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Antioxidant and Antibacterial Activity of Algerian Propolis on resistant *Staphylococcus aureus* Isolated from Goat Clinical Mastitis.

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Abstract:

Antibiotic resistance is a major concern in human and veterinary medicine and poses a serious challenge to public health. Mastitis in goats is a frequent pathology that create a lot of problems in dairy farming, and its management imposes the use of ATB for treatment and prevention. However bacterial resistance, especially of *S.aureus*, a major mastitis pathogen, accelerates research for new drugs. Propolis is a natural bee product with various biological activities, such as antioxidant and antibacterial activity. The aim of this study is to assess antioxidant and antibacterial activities of Algerian propolis against *S.aureus* from goat mastitis. Five samples of propolis from different regions of Algeria were tested for antioxidant activity by the DPPH method using their ethanolic extracts. (AOA), and both disk diffusion and microdilution methods for antibacterial activity (ABA). Results show good antioxidant activity with IC 50 in the range of 0.167-1.5 mg/mL. And for antibacterial activity, all twenty strains were sensitive to ethanolic extracts of propolis (EEPs), with MICs ranging from ≤0.039 to 1.25 mg/ml, noting the high sensitivity of multidrug-resistant strains to EEPs (MICs: 0.039-0.312 mg/ml). Results suggest that propolis have a strong effect on *S.aureus*, promoting to the udder, reducing of oxidative stress (OS) and inhibiting bacterial growth.

Key words: Algerian propolis, Antioxidant activity, *S. aureus*, goat mastitis, Antibacterial, MIC.

Introduction:

Historically, humans have lived for extended periods without the discovery of antibiotics (ATB). This was made possible by using natural remedies, showcasing a harmonious relationship between man and his environment without using chemicals. This reality underscores the importance of nature in promoting alternative health solutions.

In our modern era, these precious drugs have become a staple in veterinary medicine and are widely used to treat or even enhance animal production However, a gradual decline in

discovery and development is noted, and at the same time, the evolution of drug resistance has been observed since 1970 (Hutchings *et al*., 2019). In goats dairy farming, antibiotics were served for the treatment and prevention of mastitis, an intramammary infection (IMI), a major problem that can have serious consequences for the health and welfare of the goats (Machado, 2018). This could result in significant losses for farmers in terms of milk production and quality, as well as additional costs for animal treatment, discarded contaminated milk, and animal culling . However, the development of Antibiotics resistance, especially among methicillin resistant staphylococci, increases the probability of treatment failure and threatens public health (Rubin JE *et al.*, 2011). *Staphylococcus aureus* is the most incriminated bacteria in goat clinic mastitis (Bergonier *et al.*, 2000), and by its toxins and other virulence factors cause a severe inflammation of the goat udder with fever, swelling, pain, oedema, loss of appetite, firm consistency, and frequently leading to gangrenous mastitis (Machado GP, 2018). In addition, contaminated dairy products threaten public health due to the presence of toxins and the risk of food poisoning (Acosta *et al*., 2018). *S.aureus* has developed antibiotic resistance to many drugs because this bacteria has a high potency for adaptation and acquisition of resistance genes through horizontal transfer (Haag *et al*., 2019; Peton and Le Loir. 2013), and consequently, new expensive antibiotics are needed (Tommasi *et al*., 2015) In addition, the problem of antibiotic residues in milk is a matter of concern, Virto *et al*., (2022) reported the presence of residues of antibiotic in cow and goat milk in several countries around the world after mastitis treatment or prevention, besides their presence in the commercial dairy products that could lead to human health problems or the spread of resistant bacteria. Oxidative stress (OS) is an imbalance between the production of reactive oxygen species (ROS) and and their safe disposal and repair, resulting cell damage (Turk. R, 2017).

Propolis is a resinous material produced by honey bees known for its biological activities. Its anibacterial effect against several species was studied in most studies against reference strains (Bittencourt *et al*., 2015; Boisard *et al.*, 2015; Choi *et al*., 2006; Bankova *et al.*, 1996). Moreover, the antioxidant activity of propolis, attributed to its composition rich in bioactive molecules, exhibits various beneficial health effects (Braakhuis, 2019).

Despite the propolis effect on a large number of gram-positive and gram-negative bacteria, its uses in veterinary medicine for preventive or curative purposes remain limited and irregular compared with the human health products that most companies and researchers focus on (Santos *et al*., 2019). The aim of this study is to assess the antioxidant and antibacterial activity of Algerian propolis on resistant *S. aureus* isolated from goat Clinical mastitis.

Material And Methods:

1-Propolis Samples :

Propolis samples were collected during the late summer of 2021–2022, from five different regions in Algeria. The raw propolis was provided by bee keepers Directly. Table 1 represents the sample characteristics and their origins. The samples were frozen at -20 °C until further analysis.

Tables and figures:

Table 1: Propolis geographic origin

Samples	Origin	Location	Year
1	Guelma	36°30′N/7 26°′E	2021
2	Tlemcen	34°53′N/1°19′W	2021
3	Blida	36° 28′N/2°38′E	2021
4	Tipaza	36°35′N/2° 26′E	2022
5	Djelfa	34°10′N/3°30′E	2022

2-Propolis Preparation and Extraction:

The frozen samples were crushed by using an electric grinder, and 20 grams of propolis powder were dissolved in 200 ml of 80% (W/V) ethanol (Sigma Aldrich-Germany). The mixture was handly shaken for five minutes. The extraction was performed in sterile flasks for seven days at room temperature in a dark place with daily shaking.

