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In Vitro Studies to Evaluate the *Antistaphylococal Aureus* Activities of Propolis: A Wonder Honey Bee Product in Comparison with Antibiotics: Ampicillin and Amoxycillin

Anita Devi[1]*, Neelima R Kumar[1] and Jaspreet Kaur[2]

[1]Department of Zoology, Panjab University, Chandigarh-160014, INDIA
[2]Department of Biotechnology (UIET) Panjab University, Chandigarh-160014, INDIA
*E-mail: *anitakadian23@gmail.com*

Abstract

Aims: *Propolis: A wonder honey bee product is a natural resinous substance called Bee Glue, collected by bees from the exudates and secretions of plants. The aim of the present study was to determine the antistaphylococal aureus activities of propolis.*

Methods and Results*: The antibacterial activity of propolis was determined by well diffusion and Macro dilution methods using ethanolic, methanolic and water extracts. Different extracts of Propolis at the concentrations of 15mg/ml, 30mg/ml, 45mg/ml, 60mg/ml and 75mg/ml were prepared and 50ul was added to the wells. After growth, zones of inhibition were determined and compared with antibiotic Ampicillin (10µg) and Amoxycillin (30µg). The ethanolic extract of propolis showed maximum inhibition (14.3mm) as compared to the methanolic (12.6mm) and water extract (10.7mm). The zone of inhibition of the positive control was found to be maximum (15.9mm) for Ampicillin and (17.8mm) for Amoxycillin.*

Conclusions: *Therefore from this study we can conclude that propolis can be used as a potential natural product for antibacterial therapeutics in the world of emerging drug resistance in microbes.*

Significance and Impact of the Study: *The antimicrobial properties warrant further studies on the clinical applications of propolis and some other honey bee products against microorganisms.*

Keywords*: Staphylococcus aureus, Propolis, Drug resistance, Ampicillin, Amoxycillin*

1. INTRODUCTION

Most of the drugs used currently to treat microbes and other infections are derived from natural sources including ethanomedicinal plants. They provide new sources of therapeutic agent against multiplying drug resistant bacteria such as *Staphylococcus aureus*. This is a major cause of one fifth of all hospital acquired infections (Cepeda *et al*., 2005). The continuing rise in its infection rates and its spread worldwide has lead to calls for action to control infection and develop *anti staphylococcal* agents and vaccines. Several natural products have been identified and their usage documented. One such natural product is the wonder honey bee product propolis, a sticky dark colored resinous material which is derived from plant exudates and collected by honey bees. Despite the availability of numerous evidence related to the antimicrobial activity of propolis, information concerning the antibacterial activity of Indian propolis and factors that might affect the antibacterial action of propolis is still quite limited. Therefore, *Staphylococcus aureus*, a pathogen frequently reported to produce food poisoning all over the world was used as a test organism in the present study.

2. MATERIALS AND METHODS

2.1 Collection and preparation of the propolis extract

Propolis of *Apis mellifera* was collected from Langstroth hive placed in the field of *Brassica campestris* at an apiary in village Tierra near Chandigarh and it was collected by scrapping from the frames with the help of the hive tool.

For the extraction of propolis the sample (10g) was cut into small pieces, ground and subsequent solvent extraction were done using ethanol, methanol and water separately and were labeled as EEP, MEP and WEP. The volume was made to 40ml and it was kept for 5 days with occasional shaking. It was filtered through a Whatman No.41 filter paper and then dried.

1.2 Microorganisms

S.aureus was procured from IMTECH (Institute of Microbial Technology) Sector–39, Chandigarh, India. The organism was maintained in suitable media (agar plates at 4^0C). The organism was checked biochemically prior to storage at -30^0C. *S.aureus* was grown in BHI broth for further experiments.

1.3 The antimicrobial susceptibility test

Antibacterial activity was determined by well diffusion method in compliance with the rules of the National Committee for Clinical and Laboratory Standards (NCCLS). The test organism was inoculated on Mueller Hinton agar plates and spread uniformly to form a lawn. Wells (6mm diameter) were cut into the agar aseptically. Into each well (50µl) added different concentrations of propolis extracts (15mg/ml, 30mg/ml, 45mg/ml, 60mg/ml and 75mg/ml). Ampicillin (10µg) and Amoxycillin (30µg) discs were taken as positive control. The plates were incubated at 37°C for 24 h. The zones of inhibition were then recorded.

