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Propolis extract effect against methicillin resistant Staphylococcus aureus MRSA

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Abstract

The Methicillin-resistant Staphylococcus aureus (MRSA) bacteria have been considered as a main cause of nosocomial infections in hospitals, there is a real therapeutic interest to investigate natural compounds or extracts which are able to limit this resistance. The aims of this study are the MRSA strains detection and the assessment of their resistance profile against diverse antibiotic families. Besides, the study aims to assess the antibacterial activity of the propolis against these multi-resistant strains. To achieve this purpose, 44 S. aureus strains were isolated and identified by standard tests. Both Antibiotic sensitivity and antibacterial activity of propolis were determined using the Mueller-Hinton agar diffusion method. A low rate of MRSA (20.45%) has been revealed compared to those noted by the 13th and 15th evaluation reports of the Algerian network for observing bacterial resistance to antibiotics (respectively 32.67% and 47.33%). The resistance profile analysis of MRSA against antibiotic confirmed the multi-resistant nature of these bacteria to several antibiotic families, especially aminosides and macrolides. The studied propolis showed a large antibacterial effect against all our MRSA multi-resistant strains. This effect is related to its high total polyphenols and flavonoids levels. Therefore, propolis exhibited real potential in an alternative fight against staphylococcal infections.

Key words: *nosocomial infection, resistance phenotype, MRSA (meticillin-resistant S. aureus), EEP (ethanol propolis extract).*

1. Introduction

Staphylococcus aureus known also as positive coagulase *Staphylococcus* is a pathogen Gram positive, ubiquitous commensal bacterium of the skin and mucous membranes in humans and animals, frequently found in multiple infections of nosocomial and community origins [25]. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a major public health challenge in several healthcare settings around the world. MRSA is a common cause of epidemics and has converted to endemic in various areas where it raises the load of morbidity, mortality and the care cost associated with nosocomial infections [3, 25]. They were first described; in continental Europe, then in America and elsewhere in the world; as a threat especially in hospitals. But nearby the 2000 years, new clones were described as responsible for community infections. The emergence of these community strains was first described in USA, then in several countries around the world. Unlike the other Maghreb countries, Algeria has the highest MRSA prevalence (44%) comparing to Tunisia (18%) and Morocco (19%) [2,28]. In addition, MRSA is so often resistant to various other families of antibiotics, making it more difficult to choose the best therapeutic option. Their resistance is related to their high genomic plasticity that can be acquired or provided by a plasmid or other mobile elements (staphylococcal chromosomal cassette, transposons, bacteriophage, and insertion sequences) [1, 6]. Thus, there is a real therapeutic interest to investigate natural compounds or extracts that are able to limit

this resistance. In this context we suggest a mysterious product of the hive rich in active components with interesting pharmacological properties, including antioxidant, anti-inflammatory but especially natural antibiotics: it is propolis, which is still unknown to the general public, but it has a great future [3]. Propolis is plant mastic, made from resins harvested from the stems and shoots of certain trees and balsamic plants. Bees bring it to the hive, add it up and probably modify it partly by the contribution of some of their own secretions (mainly salivary wax and secretions) [12, 18]. Its composition is extremely complex and variable; more than 300 different components of propolis have been almost identified [22]. Nevertheless, it is constantly and relatively stable to find: resins (50 to 55%), waxes (30 to 40%), volatile oils (5 to 10%), pollen (5%), amino acids, vitamins, trace elements, fatty acids and flavonoids (the main one being galangin) [22, 26].

2. Materials and methods

Bacterial strains

Our current study focused on 44 strains of *Staphylococcus aureus* isolated and identified in bacteriology laboratories of the hospital of Ain fakroun, city of Oum El Bouaghi (Algeria) during the year 2015. These strains derived from various pathological products mainly different pus, urine and blood culture. Two other *S. aureus* reference strains were also used: *S. aureus* ATCC 43300 and *S. aureus* ATCC 252923. The isolation and identification of *S. aureus* strains was performed by conventional techniques: microscopic morphological examination, isolation on Chapman agar medium, presence of catalase and demonstration of free coagulase.

The sensitivity of *S. aureus* strains was determined by the Muller-Hinton agar diffusion method according to the recommendations of the antibiogram committee of the French Microbiology Society (CA-SFM 2015) [13]. The *S. aureus* resistance to penicillin M (oxacillin) was investigated using a cefoxitin disk (30 µg); in addition to the oxacillin disk itself under standard conditions. The interpretation criteria are: ≥ 27 mm, sensitive to oxacillin; <25 mm, resistant to oxacillin [9, 13].