After the extraction period, the ethanolic extract of propolis (EEP) was filtered with Watman paper N 1. The obtained extracts were left in the freezer for over a night to precipitate wox. The solvent was evaporated under reduced pressure at 45 °C in a rotary evaporator (STUART RE300). Finally, evaporation was completed to dryness in sterile Petri dishes at 45 °C. The EEP was covered with aluminum paper to avoid light deterioration and conserved in a dark, cool place.

3-Antioxidant Activity Assay:

The antioxidant assay was performed according to the method of Ozdal et *al.* 2019 with slight modifications. The radical scavanging activity (RSA) and the IC50 value for each propolis extract were determined. The 1,1-diphenyl-2-picrylhydrazyl stock solution of 0.1 mM was prepared by dissolving 04 mg in 100 ml of absolute ethanol, and the solution was left in a dark place in a room temperator for two hours. The DPPH scavanging activity was screened for each

EEP at the concentrations : 0.1–2 mg/mL. The reaction mixture contained 200 µL of EEP solution and 2000 µL of DPPH. The mixture was vortexed and kept for 30 minutes in the dark. The decrease in absorbance was measured at 517 nm. The blank sample was 200 µL of absolute ethanol added to 2000 µL of DPPH solution. Ascorbic acid at concentrations of 0.05, 0.2, 0.3, 0.4, and 1 mg/mL was used as a reference standard. The percentage of inhibition (PI) was calculated as the percentage of reduced DPPH radical. It was calculated as below:

$$\mathbf{PI = (A_{blank} - A_{sample}) / A_{blank} \times 100\%} \quad (1)$$

where: A_{sample}—absorbance of propolis sample; A_{blank}—absorbance of blank sample

The assay was performed in triplicate.

4-Microbial Strains Isolation And Identification:

19 *Staphylococcus aureus* clinic starins were isolated at the Veterinary Institute of Tiaret University, Algeria. Samples of milk from goat's mastitic udder were obtained according to the recommendations of the National Mastitis Council's methods (National Mastitis Council, 1990) and transported under cooling conditions (+4 °C) into the laboratory. Blood Agar and Chapman agar were used for the isolation of *S. aureus* from milk samples. Identification was performed using biochemical tests: Gram staining, catalase, coagulase, and the DNAse tests. The confirmation of identification was carried out by Malddi-Toff. *S. aureus* ATCC 6538 was used as a reference strain. Isolates were conserved in BHI+25% glycerol at -20 °C.

5-Antibiotic Susceptibility Test:

The antibiotic susceptibility was evaluated by disc diffusion method according to CLSI Guidelines (2020). Briefly, 0.5 mcf inoculum was prepared from an18 hours culture of *S.aureus* in nutrient agar. Inoculation was performed by Kerby method on Muller Hinton agar (MH). The following antibiotic discs (HIMIDIA, India) were used: Penicillin(10µg), Amikacin (30 µg), Bacitracin (10 µg), Cefoxitin (30 µg), Gentamicin (10µg), Ciprofloxacin (5µg), Tetracyclin (30µg), Sulpham/Trimethoprim (25µg), Clindamycin (2µg), Erytromycin(15µg). After 24 h of incubation, the inhibition zone (**IZ**) was measured.

6-Antimicrobial Tests for EEPs :

The antimicrrobial susceptibility tests for different EEPs was performed using two methods :

A- Disc Diffusion Method :

A steril paper disc of 6 mm (HIMEDIA-India), was charged with 1 mg of diluted EEP. The discs were dried at 45 °C. A control disc charged with ethaol (80%) was used. Petri dishes of MH agar were plated with 0.5 mcf inoculum as specified in **Section 5**. The innoculated petri dishes were incubated for 24 h at 37 °C. The inhibition zone (**IZ**) was measured in mm.

B- Microdillution Method :

In order to evaluate Minimal imhibitory concentration (MIC) values for each EEP sample, the microdilution method as described by CLSI (2012) was used. Briefly, the density of the bacterial cells was adjusted to a final concentration of 10^5 CFU/mL in Muller Hinton broth (MHB). The cell suspension was mixed in 1:1 ratio with two-fold serial dilutions of EEP (0,039–5mg/mL) for each sample in MHB, with one well as negatif control, and other for 5% ethanol without propolis. The concentration of ethanol (the solvent) was kept constant (5% v/v) in each well. The culture was incubated at 35 °C for 20–24 h. The Triphenyltetrazulium chloride 1% (TTC) reactif was added to each well (10 µL), and the microplaque was reincubated for two hours at 35 °C. The positive culture wells will show a red color. The MIC value was determined as the lowest concentration, which had no color change, and consequently no visible growth. The most active samples from disc diffusion method were chosen for MIC assessement.

Statistical analysis :

Experiments in this study were completed in triplicate and data was expressed as the means ± SD. For the antibacterial activity meseaured as MICs, the difference between propolis samples were analyzed by SPSS software (Version 18), using one-way analysis of variance (ANOVA) followed by Tukey's test. The p value at the level of 0.05 was considered as statisticaly significant. GraphPad Prism® software was used for antioxidant activity analysis and Figure creation.

Results and Discussion :

The percentage of inhibition for three concentrations (0.2, 0.8, 1.6 mg/mL) of each EEP is illustrated in Figure 1. All ethanolic extracts of propolis (EEPs) showed a significant antioxidant activity (Figure 1). The IC 50 values (Figure 2) were in the range : 0.118 - 1.50 mg/ml. It's well known that more IC 50 is low, more the antioxidant capacity (AOC) is high. So Djelfa sample is the highest **AOC** among all EEP's (0,118 mg/ml). Samples from Tlemcen, Blida and Tipaza had close IC 50 values: 0.476 mg/ml, 0.486 mg/ml, 0.524 mg/ml, respectively. And finally, Guelma sample has the highest IC50 (1.50 mg/ml) and consequently the lowest AOC. Asorbic acid has an IC 50 of 0,051mg/ml. The analysis of AOA indicates that is dose and sample dependent.

