Physical properties, yield of extraction and pH values: unpredictable differences in pH values of the extracts of different propolis samples shows no relation to their geographic origin, the species or subspecies of bees, the yield of extraction and the quality of propolis. According to previous studies, there were extensive differences in pH measures of different EEP extract but PV and WEP extracts had slight differences.

The pH values of ethanol-extracted propolis samples in other countries were acidic between 4.2 and 5.3. pH values of our study were also acidic between 5.12 and 5.48.

Yield of extracts for different countries shows many differences. (Maximum 61.3% W/W) and like our results, those raw propolis samples that were pure and sticky had higher yields. (Garedew *et al*., 2004; Bankova *et al*., 2000; Popova *et al*., 2005).

Antimicrobial activity: There were no significant differences between antimicrobial activities of samples from various regions. Antimicrobial activities of propolis extracts increased with higher propolis concentrations and were significant as decreases in bacteria and fungi colonies (Kujumgiev *et al*., 1999; Sforcin *et al*., 2000; Bankova, 2005; Popova *et al*., 2005).

In Urmia and Maragheh samples, MIC values for antifungal activity were higher than antibacterial activity.

Such comparative studies can be valuable with respect to propolis standardization and practical application in therapy. They will allow scientists to connect a particular chemical propolis type to a specific of biological activity and formulate recommendations for the practitioners. This could help the general public to make more efficient use of the beneficial properties of propolis.

REFERENCES

Bankova, V., S. Castro and M. Marcucci, 2000. Propolis: Recent advances in chemistry and plant origin. Apidologie, 31: 3-15.

Bankova, V., 2005. Recent trends and important developments in propolis research. Institute of Organic Chemistry whit Centre of Phytochemistry, Bulgarian Academy of Sciences 1113 Sofia, ecAM., 2: 29-32.

Garedew, A., E. Schemolz and I. Lamprecht, 2004. Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: An *in vitro* approach. Free University of Berlin, Institate of Zoology, KoniginLuise-Strasse. 1-3,D-14195 Berlin, Germany, Institute of Biology, Free University of Berlin, Grunewaldstra, 2004; B-34, D-121, 5 Berlin, Germany.

Kujumgiev, A., I. Tsvetkova, Y. Serkedgieva, V. Bankova and R. Christov, 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Ethnopharmacology, 64: 235-240.

Marcucci, M.C., F. Ferreres, C. Garcia-Viguera, V. Bankova, S.L. Decastro, A.P. Dantas, P.H.M. Valente and N. Paulino, 2001. Phenolic compounds from Brazilian propolis with pharmacological activities. Ethnopharmacology, 74: 105-112.

Pietta, P.G., C. Gardana and A.M Pietta. 2002. Analytical methods for quality control of propolis. Fitoterapia, 73 suppl., pp: S7-S20.

Popova, S., O. Kaftanooglu and V. Bankova, 2005. Antibacterial activity of turkis propolis and its qualitative and quantitative chemical composition. Phytomedicine, 12: 221-228.

Sforcin, J.M., A. Fernandes, C.A.M. lopes and V. Bankova, 2000. Seasonal effect on Brazilian propolis antibacterial activity. Ethnopharmacology, 73: 243-249.