Propolis

The source of studied propolis was obtained from Jijel (Algeria); the ethanol extract of propolis (EEP) was prepared in the phyto-pharmacology laboratory of Biology Department in the University of Jijel; Algeria. The cut Propolis into small pieces was submerged in ethanol (95%) for 15 days. After filtration, the solvent was evaporated at 79 ° C using a rotary evaporator (Evaporator E100). The residue was macerated in methanol (70%) overnight. After evaporation, the obtained extract is called crude extract of propolis.

Two EEP supports of 6 mm in diameter each were used: discs and wells each received 10 µl of the test solution. The dishes were incubated at 37 ° C for 24 hours. The biological activity is manifested by the appearance of an inhibition halo of microbial growth around the well and the disks containing the extract to be tested.

The sensitivity of isolated MRSA strains to propolis is evaluated by the diffusion technique in Muller Hinton agar medium in petri dishes according to the recommendations of (CA SFM 2015) [13].

3. Results

A prevalence of **0.34%** was provided by isolating **44** *S. aureus* strains on **12600** samples, from pus (61.36%), urine (15.90%), vaginal samples (13.63%), as well as care equipment and blood culture with a relatively low frequency (2.27%) (Figure 1).

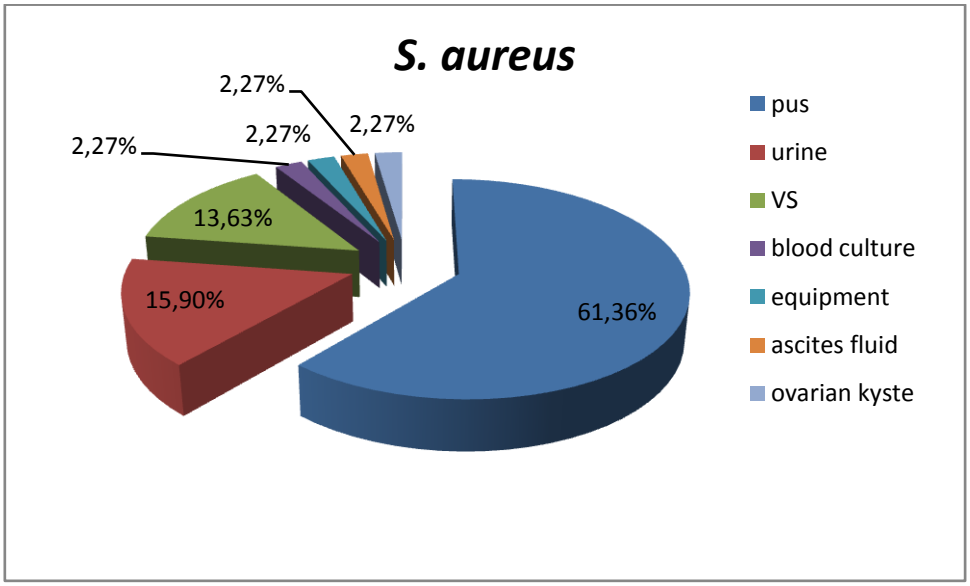


Figure 1: Distribution of *S. aureus* strains according to samples.

MRSA rate

The study of the antibiotic sensitivity detected 09 strains resistant to methicillin MRSA among the 44 isolated strains of *S. aureus*, providing a prevalence of (20.45%). These MRSA strains were mainly isolated from pus (55.55%), urine (22.22%) and vaginal samples (11.11%) (Figure 2).