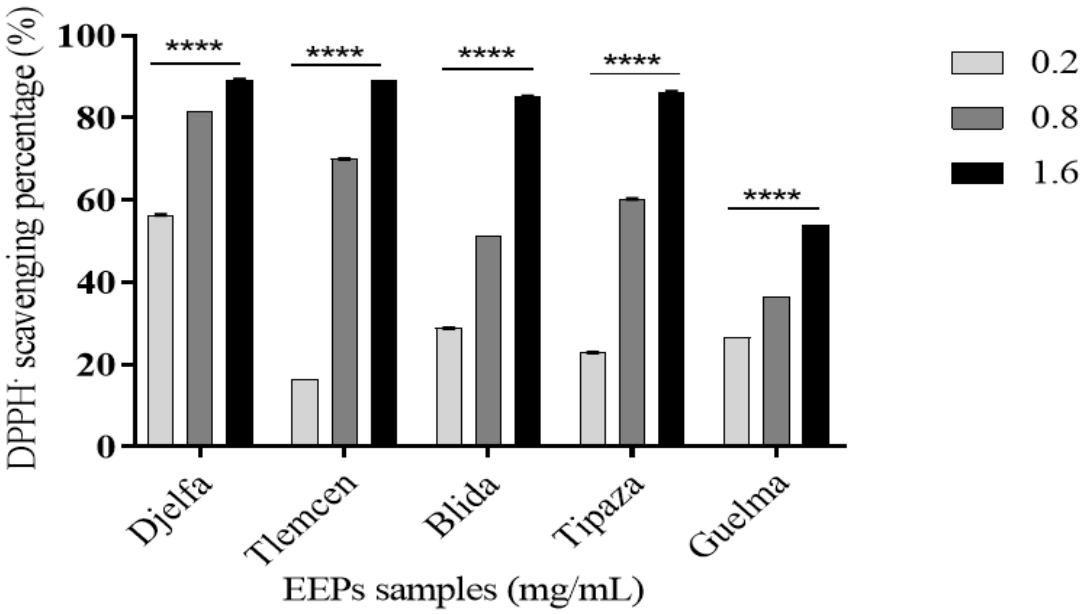

Figure 1 : Distribution of antioxidant activity (%) of propolis samples among Algerian regions by using DPPH test**,** and three concentrations of EEP : (0.2, 0.8, 1.6 mg/ml). Two-way ANOVA (*P*<0.001) followed by *Tukey* test multiple comparison between the groups was employed to asses differences between groups, bars from different samples of the same concentration not sharing the same letters differ significantly. While asterisks (****) indicate significative difference between the concentrations of EEPs of the same sample.

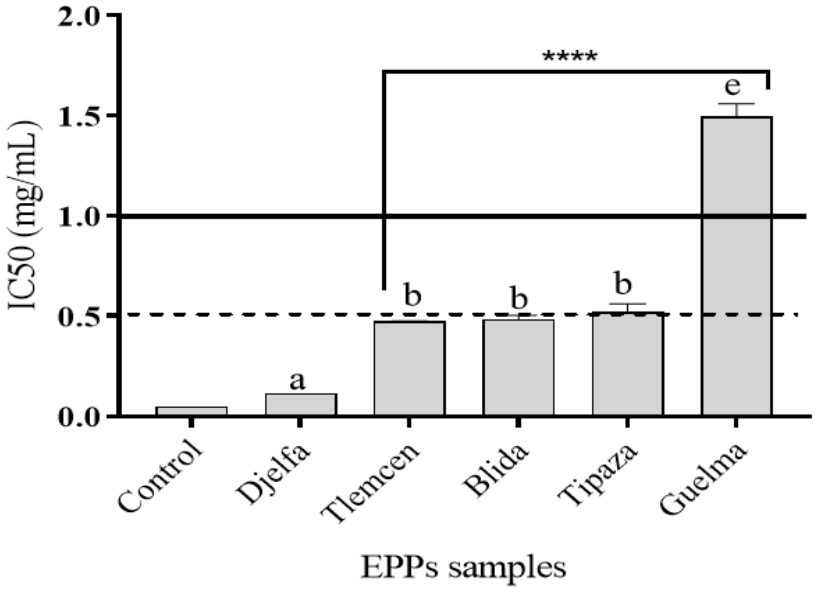

Figure 2 : IC50 (mg/mL) of EEPs from Algerian regions. Ascorbic acid (control) was used as positive control. One-way ANOVA was used followed by *Tukey* test multiple comparison

between the groups, bars not sharing the same letters differ significantly $P<0.05$. When it followed by *Dunnett* multiple comparison test to the control positive, bars superscript asterisks differ significantly $P<0.01$.

Antibiotic sucseptibility :

89% of *S.aureus* strains were resistant (17/19), multiresistance was estimated at 15,78% (3/19). Penicillin is the most concerned antibiotic in terms of resistance; thus, 57.89% (11/19) of isolates were resistant to penicillin. Other resistance to tetracyclin was significant with 36% (7/19) and bacitracin (26%) (5/19). A low resistance was noted: 5-15% for cefoxitin, clindamycin, and amikacin. gentamicin, sulfam/trimethprim, and erytromycin showed 100% efficiency. Three (3) methicillin resistant *S.aureus* (MRSA) strains were identified by disc diffusion technique(resistance to cefoxitin).

