Bionature, 40(1) 2020 : 4-8 ISSN: 0970-9835 (P), 0974-4282 (O)

ANTIBACTERIAL ACTIVITY OF DIFFERENT ETHANOLIC EXTRACT OF ALGERIAN PROPOLIS AGAINST *Staphylococcus aureus*

ABDELLATIF BOUDRA[1*], IDIR BENBELKACEM[1] AND MOHAMED AMINE AISSA[2]

[1]Department of Animal Health, The Veterinary Institute, University of Tiaret, 14000, Algeria.
[2]Laboratory of Microbiology II, The Veterinary Institute, University of Tiaret, 14000, Algeria.

Email: afboudra@gmail.com

Received: 29 January 2020
Accepted: 06 April 2020
Published: 12 April 2020

Original Research Article

ABSTRACT

The antimicrobial activity of propolis extracts is well documented. Nevertheless, the properties of the Algerian propolis are poorly investigated. This fact could be due to the diversity of the climate and the geographic areas from which propolis is usually harvested. The present work is a contribution to evaluate the antimicrobial susceptibility testing of propolis varieties with high total polyphenols and flavonoids contents, chosen beforehand from an analysis made of 10 propolis samples. The disk diffusion method was employed to determine the antistaphylococcal activity of three EEAP (ethanolic extract of Algerian propolis) samples, at different concentrations (5%, 10, 20 and 30%), against a reference bacterium strain of *Staphylococcus aureus*, the most redoubtable agent in osteomyelitis. Inhibitory zone diameters for the tested bacterium were 14.99 mm for EEAP at 5% concentration, 16.41 mm at 10%, 18.08 mm at 20% and 19.16 mm at 30%, respectively. Our *in vitro* study revealed that EEAP with high polyphenols and flavonoids contents exhibit a strong antibacterial activity, and it may be indicated as an alternative solution for the treatment of staphylococcal osteomyelitis.

Keywords: Algerian propolis; *Staphylococcus aureus;* osteomyelitis; inhibition zone.

INTRODUCTION

Staphylococcus aureus is the most important bacterial pathogen isolated from osteomyelitis cases [1]. Treatment of the osteomyelitis infections is problematic due to various factors, including the low bioavailability of antibiotics in bone tissue, increasing resistance to antibiotics in bacterial pathogens, and biofilm properties. In many cases, physicians are left with surgical debridement is the only option to eliminate invasive bacteria to obtain a sterile site [2].

In the light of the aforementioned problems and concerns, it is necessary to develop alternative noninvasive treatment to limit the occurrence of staphylococcal osteomyelitis.

Bee natural antibacterial products including propolis represent a rich source of antimicrobial agents and are less toxic compared to most conventional therapies. In this context, Rezende [3] has previously reported the antimicrobial activity of propolis against Gram-positive bacteria and yeasts. Nevertheless, the chemical composition of the propolis depends on its floral origin, climate and geographical conditions [4].

Generally, propolis is composed of 50% vegetable resin and balm, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% of several substances including organic debris [5]. Different pharmacological properties of propolis were reported, as a local anesthetic effect, stimulation of cartilage formation and osteointegration enhancement in autoclaved allograft [6-10].

In this context, the present study aimed to evaluate the *in vitro* antibacterial activity of several propolis samples collected from different Algerian ecosystems.

MATERIALS AND METHODS

Propolis Samples

Ten propolis samples harvested from different floral and geographical origins were analyzed and classified based on the concentration of polyphenols and flavonoids. As described previously [11], the polyphenols concentrations were determined using *Folin coicalteu* method, however flavonoids concentrations were determined by the *Woisky* and *Salatino* method. All analyses were made at a specialized laboratory (*CARI ASPL®, Belgium*). Total polyphenols and flavonoids contents of the ten analyzed samples are presented in Table 1.

Preparation of Inoculum

The antimicrobial activity of the selected propolis samples was evaluated using a *Staphylococcus aureus* reference strain (ATTC 25923).

Before the experiment, the bacterial strain was inoculated onto the surface of Mueller-Hinton agar media, the inoculum suspension was obtained by taking isolated colonies from 24 h cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl), and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard.

Disk Diffusion Method

Sterile paper discs were soaked in a solution of propolis extracts (EEAP6, EEAP8, EEAP10) at different concentrations (5, 10, 20 and 30%), kept at room temperature for 2 hours to allow diffusion of the solution and then placed on the surface of Mueller-Hinton agar plates for aerobic incubation at 37°C for 24 h [12].