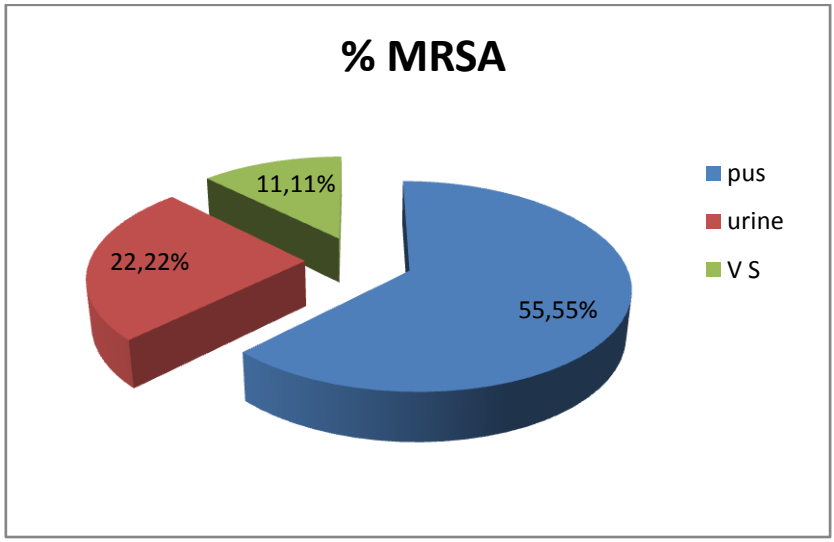
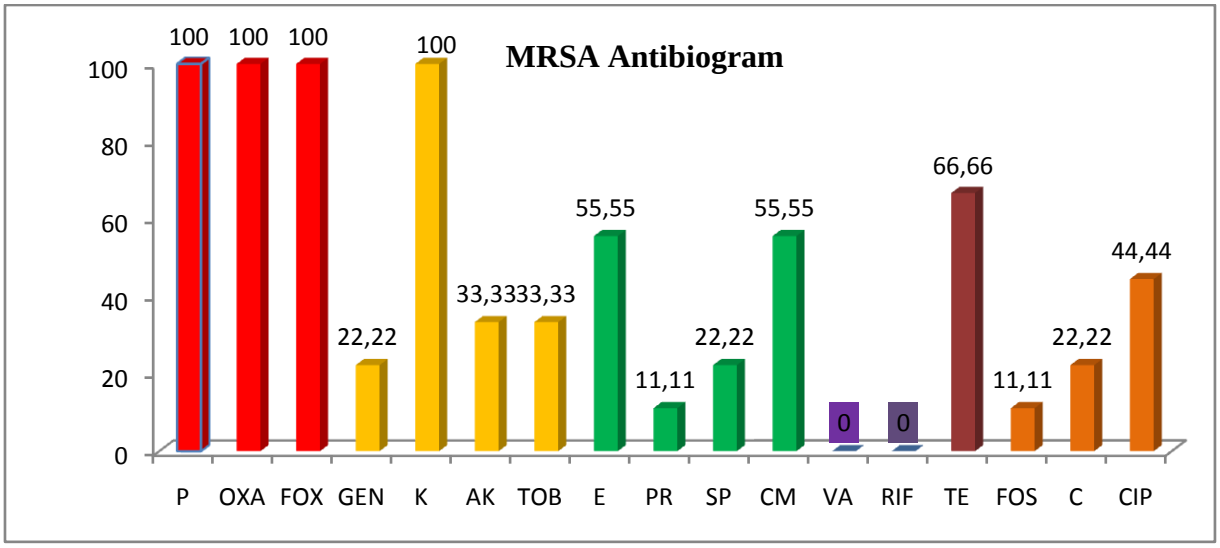


Figure 2: Distribution of *S. aureus* strains resistant to methicillin according to samples.

The resistance evaluation of the MRSA strains to antibiotics revealed the multi-resistance of isolated strains.



tams: Penicillin G (P) Oxacillin (OX) Cefoxitin (FOX)
nosides: Gentamicin (GEN) Kanamycin (K) Amikacin (AK) Tobramycin (Tob)
: Macrolides: Erythromycin (E) Spiramycin (SP**) Lincosamides**: Clindamycin (CM) **Streptogramin** pristinamycin (PR)
Glycopeptides: Vancomycin (VA)
acycline (TE

Other: Fluoroquinolones Ciprofloxacin (CIP) Chloramphenicol (C) Fosfomycin (FOS) Rifamycin (Rif)