Antimicrobial activity of EEP :

The results of antibacterial activity are summarized In the Table 02 (Inhibition Zone), and MICs in Table 3.

All EEPs have exhibited antibacterial activity against tested strains. The most active EEP by disc diffusion method was Djelfa EEP (Mean IZ: 13.98±0.67mm), the weakest EEP was Guelma EEP (mean IZ: 7.12± 0.43mm) and it was not tested for MICs determination. The minimal inhibitory concentrations (MICs) were in the range: ≤ 0,039-1.25 mg/mL, and mean MICs were: Djelfa EEP has mean MIC estimated at 0.167±0.13 mg/mL, other mean values were: 0.453±0.29, 0.529±0,23, 0,564±0.42 mg/mL for Tlemcen, Blida, and Tipaza samples, respectively. Significant difference were found between samples from different geographic origins (p=0.002). The Tukey's test indicates that the Djelfa sample show a significant difference from Blida sample(p=0.08), and from Tipaza propolis(p=0.03).

Minimal bactericidal concentration (MBC) was ranging from 0.312 to 5mg/mL, or higher sometimes. Moreover, the EEP DJ seems to be the most active sample against *S.aureus* isolates, with the lowest MICs and MBCs. MRSA strains showed a high sensitivity to EEPs (C13-C15-C17), with MICs in the range: ≤0,039-0,312mg/mL. Djelfa EEP was the most active against them (MIC of ≤0.039mg/mL).

Table 2: IZ in mm. D: Djelfa, TLM:Tlemcen, BD:Blida, TPZ:Tipaza, Guelma:GLM

Strains	DJ	TLM	BD	GLM	TPZ
C1	19 ±2.12	10.5±0.70	10.5± 0.70	7.5± 0.70	10.5± 0.70
C2	15.75±0.35	11±00	10± 00	7± 1.41	11.5± 0.70
C3	14.5 ±0.70	10± 00	9.5± 0.70	6.5± 0.70	8.5± 0.70
C4	13.5 ±0.70	9.5± 0.70	11± 1.41	6± 00	11±2.82

C5	19±1.41	13.5± 0.70	12± 1.41	10.5± 0.70	11.5± 0.70
C6	14±00	9.25± 0.35	8.5± 0.70	7.5± 00	9± 00
C7	14±00	9.5± 0.70	8.75± 0.35	8± 00	9± 00
C8	12.5±00	8.15±0.91	8± 00	8± 0.70	7.75± 0.35
C9	13.25±0.35	10.4± 9.14	8.85± 0.21	7.5± 0.70	9.5± 0.70
C10	11±1.41	7.5± 0.70	8± 1.41	6± 00	8± 1.41
C11	13±0.70	8.5± 0.70	8± 00	7.4± 0.14	8.75± 0.35
C12	12.5±0.70	7.75± 1.06	7.25± 0.35	6± 00	9± 00
C13	13.5±0.70	9.5± 0.70	8.25± 0.35	8± 1.41	9± 00
C14	12±1.41	8.75± 0.35	8± 00	7.25± 0.35	7± 1.41
C15	13.5±00	8.75± 0.35	7.75± 0.35	6.5± 0.70	12± 2.82
C16	14±00	9.1± 0.14	9.4± 0.14	7±00	13.4± 0.28
C17	14.35±0.49	9± 00	10± 0.70	7.5±0.70	12.5± 0.70
C18	13.75±0.35	9± 0.70	8.9± 0.14	6.25±0.35	9.75± 0.35
C19	13±1.41	8.25± 1.06	7.5± 0.70	6± 0.00	8± 00
ATCC 6538	13.5± 0.70	95± 0.70	8± 00	6± 00	8.5± 0.70
MEAN+SD	13.98±0.67	9.37± 0.53	8.9±0.48	7.12± 0.43	9.7075± 0.73

Table 3: MICs /MBCs in mg/mL

	MIC				MBC			
Strains	DJ	TLM	BD	TPZ	DJ	TLM	BD	TPZ
C1	0.156	0.625	0.625	1.25	2.5	1.25	5	2.5
C2	≤0.039	0.156	0.625	1.25	0.312	5	5	1.25
C3	0.312	0.156	0.625	1.25	1.25	1.25	5	5
C4	0.312	0.312	1.25	1.25	1.25	5	>5	1.25
C5	0.312	0.625	0.625	0.312	1.25	2.5	5	5
C6	0.312	0.625	0.625	0.625	1.25	2.5	5	>5
C7	0.312	0.625	0.625	1.25	1.25	1.25	5	>5
C8	0.625	1.25	1.25	1.25	1.25	2.5	>5	5
C9	0.156	1.25	0.625	0.625	1.25	>5	5	5
C10	≤0.039	0.625	0.625	0.156	2.5	5	5	2.5
C11	0.078	0.625	0.312	0.312	1.25	5	2.5	5
C12	0.078	0.625	0.625	0.312	1.25	>5	2.5	5
C13	≤0.039	0.078	0.156	0.156	2.5	2.5	2.5	1.25
C14	≤0.039	0.078	0.312	0.156	2.5	2.5	5	2.5
C15	≤0.039	0.156	0.312	0.312	1.25	0.312	5	0.625
C16	0.078	0.078	0.156	0.156	1.25	5	2.5	0.625
C17	≤0.039	0.078	0.156	0.156	1,25	>5	5	1.25
C18	0.312	0.156	0.156	0.312	1.25	1.25	2.5	0.625
C19	≤0.039	0.312	0.625	≤0.039	2.5	1.25	5	0.625
ATCC 6538	≤0.039	0,625	0.312	0.156	2.5	2.5	2.5	2.5