Table 1. Mean±SD values for polyphenols and flavonoids contents in the collected propolis [11]

Samples	Polyphenols (mg/g of propolis)	Flavonoids (mg/g of propolis)
EEAP 1	19.00±0.71^{a}	6.50±0.71^{a}
EEAP 2	49.70±2.12^{b}	23.25±1.06^{b}
EEAP 3	14.20±4.81^{ac}	5.60±0.42^{ac}
EEAP 4	35.25±5.59^{d}	15.95±0.07^{d}
EEAP 5	39.55±4.03^{d}	15.90±0.85^{d}
EEAP 6	76.10±0.71^{e}	42.85±0.49^{e}
EEAP 7	7.30±1.41^{c}	2.00±0.14^{f}
EEAP 8	71.55±1.48^{e}	38.70±0.28^{g}
EEAP 9	20.25±4.45^{ac}	5.80±0.14^{ac}
EEAP 10	99.80±8.06^{f}	54.50±1.41^{h}

Means followed by the same letter in the column are not significantly different (p > 0.05)

The zones of inhibition were measured in millimeters. Positive growth controls with an antibiotic disk (amoxicillin) was included.

Statistical Analysis

To clearly define the difference between the three zones of inhibition of the three varieties of propolis extract with different concentrations (5, 10, 20 and 30%), we compared each concentration of the three varieties by adopting ANOVA as an analysis method.

RESULTS

The antimicrobial activity of the three varieties of propolis rich in polyphenols and flavonoids EEAP 6,8 and 10 showed that the widest inhibition zone was 16 mm for EEAP10 at a concentration of 5%, at 10% 17.5 mm for EEAP8 at 20% of 18.5 mm for EEAP8 and 10 (Fig. 1).

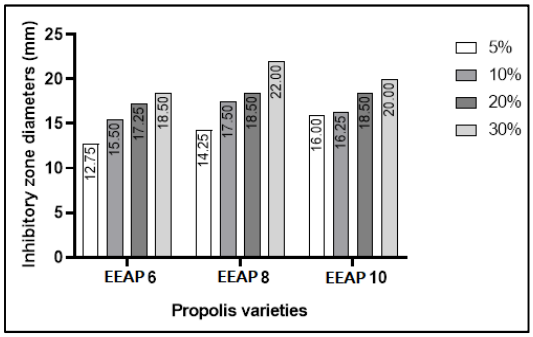

Fig. 1. Inhibitory zone diameters (mm) of three varieties of extracted propolis at different concentrations against *Staphylococcus aureus*

At 30% of 20 mm for EEAP 10 and that of amoxicillin disc was 22 mm. However, the results of the antimicrobial activity of the three propolis samples remain fairly close, and the difference was not significant at the 5% significance level; at 5% concentration ($p< 0.37$), 10% ($p< 0.24$), 20% ($p<0.354$) and 30% ($p< 0.534$).

DISCUSSION AND CONCLUSION

The treatment of osteomyelitis is difficult because of various factors, including the low bioavailability of antibiotics in bone tissue, increased antibiotic resistance, and the biofilm properties of bacteria [13]. The antimicrobial activity of propolis against *Staphylococcus aureus* has already been mentioned [5], the causative agent of osteomyelitis [13]. Our study aimed to assess and measure the antistaphylococcal activity using an Algerian product.

The antimicrobial activity of different propolis concentrations (5, 10, 20 and 30%) against *Staphylococcus aureus* by the disk diffusion method showed sensitivity towards the three varieties of the EEAP. The inhibitory zone diameters were measured and the obtained results were of the order of 14.99 ± 1.62 mm for the concentration of 5% ($p < 0.37$), and of 16.41 ± 1.01 mm for the concentration of 10% ($p < 0.24$), at 20% concentration an area of 18.08 ± 0.72mm ($p <0.354$) and 19.16 ± 0.76 mm at 30% concentration was recorded ($p <0.534$). The zone of inhibition for the antibiotic disk being 22 mm.

At 5% propolis concentration, *Staphylococcus aureus* were sensitive by the presence of the inhibition halos estimated at 16 mm for EEAP 10, 14.25 mm for EEAP 8 and 12.5 mm for EEAP 6. At 10%, the bacterium was sensitive. Hence, the appearance of an inhibition zone of 15.5 mm for EEAP 6, 17.5 mm for EEAP 8 and 16.25 for EEAP 10, for the same concentration Niken et al. [6] recorded a zone of inhibition of 12 mm.

At a concentration of 20%, the inhibitory halos were higher than that recorded by Niken et al. [6], which was 14 mm at the same concentration; and significantly higher compared to the results reported by Nedji et al. [14], which was 12.25 mm at a concentration of 100%. Nevertheless, the propolis used by Nedji et al. [14] contained a higher content of polyphenols and flavonoids than that found in our study.

Our results were slightly elevated at 30% concentration compared to those found by Niken et al. [6], which was 17 mm. Our values were of the order of 18.5 mm, 20 mm for EEAP 6, EEAP 8 and EEAP 10, respectively.

These results were in agreement with Taylor et al. [15], and Hernández-Pérez et al. [16], who found that the effectiveness of propolis extract is proportionally increased with respect to concentration. The antimicrobial propolis effectiveness could be due to the phenolic acids and flavonoid components of propolis, which detach the cytoplasmic membrane from energy transduction, which alters the bacterial viability. The antimicrobial action of propolis could be attributed to these effects on the bioenergetics status of the membrane [17]. The presence of polyphenols is associated with the pharmacological and biological properties of propolis [18].