Figure 3: Sensitivity of MRSA to antibiotics

All isolated MRSA strains showed cross-resistance to β-lactams and associated resistance with other families of antibiotics (Figure 3). The resistance of our MRSA to aminosides exhibited three different phenotypes, involving three inactivating enzymes. All MRSA strains (100%) were resistant to kanamycin. 4 strains had a K-phenotype (44.44%), due to the production of the enzyme Aminosides-phosphotransferase APH (3 ') - III. A rate of 33.33% MRSA strains were resistant to kanamycin and tobramycin, the KT phenotype expressed by the production of the enzyme Aminoside-Nucleotidyltransferases ANT (4 ') (4' ') - I. While 22.22% MRSA strains expressed a KTG phenotype, they were resistant to three antibiotics (kanamycin, tobramycin, gentamicin), due to Aminoides-acetyltransferases enzyme (AAC) APH (2 '') - AAC (6 ') - APH (2' '). Concerning MLS (Macrolide Lincosamides and Streptogramins) a similar resistance rate of 55.55% was recorded with both Macrolides (erythromycin) and Lincosamides (Clindamysin) while a low level of 11.11% was recorded with Streptogramins (pristinamycin). In addition, for the other antibiotics, the levels were 66.66% for tetracyclin, 44.44% for ciprofloxacin, 22.22% for chloramphenicol and a lowlevel for fosfomycin (11.11%). Furthermore, no resistance was detected for the two antibiotics vancomycin and rifampicin. They can therefore be good alternatives therapy.

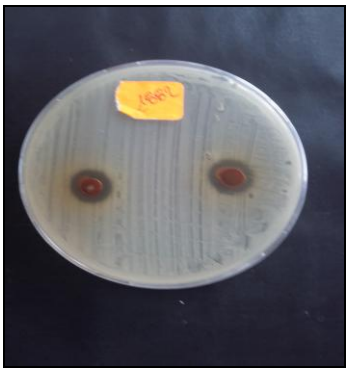
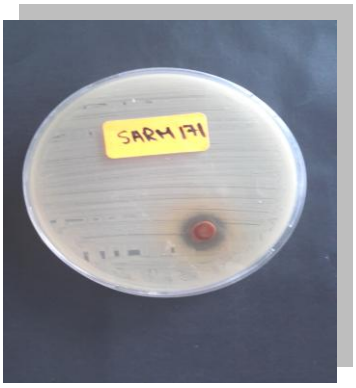
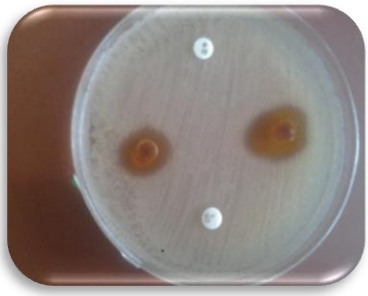
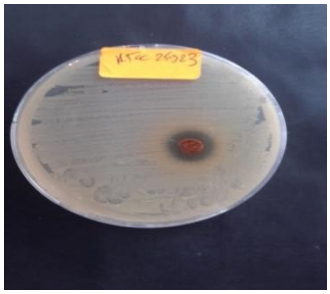
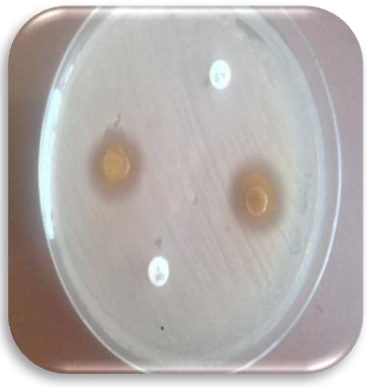
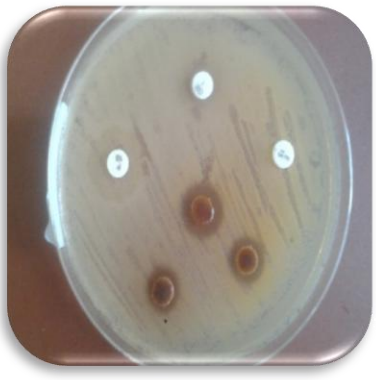
		
MRSA 1882	**MRSA 171**	**MRSA BIO**
		
ATCC43300	**ATCC43300**	**ATCC 252923.**
		
MRSA 215 **ATB: P OX**	**MRSA 171** **ATB: P OX E propolis**	**MRSA BIO** **ATB: AMP OX** **Propolis**

Figure 4: Antibacterial activity of propolis against MRSA strains and ATCC strains

The results detailed in Figure (4) showed that the tested propolis had an antibacterial effect against all MRSA multi-resistant studied strains.