Discussion:

Antioxidant activity:

Numerous studies had assessed the antioxidant activity of propolis worldwide (Choi *et al.*, 2006; Piccinelli et *al.*, 2013; Machado *et al.,* 2016; AL-Ani *et al.,* 2018; Kurek-Górecka *et al.*, 2022). Propolis is a complex resinous matrix that contains a large number of biologically active metabolites (Popova *et al.,* 2017). The DPPH assay is a famous method used to evaluate antioxidant activity, based on electron donation mechanism, and the ability of scavenging a stable free radical (DPPH), resulting in its reduction by the antioxidant In the present study, propolis samples from the East, West and Central and sub-saharan regions of Algeria are tested and compared for their antioxidant activity. The IC50 values were determined as the quantitie of EEP that result in a 50% reduction of DPPH.

Samples that have a lower IC 50 were correlated with higher antioxidant potential (Mohtar *et al.,* 2020). In this study, all samples exhibit antioxidant activity. The IC 50 was ranging between 0,118 and 1.5mg/ml. Djelfa and Telmcen samples showed the highest percentage of DPPH inhibition (89%), and Guelma propolis showed the lowest DPPH inhibition (56%). In a comparative study of the antioxidant activity of propolis from different regions around the world (Poland, Urguay, Turkey, Romania), Kurek-Górecka *et al.*, (2022) found an IC 50 ranging from 0,325 to 0.630 mg /mL. In a previous study Machado *et al.,* (2016) reported an IC50 of 163.00-273.46 mg/mL for ethanolic extract of brown propolis from Brazil and lowest values for green and red propolis (31.80-157.39 µg/mL). Piccinelli *et al.,* (2013) reported the antioxidant activity of Algerian propolis from Bejaia, and found an IC50 of 32-600 µg/mL, they Correlated antioxidant activity with his composition rich with polyphenolic compounds such as caffeic acid esters and flavanones, kaempferol , and galangin, and the low AOC in some samples was attributed to less polyphenol in EEP and high content of labdane diterpenes. AL-Ani *et al.,* (2018) analysed the AOC of European propolis from Germany, Ireland, and the Czech Republic and found that it have a highest AOC with IC50 : 26.45-36.40 µg/mL. Grecka *et al.,* (2019) found a lowest IC 50 in the range 11-75.06 µg/mL for the Polish EEP.

Flavonoids and phenolic acids are the most secondary metabolites in propolis samples that exherb a beneficial effects as antioxidants and prevent oxidative damage of DNA stimulated by ROS.

Over the last two decades, accumulating evidence has highlighted a significant relationship between inflammation and oxidative stress (OS). These two processes mutually reinforce each other, creating a vicious cycle that can sustain and amplify the inflammatory response (Lugrin *et al.,* 2014). Mastitis may lead to an increased formation of free radicals in milk, resulting in OS (Abd Ellah. 2013 ; Gu *et al.,* 2009). The elevated production of ROS in the milk of mastitis

is linked to an increased number of inflammatory cells, such as macrophages and neutrophils, and consequently a massive release of cytokines (TNF-α, IL-1β, IL-6, IL-8) and other molecules like nitric oxide (NO) (Notebaert *et al.,* 2008; Turk.R, 2017). IMI are associated with a reduced milk antioxidant capacity and a decrease in catalase, lactoperoxidase, glutathione peroxidase, or superoxide dismutase activity (Novac and Andrei. 2020). Moreover, total antioxidant capacity (TAC) was found to be lower in milk from infected udders, particularly in *S. aureus* infection. Additionally, markers of oxidative stress, as well as oxidant compounds, were present at higher concentrations in *S. aureus* udder infections compared to other pathogens or uninfected milk (Novac *et al.,* 2022). Thus Antioxidant may have a beneficial effect on udder immunity, and limit the damage caused by ROS. These evidences emphasize the importance of antioxidant level in the udder and the importance of free radicals reducing after accumulating in a high amount during *S.aureus* IMI, but further studies are needed to confirm that.

Antibiotic susceptibility test revealed a high resistance rate estimated at 89% (17/19) of *S. aureus* isolates, and 57.89 % to penicillin, and 37% of resistance to Tetracyclin. Nelli *et al.,* (2022) found a percentage of 64,3% of resistance in *S. aureus* isolates (18/28) in Greece, with less resistance to penicillin(39,3 %). The overall increase in milk production by dairy goats has resulted in a growing use of antimicrobials to treat or prevent mastitis in these animals, However, the availability of drugs registered for goat use is limited (Virto *et al.,* 2022). Antibiotic resistance came to complicate the situation and gave veterinarians limited therapeutic choices.

Antibacterial activity (**ABA**) of propolis extract on *S.aureus* was Studied in several studies (Choi *et al.,* 2006; Popova *et al.,* 2017; AL-Ani *et al.,* 2018; Wang *et al.,* 2021; Grecka *et al.,* 2019; Bouchelaghem *et al.,* 2022; Zohdi *et al., 2024*), most times against reference strains, and rarely against clinical isolates. Wild isolates, could be caracterized by an arsenal of virulence factors and resistance genes to several ATB due to the frequency of ATB use especially as a growth-promoting factor (Palma *et al.,* 2020), subsequently stimulating the pression of resistant mutants selection, and inducing resistance to ATB. The result of antibacterial activity (ABA) obtained in this study showed that Algerian propolis exhibits a strong **ABA** on *S. aureus* isolated from goat clinical mastitis.