Our in vitro study has confirmed that the Algerian propolis (*Laghouat, Tiaret*) has a very important anti-staphylococcal infectious activity action even at a concentration of 5% against the *Staphylococcus aureus* causative agent of osteomyelitis.

In conclusion, our *in vitro* study has confirmed that the Algerian propolis (*Laghouat, Tiaret*) has a very important anti-staphylococcal infectious activity action even at a concentration of 5% against the *Staphylococcus aureus* causative agent of osteomyelitis. The satisfactory antistaphylococcal activity of the three different samples of Algerian propolis suggests that the propolis may be used as an alternative treatment of osteomyelitis, limiting consequently the use of invasive methods and the application of antibiotics that could increase the risk of antibacterial resistance. However, before any *in vivo* application of the propolis or its derived antimicrobials components, it will be necessary to conduct toxicity tests on bone cell cultures.

AUTHOR CONTRIBUTIONS

Author AB contributed to the design and implementation of the research, authors AB, IB and MAA the analysis of the results and the writing of the manuscript. All authors discussed the results and contributed to the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hatzenbuehler J, Pulling TJ. Diagnosis and management of osteomyelitis. Am. Fam. Physician. 2011;84(9):1027–1033.
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N. Engl. J. Med. 2004;352(1):95-97. DOI: 10.1056/NEJM200501063520120
3. Rezende GPSR, Pimenta FC, Costa LRRS. Antimicrobial activity of two Brazilian commercial propolis extracts. Braz. J. Oral. Sci. 2006;5(16):967-970. Available:https://doi.org/10.20396/bjos.v5i16.8641876

4. Lahouel M, Boutabet K, Kebsa W, Alyane M. Polyphenolic fractions of Algerian propolis reverses doxorubicin-induced acute renal oxidative stress. Afr. J. Pharm. Pharmacol. 2010;4(10):712-720.
5. Júnior AF, Balestrin EC, Betoni JEC, Orsi Rde O, Cunha MLRS, Montelli AC. Propolis: Anti-*Staphylococcus aureus* activity and synergism with antimicrobial drugs. Mem. Inst. Oswaldo. Cruz, Rio de Janeiro. 2005;100(5):563-565.
6. Niken P, Phalimahtussadiyah R, Zakiah U. Properties and flavonoids content in propolis of some extraction method of raw propolis. Int. J. Pharm. Sci. 2014;6(6):338-340.
7. Boudra A, Hamdi M, Amara K. Role of propolis in osteointegration enhancement in allograft autoclaved implanted in femur of dogs. J. Veterinar. Sci. Technolo. 2014;2(5): 1-3.
DOI: 10.4172/2157-7579.1000173
8. Ezgi A, Hasan Y. Evaluation of the effect of propolis on implant stability by resonance frequency analysis and removal torque test. Biomed. J Sci & Tech. Res. 2017;5(1):1-6.
DOI: 10.26717/BJSTR.2017.01.000477
9. Grange JM, Davey RW. Antibacterial properties of the propolis (bee glue). J. R. Soc. Med. 1990;83:159-160.
10. Parolia A, Thomas S, Kundabala M, Mohan M. Propolis and its potential uses in oral health. Int. J. Med. Med. Sci. 2010;2(7):210-215.
11. Boudra A, Benallou B, Meliani S, Hamdi M. Comparison of ethanolic extract yield, ph, polyphenols and flavonoids in Algerian propolis collected from various geographic regions, Bionature. 2019;39(1):1-6.
12. Hendi NK, Naher HS, Al-Charrakh AH. *In vitro* antibacterial and antifungal activity of Iraqi propolis. J. Med. Plants Res. 2011;5(20):5058-5066.
13. Olson ME, Horswill AR. *Staphylococcus aureus* osteomyelitis: Bad to the bone. Cell Host & Microbe. 2013;13(6):629-631.
DOI: 10.1016/j.chom.2013.05.015
14. Nedji N, Loucif-Ayad W. Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition. Asian. Pac. J. Trop. Dis. 2014;4(6):433-437.
DOI: 10.1016/S2222-1808(14)60601-0
15. Taylor RSL, Manandhar NP, Hudson JB, Towers GHN. Antiviral activity of Nepales medicinal plants. J. Ethno. Aharmacol. 1996;52:156-163.
DOI: 10.1016/0378-8741(96)01409-2.
16. Hernández-Pérez M, López-García RE, Rabanal RM, Darias V, Arias A. Antimicrobial activity of *Visnea mocanera* leaf extracts. J. Ethnopharmacol. 1994;41(1-2):115-119.
17. Kim YH, Chung HJ. The effect of Korean propolis against foodborne pathogens and transmission electron microscopic examination. N. Biotechnol. 2011;28(6):713-718.
DOI: 10.1016/j.nbt.2010.12.006
18. Gülçin I, Bursal E, Sehitoğlu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem. Toxicol. 2010;48(8-9):2227-2238.
DOI: 10.1016/j.fct.2010.05.053