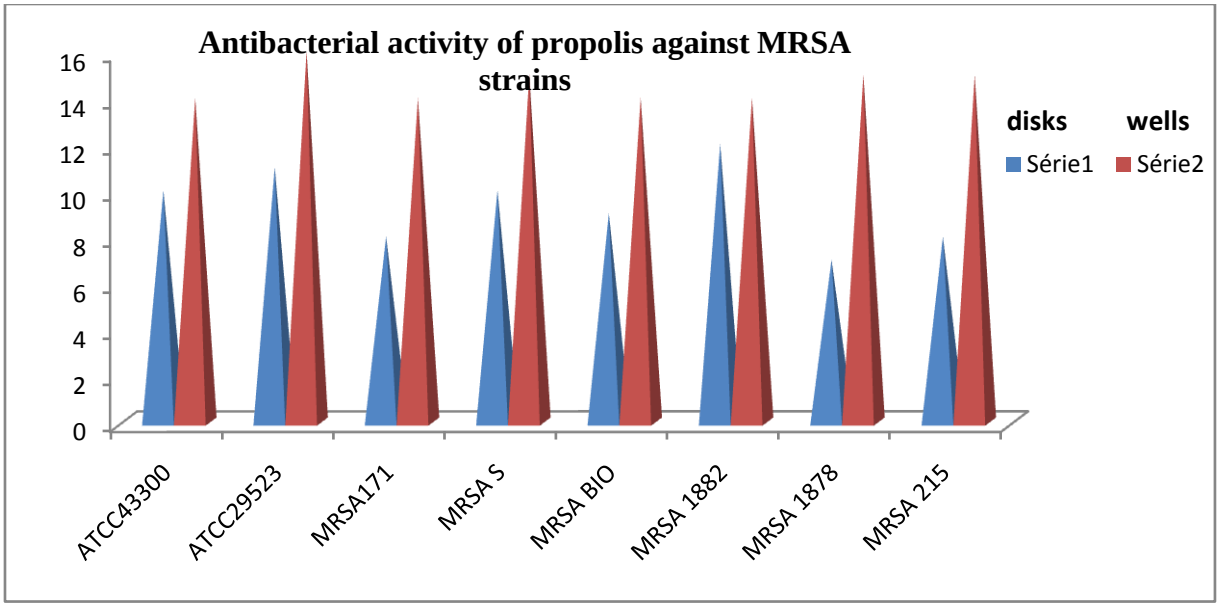


Figure 5: Antibacterial activity of propolis against MRSA strains according to two methods wells and disks

The antibacterial activity of propolis was studied according to two systems: disks and wells. According to the noted results (Figure 5), inhibition zones with wells method were larger than those of the disks.

4. Discussion

The main rates of *S. aureus* and MRSA were isolated from pus, respectively 61.36% and 55.55%. Definitely, *S. aureus* is a major bacterial species in medical pathology. It is one of the main etiological agents for superficial and deep suppurated infections [11]. Certainly, the rate of MRSA noted in this study (20.45%) was lower than that reported by the 13th and 15th evaluation report of the Algerian survey network for the bacterial resistance against antibiotics [19, 20]. According to both reports, the global analysis of *S. aureus* species data produced a resistance percentage of MRSA between 32.67% and 47.33%. Hence, this rate is relatively stable from one year to the next (between 35% and 45% since 2005) [19, 20].

Besides, the analysis of the antibiotic resistance profile of MRSA confirmed the multi-resistant nature of these bacteria against different families of antibiotics. Isolated MRSA strains were completely resistant to penicillin G, oxacillin and cefoxitin.

S. aureus has developed different mechanisms of antistaphylococcal resistance. More than 90% of *S. aureus* strains produce penicillinase [5, 6]. MRSA strains developed a cross-resistance between penicillins M (methicillin, oxacillin) and other β-lactams by producing PLP2a proteins that linked penicillin (PLP) and had low affinity for its compounds. In fact, the gene coding PLP2a, mecA, is carried by a chromosomal element that also contains other resistance genes against other antibiotics (aminosides and macrolides), which explains the multi-resistance profile of MRSA [1, 6].

On behalf of aminosides, the main resistance level of our MRSA was kanamicin (phenotype K) with 44.44% followed by phenotype KT 33.33%. However a relatively low resistance rate against

gentamycin phenotype KTG 22.22% has been noted. Thus, this result supported those of several authors who reported that about 80% of MRSA were phenotypic (KT) and that the gentamicin resistance frequency among MRSA increased during the 1970s and 1980s, reaching about 90% of strains around 1990 [1]. Since the beginning of the 1990s, the tendency has reversed, with gentamicin resistance frequencies of 50 to 60% between the years 1995 and 1998, of 11 to 20% in 1999 and 2000. Therefore, gentamicin is the most often active aminside on *S. aureus* [1, 6].