In a study on the antimicrobial activity of algerian propolis from the East of Algeria using disc diffusion method, Nedji and Ayad. (2014) reported IZ of 9.75-12.25 mm against *S.aureus*, these results are consistent with the present study. Zohdi *et al.,* (2024) reported the ABA of

EEP from Malaysia on reference strain (ATCC 25923), obtaining IZ :10-10.50 mm, and MIC 1562 µg/mL and MBC 3125 µg/mL.

Wojtyczka et ***al***., 2013 found MICs in the range of 0.39-0.78 mg/ml for polish propolis against *S.aureus* clinical isolates, and MBC between 0.78 and 3.13mg/ml which is in Agreement with the current study. Drago *et al.,* (2007) reported a MICs ranged from 0.084 to 5.35 mg/ml for *S.aureus* isolated from human patients, and MBCs of 1.34-5.35 mg/ml. Results in this study are in agreement with Grecka *et al.,* 2019, their study was on Polish propolis, and the assessed MICs were in the range: 32-1024 µg/mL for cilnical isolates of *S.aureus* from human patients with different infections, and 128-512 µg/mL for reference strains. The obtained MBCs ranged from 512 to > 2048 µg/mL for clinical strains and from 128 to >4096 µg/mL for ATCC strains. They found a high sensitivity of Multi Drug Resistant (MDR) *S.aureus* to EEPs, with MICs between 64 and 512 µg/mL. Nevertheless, in our study, MDR were more sensitive to EEPs (MICs: ≤0,039-0,312mg/ml). These findings suggest that EEPs could be considered as a remedy for the treatment of goat mastitis caused by *S.aureus*.

Moreover, Grecka *et al.,* 2019, reported that when associated with ATBs, EEP decreases the MICs of most ATB, indicating a synergy for Teteracyclin, Gentamicin, Kanamycin, Amikacin, and Fusidic acid, and it is noted that the mode of action of these ATBs is inhibition of protein synthesis. Zhang et *al.,* 2022 found MIC of 50 µg/mL for MRSA in a study on chinese red propolis.

Assumpção *et al,*. (2022) tested the EEP on two *S.aureus* isolated from subclinical mastitis, and two reference strains, and reported highest MIC's 1.25-2.5mg/ml, with MBC's 5mg/ml or higher. Generally all *S.aureus* strains showed 100% sensitivity to four EEP from different regions of Algeria. We noted that MBCs had highest values compared to MICs, except the EEP from Djelfa, thus, we conclude that they have a bacteriostatic activity and the EEP from Djelfa a bactericide activity.

The antibacterial proprieties of propolis extracts are related to the possible synergistic effect of its components, on the collection region and the races of the honey bees (Bittencourt *et al.,* 2015). Some studies revealed that propolis exerts synergistic effects with antibiotics (Grecka *et al.,* 2019; Bouchelaghem *et al.,* 2022), acting on the bacterial wall structure and ribosome function. The mechanism of propolis ABA seems to be related to specific components. The potent bacteriostatic and bactericidal effects of propolis can be associated with their combined action, manifested by an inhibition of protein synthesis and bacterial growth by stopping cell division. The activity could be associated mostly with the high content of flavonoids such as galangin, pinocembrin, and pinobanksin, considered antimicrobial agents. Galangin and caffeic

acids are enzymatic inhibition factors causing the inhibition of bacterial growth. In addition, other active substances in propolis may disorganize the cytoplasmic membrane and cell wall, leading to bacteriolysis. Flavonoids affect the bacterial membrane functions and cause permeability alteration within the inner microorganism membrane (Popova *et al.,* 2017; Wang *et al.,* 2023). Other mechanisms of ABA are decreasing cell mobility, and reduction of adenosine triphosphate (ATP) production (Przybyłek and Karpiński, 2019).

There is an increasing need to discover and develop alternative therapies for the treatment of mastitis caused by multidrug-resistant bacteria. Amarante *et al.,*(2019) reported in their study that EEPs contained a high amount of coumaric and cinnamic acids and exhibited high antimicrobial activity against *Staphylococcus sp* and *S.aureus* strains. MBCs were in the range 34.3 and 137.5µg/mL.

Antibacterial activity of EEPs against *S.aureus* is realted to propolis origin, chemical composition (Przybyłek and Karpiński, 2019). Bankova et *al.,* 1996 reported the antibacterial activity of diterpenic Acids from Brazilian propolis. Salomão *et al.,* (2008) analyzed propolis from different regions of Brazil and found that high activity on *S.aureus* of Brazilian propolis. Extracts were positively correlated with its content of P-coumaric acid (PCUM) and derivatives. . Bittencourt *et al.,* (2015) correlated the strong activity of Brazilian propolis to the high content of the molecules: Tetradecanal, γ-palmitolactone and ethyl hydrocinnamate.

Propolis is a matrix that researchers reported the presence more than 300 components belonging to the flavonoids, terpenes, and phenolics (Huang *et al.,* 2014). Using propolis in therapies is reported since ancient times (Kuropatnicki *et al.,* 2013). Mastitis in goats is a growing concern to goat farming and a risk for public health, in the light of the rising of antbiotic resistance worldwide, propolis may be used in combination with ATBs to increase efficacy and lower minimum inhibitory concentrations as previously reported. Additionally, the problem of antibiotic residues in milk could be avoided using propolis.