1.4 Determination of Inhibitory Concentrations:

Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the propolis extract which inhibited the growth of the tested microorganisms. The minimum inhibitory concentration (MIC) of propolis was determined using the broth dilution method. For this a series of tubes were prepared with broth to which 50µl of various concentrations of propolis were added viz., zero (negative control), 15mg/ml, 30mg/ml, 45mg/ml,60mg/ml and 75mg/ml. Ampicillin and Amoxycillin were taken as the positive control. The tubes were then inoculated with 0.5ml of culture suspension (5×10^6 cfu). After incubating overnight at 37°C, O.D at 600 nm was taken.

3. RESULTS AND DISCUSSION

Multiple drug resistance in bacterial pathogens is a continuing problem throughout the world. Therefore there is an established need to develop new antimicrobial agents to combat these pathogens. In our study, we found that propolis extracts contain antibacterial compounds that we have demonstrated have a potential use against *staphylococcus* and other nosocomial pathogens.

The aim of the present study was to determine the *antistaphylococal* activities of propolis a natural honey bee product**.** It contain variety of constituents and differ greatly due to the variations in their geographic and botanical origins (Moreno, Isla, Sampietro and Vattunon, 2000; Kumazawa, Hamasaka and Nakayama, 2004). The pharmacological properties of this natural product are due to presence of chemical compounds such as polyphenols, sesquiterpine, quinines, coumarine, steroids, amino acids and inorganic compounds (Bankova, Castro and Marcucci, 2000). The ethanolic extract of propolis is responsible for all the therapeutic potentialities like antibiotic (Koo *et al.,* 2000), anti-inflammatory (Wang and Mineshita 1993), antioxidative (Moreno *et al*., 2000) antiviral (Marcucci, 1995; Kujumgiev *et al*., 1999), antifungal (Kujumgiev *et al*., 1999). *S.aureus*, a gram positive bacterium that inhabit human respiratory tract and on the skin is a common cause of skin infections, respiratory diseases and systemic infection. It is frequently reported to produce food poisoning all over the world and therefore was used as a test organism in the present study.

The zones of inhibition were recorded for all the plates and that also included the size of the well. As shown below, it was observed that among all the propolis extracts viz; ethanolic (EEP), methanolic (MEP) and water extract (WEP), the ethanolic extract was found to have maximum zone of inhibition at all the concentrations studied. It showed EEP is most effective. The positive control of Ampicillin and Amoxycillin had maximum zone of inhibition and comparable inhibition by propolis could be observed at 75mg/ml. This shows inhibitory activity of honey bee propolis against *S.aureus* grown under culture conditions.

Table 1: Well diffusion assay for *in vitro* studies against *S. aureus* by using different concentrations of propolis.

Propolis Extract	15mg/ml	30mg/ml	45mg/ml	60mg/ml	75mg/ml
EEP	11.8mm	12.2mm	12.5mm	13.7mm	14.3mm
MEP	8.7mm	9.8mm	10.3mm	10.6mm	12.6mm
WEP	6.8mm	7mm	7.7mm	9.2mm	10.7mm

Table 2: Results of broth dilution assay at different concentrations of propolis extract;

Determination of Inhibitory Concentrations	Optical Density at (600nm)
Media only	0.03
Media + S.aureus	0.76
Media+S.aureus +15mg/ml propolis	0.57
Media+S.aureus +30mg/ml propolis	0.28
Media+S.aureus +45mg/ml propolis	0.13
Media+S.aureus +60mg/ml propolis	0.09
Media+S.aureus +75mg/ml propolis	0.06

From Table No.1 it was observed that the antibacterial activity was found to be increasing with increasing concentration and was maximum in ethanolic extract (14.3mm), than methanolic (12.6mm) and least in water extract (10.7mm) at 75mg/ml of propolis extract. The zone of inhibition of the positive control was found to be maximum (15.9mm) for Ampicillin and (17.8mm) for Amoxycillin. This shows therapeutic potentiality of honey bee product propolis at different concentration studied.

From Table No. 2 the inhibitory concentration was determined by using broth dilution method. It was observed that after incubating overnight at 37^0C, the O.D at 600nm was taken and showed, by using varying concentration of propolis extract the growth of the *S.aureus* decreases with the increasing concentration of propolis.

4. CONCLUSION

From the studies it is clear that among all the solvents used for propolis extraction ethanol was found to most effective and

showed *antistaphylococal* activity in the world of emerging drug resistance in microbes and therefore the antimicrobial properties warrant further studies on the clinical applications of propolis and some other honey bee products against microorganisms.

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