The focal MRSA resistance mechanism against aminosides is enzymatic. The enzymes APH (3 '), ANT (4') and APH (2 ') - AAC (6') respectively inactivate kanamycin (K phenotype), kanamycin and tobramycin (KT phenotype), and kanamycin, tobramycin and gentamicin. (KTG phenotype) [5,14].

In addition, an erythromycin resistance rate of 55.55% was noted, correspondingly to the result reported by Batard et al. 2005, which noted a level of 40 to 50% of hospital strains of *Staphylococcus* species that were resistant to erythromycin. However it remains higher than what was reported in the 15th national report of antibiotic resistance survey (32.81%) [20]. The results showed that the resistance to clindamycin was associated with that of erythromycin (55.55%). According to several authors, typically erythromycin and clindamycin resistant strains have a constitutive expression *erm* gene (MLSgB phenotype) that confers resistance to Macrolides, Lincosamides, and Streptogramins B. Besides, Pristinamycin retained bacteriostatic activity on our MRSA strains, 88.89% were sensitive to this antibiotic. Though, the resistance rate of our strains to the same antibiotic was 11.11%, it remained a slight high compared to that noted nationally (2.70%) [20] and that reported in a Tunisian study which noted no resistance to this same antibiotic [21]. The most common resistance mechanism of MRSA to MLSb macrolides, lincosamides, synergistin B is the modification of the 23S rRNA target by methylation [14]. Several genes for MLS resistance have been noted in *S. aureus* [1]. The *erm* genes (A, B or C): erythromycin ribosome methylase exchange cross-resistance to macrolides, lincomycins and streptogramins B. Their expression can be either inducible or constitutive [1, 5]. Although *erm* genes exchange streptogramin B resistance, pristinamycins retain bacteriostatic activity on *S. aureus* due to the synergism between streptogramin A and B [1].

For other antibiotics, the resistance levels were 66.66% for tetracycline, 44.44% for ciprofloxacin, 22.22% for Chloramphenicol and a relatively low level for fosfomycin 11.11%.

Fluoroquinolones have the advantage of high bactericidal activity, good tissue diffusion and excellent bioavailability. Inappropriately, their strong ability to select resistant mutants has shown high levels of resistance in fluoroquinolone MRSA within a few years. The ciprofloxacin resistance rate recorded in this study (44.44%) is lower than that reported by Leclercq et al. 2003 in France, which indicated that approximately 60 to 70% of MRSA strains were resistant to fluoroquinolones [15]. Like fluoroquinolons, rifampicin and fosfomycin cannot be used as mono-therapy due to the fast emergence of resistant mutants. In France, approximately 90% of MRSA are sensitive to these two antibiotics [15].

Vancomycin and rifamycin retained reputable activity on our MRSA strains. In fact, glycopeptides(vancomycin and teicoplanin) are the first-line treatment for resistance to meticillin or allergy to penicillins [14].

The study of the antibacterial activity of propolis with respect to our MRSA strains showed that EEP indicated a high antibacterial activity against our

MRSA strains. Previous studies have emphasized on the antibacterial action of propolis [24]. Its antibacterial spectrum is very broad, acting on multiresistant bacteria such as MRSA [4, 16, 24]. This property is mainly due to flavonoids and phenol acids, particularly galangine, pinocembrin, caffeic, ferulic and salicylic acids [8, 18]. However, the mechanism of action is still poorly understood. Japanese researchers believe that the inhibition of bacterial growth is due to the destruction of their wall thus preventing their cell division [22]. Other mechanisms are noted, such as disruption of the cytoplasm and inhibition of bacterial RNA polymerase due to the loss of their ability to bind to DNA [12]. The propolis antibacterial activity was larger with the well method than the discs. This result is reliable with the report of SaeedEshraghi(2008), who noted larger antibacterial activity with the well method as it allows a better diffusion of PEE [7].

Several *in vitro* studies have also revealed a real synergism between propolis and antibiotics [16, 24]. Therefore, these propolis extracts could be a real potential in an alternative fight against staphylococcal infections.

5. Conclusion

MRSA endures to be a global concern and a real public health problem in our country. The high level of MRSA resistance; and to other families of antibiotics such as aminosides, justifies the necessity for careful monitoring of the diffusion of these strains. The importance of propolis is mainly due to its high content of bioflavonoids; that are natural substances with a natural antibiotic activity. It appears from this current study that propolis is a very interesting product that may have therapeutic possibilities due to its antibacterial effect especially toward GRAM positive bacteria and even on multi-resistant bacteria such as MRSA.

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