Conclusion:

Mastitis due to *S.aureus* poses a serious problem to goat farming, besides antibiotic residues in dairy products after treatment. The antibiotic resistance is growing to several antimicrobial drugs. Results showed the efficiency of Algerian propolis in the inhibition of bacterial growth in vitro and antioxidant activity could help to control the bad effects of the infection and limit the damage caused by ROS.

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References:

- Abd Ellah, M. R.et al., 2013. Role of free radicals and antioxidants in mastitis. *Journal of Advanced Veterinary Research*, *3*(1), 1-7.

- Acosta, A. C., Oliveira, P. R. F., Albuquerque, L., Silva, I. F., Medeiros, E. S., Costa, M. M., ... & Mota, R. A. (2018). Frequency of Staphylococcus aureus virulence genes in milk of cows and goats with mastitis. *Pesquisa Veterinária Brasileira*, *38*, 2029-2036.

- Al-Ani, I., Zimmermann, S., Reichling, J., & Wink, M. (2018). Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. *Medicines*, *5*(1), 2.

- Amarante J.F., Ribeiro M.F., Costa M.M., Menezes F.G., Silva T.M.S., Amarante T.A.B., Gradela A. & Moura L.M.D. 2019. Chemical composition and antimicrobial activity of two extract of propolis against isolates of Staphylococcus spp. and multiresistant bacterials. Pesquisa Veterinária Brasileira 39(9):734-743.

- Assumpção YDM, Barbosa EV, Pereira RFA, et al., 2022. Comparative analysis between in vitro performances of the hydroalcoholic extract of green propolis and *Baccharis dracunoculifolia* against *Staphylococcus aureus. J. of Advan Vet Resea.*

- Bankova V, Marcucci MC, Simova S, Nikolova, N., Kujumgiev, A., & Popov, S. (1996). Antibacterial diterpenic acids from Brazilian propolis. *Zeitschrift für Naturforschung C*, *51*(5-6), 277-280.

- Bergonier D, De Crémoux R, Rupp R, et al.,2000. Mastitis of dairy small ruminants. Vet research 34(5) : 689-716.

- Bittencourt, ML, Ribeiro, P. R., Franco, R. L., *et al.*,2015. Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. *Food Resea Int 76* : 449-457.

- Boisard S, Le Ray AM, Landreau A, *et al*., 2015. Antifungal and antibacterial metabolites from a French poplar type propolis. *Evidence-Based Complementary and Alternative Medicine*, (1) : 319240.

- Bouchelaghem, S.; Das, S.; Naorem, R.S.; Czuni, L.; Papp, G.; Kocsis, M, et al., 2022. Evaluation of Total Phenolic and Flavonoid Contents, Antibacterial and Antibiofilm Activities of Hungarian Propolis Ethanolic Extract against Staphylococcus aureus. Molecules, 27, 574.

- Braakhuis, A. (2019). Evidence on the health benefits of supplemental propolis. *Nutrients*, *11*(11), 2705.

- Choi, Y. M., Noh, D. O., Cho, S. Y., Suh, H. J., Kim, K. M., & Kim, J. M. (2006). Antioxidant and antimicrobial activities of propolis from several regions of Korea. *LWT-Food Science and Technology*, *39*(7), 756-761.

- CLSI 2020.Performance standards for antimicrobial Susceptibility testing. 30th ed. M100 supplement (ISBN :978-1-68440-066-9 Print).

- Drago, L., De Vecchi, E., Nicola, L., & Gismondo, M. R. (2007). In vitro antimicrobial activity of a novel propolis formulation (Actichelated propolis). *Journal of Applied Microbiology*, *103*(5), 1914-1921.

- Ferreira, L.M.d.M.C.; Souza, P.D.Q.d.; Pereira, R.R.; da Silva, E.O.; Barbosa, W.L.R.; Silva-Júnior, J.O.C.; Converti, A.; Ribeiro-Costa, R.M, et al., 2024. Preliminary Study on the Chemical and Biological Properties of Propolis Extract from Stingless Bees from the Northern Region of Brazil. Processes, 12, 700.

- Grecka, K., Kuś, P. M., Okińczyc, P., Worobo, R. W., Walkusz, J., & Szweda, P. (2019). The anti-staphylococcal potential of ethanolic Polish propolis extracts. *Molecules*, *24*(9),1732.

- Haag, A. F., Fitzgerald, J. R., & Penadés, J. R, et al., 2019. Staphylococcus aureus in Animals. *Microbiology spectrum*, *7*(3), 10-1128.

- Huang, S., Zhang, C. P., Wang, K., Li, G. Q., & Hu, F. L. (2014). Recent advances in the chemical composition of propolis. *Molecules*, *19*(12), 19610-19632.

- Hutchings, MI, Truman AW, & Wilkinson B. 2019. Antibiotics: past, present and future. *Curr opi in microbiology*, *51* : 72-80.

- Kurek-Górecka, A.; Keskin, S.; Bobis, O.; Felitti, R.; Górecki, M.; Otr˛eba, M.; Stojko, J.; Olczyk, P.; Kolayli, S.; Rzepecka-Stojko, A,2022. Comparison of the Antioxidant Activity of Propolis Samples from Different Geographical Regions. Plants, 11, 1203. https:// doi.org/10.3390/plants11091203.

-Kuropatnicki, A. K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-Based Complementary and Alternative Medicine*, *2013*(1), 964149.

- Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., & Liaudet, L, *et al.*, 2014. The role of oxidative stress during inflammatory processes. *Biological chemistry*, *395*(2), 203-230.

- Machado, B. A. S., Silva, R. P. D., Barreto, G. D. A., Costa, S. S., Silva, D. F. D., Brandao, H. N., ... & Padilha, F. F. (2016). Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic regions in Brazil. *PloS one*, *11*(1), e0145954.

- Machado, G. P. (2018). Mastitis in small ruminants. Anim. Husb. Dairy Vet. Sci : 2(4), 1-9.
- Mohtar, L. G., Messina, G. A., Bertolino, F. A., Pereira, S. V., Raba, J., & Nazareno, M. A.et al., 2020. Comparative study of different methodologies for the determination the antioxidant activity of Venezuelan propolis. *Microchemical Journal*, *158*, 105244. [CrossRef]
- National Mastitis Council, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. National Mastitis Council Inc., Arlington, VA, USA.
- Nedji, N., & Loucif-Ayad, W. (2014). Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition. *Asian Pacific Journal of Tropical Disease*, *4*(6), 433-437.
- Nelli, A.; Voidarou, C,Venardou, B.; Fotou, K.; Tsinas, A.; Bonos, E.; Fthenakis, G.C.; Skoufos, I.; Tzora, A, et al., 2022. Antimicrobial and Methicillin Resistance Pattern of Potential Mastitis-Inducing Staphylococcus aureus and Coagulase-Negative Staphylococci Isolates from the Mammary Secretion of Dairy Goats. Biology, 11, 1591.
- Notebaert S, Demon D, Berghe TV, Vandenabeele P, & Meyer E.et al., 2008. Inflammatory mediators in *Escherichia coli*-induced mastitis in mice. *Comparative immunology, microbiology and infectious diseases*, *31*(6), 551-565.
- Novac, C. Ș., Nadăș, G. C., Matei, I. A., Bouari, C. M., Kalmár, Z., Crăciun, S., ... & Andrei, S. et al., 2022. Milk Pathogens in Correlation with Inflammatory, Oxidative and Nitrosative Stress Markers in Goat Subclinical Mastitis. Animals, 12(23), 3245.
- Ozdal, T., Ceylan, F. D., Eroglu, et al., 2019. Investigation of antioxidant capacity, bioaccessibility and LC-MS/MS phenolic profile of Turkish propolis. Food Research International, 122 : 528-536.
- Palma, E., Tilocca, B., & Roncada, P. (2020). Antimicrobial resistance in veterinary medicine: An overview. *International journal of molecular sciences*, *21*(6), 1914.
-Piccinelli AL, Mencherini T, Celanoet R et al., 2013. Chemical Composition and Antioxidant Activity of Algerian Propolis. J. Agric. Food Chem. 29;61(21):5080-8.
- Popova M, Giannopoulou E, Skalicka-Woźniak K, , *et al.*,2017. Characterization and biological evaluation of propolis from Poland. *Molecules*, *22*(7) : 1159.
- Przybyłek, I., & Karpiński, T. M. (2019). Antibacterial properties of propolis. *Molecules*, *24*(11), 2047.
-Rubin JE, Ball KR, Chirino-Trejo M. 2011. Antimicrobial susceptibility of Staphylococcus aureus and Staphylococcus pseudintermedius isolated from various animals. Can Vet J. Feb;52(2):153-7.

- Salomao, K., Pereira, P. R. S., Campos, L. C., Borba, C. M., Cabello, P. H., Marcucci, M. C., & De Castro, S. L. (2008). Brazilian propolis: correlation between chemical composition and antimicrobial activity. *Evidence-Based Complementary and Alternative Medicine*, *5*(3), 317-324.

- Santos, L. M., Fonseca, M. S., Sokolonski, A. R., Deegan, K. R., Araújo, R. P., Umsza-Guez, M. A., ... & Machado, B. A. (2020). Propolis: types, composition, biological activities, and veterinary product patent prospecting. *Journal of the Science of Food and Agriculture*, *100*(4), 1369-1382.

- Tommasi, R., Brown, D. G., Walkup, G. K., Manchester, J. I., & Miller, A. A. (2015). ESKAPEing the labyrinth of antibacterial discovery. *Nature reviews Drug discovery*, *14*(8), 529-542.

- Turk, R. (2017). The role of oxidative stress and inflammatory response in the pathogenesis of mastitis in dairy cows. Mljekarstvo, 91–101.

-Vincent Peton, Yves Le Loir. Staphylococcus aureus in veterinary medicine. Infection, Genetics and Evolution (2013).

- Virto, M.; Santamarina-García, G.; Amores, G.; Hernández, I, et al., 2022. Antibiotics in Dairy Production: Where Is the Problem? Dairy, 3, 541–564.

- Wang, F., Liu, H., Li, J., Zhang, W., Jiang, B., & Xuan, H. (2021). Australian propolis ethanol extract exerts antibacterial activity against methicillin-resistant Staphylococcus aureus by mechanisms of disrupting cell structure, reversing resistance, and resisting biofilm. *Brazilian Journal of Microbiology*, *52*, 1651-1664.

- Wojtyczka, R. D., Dziedzic, A., Idzik, D., Kępa, M., Kubina, R., Kabała-Dzik, A., ... & Wąsik, T. J. (2013). Susceptibility of Staphylococcus aureus clinical isolates to propolis extract alone or in combination with antimicrobial drugs. *Molecules*, *18*(8), 9623-9640.

- Zohdi, R. M., Yaacob, N. N., Hasif, N. A. M., Adli, M. A., Payaban, M., James, R. J., & Jaapar, F. (2024). Comparative study of different Malaysian Stingless bee propolis: Physicochemical characterization, Phytochemical contents and Antibacterial activity. *Research Journal of Pharmacy and Technology*, *17*(3), 1021-1028.