© 2019 IJSRSET | Volume 6 | Issue 6 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section : Engineering and Technology
DOI : https://doi.org/10.32628/IJSRSET19662

Antibacterial Activity of Sudanese Propolis Extract Against Methicillin-susceptible and Methicillin-resistant Staphylococcus aureus isolates in Khartoum State, Sudan

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ABSTRACT

Introduction: Staphylococcus aureus is a Gram-positive round shaped bacterium frequently found in the upper respiratory tract and on the skin. The emergence of antibiotic-resistant strains of S. aureus such as methicillin-resistant S. aureus (MRSA) is a worldwide problem in clinical medicine. It is resistant to various antibiotic medications. Propolis is (bee glue) a flavonoid-rich product of honey comb, derives from the Greek pro "before" and polis "city", it exhibits antibacterial and anti-inflammatory properties which indicate that it can be an extremely powerful natural antibiotic and useful when fighting off upper respiratory infections. Materials and Methods: This was a descriptive cross-sectional study conducted at Soba University Hospital, Sudan. Following ethical consideration, 100 isolates of Staphylococcus aureus from different clinical samples, 50 samples of MSSA and 50 samples of MRSA were enrolled. subculture were used to re-identified the Staphylococcus aureus based on colonial morphology, Gram's stain, and other biochemical test were used for MSSA and MRSA detection. Perforated plastic plate's technique used for propolis collection and measurement of the inhibition zone were used for detection of sensitivy or resistant reaction. Results: All of the study MSSA samples (50/50; 100%) and MRSA (50/50; 100%) samples which cultured with a different concentrations of Al-Gelly propolis extract were shown a resistant inhibition zone while (50/50; 100%) of the study MSSA samples and (50/50; 100%) of the study MRSA samples which cultured with a different concentrations of Al-Fao propolis extract were shown sensitive inhibition zone at different sensitivity levels. The sensitivity levels of both MSSA and MRSA to the Al-Fao propolis extract was significantly correlate with the concentration of the propolis extract (P value 0.000, 0.000 respectively). The greatest effect will be a product of 20% concentration.

Keywords : Staphylococcus Aureus, Methicillin-Resistant S. Aureus, Methicillin-Suspected S. Aureus, Propolis, Bacterial Activity, Khartoum, Sudan.

I. INTRODUCTION

Staphylococcus aureus:

It is a Gram-positive round shaped bacterium frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is facultative anaerobe that can grow without the need for oxygen. Although *S.aureus* usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections, respiratory infections and food poisoning(1).

An estimated 20% to 30% of the human populations are long-term carriers of *S.aureus* which can be found as part of the normal skin flora and as a normal inhabitant of the lower reproductive tract of women (2,3,4,5,6).

S.auereus can cause a range of illnesses, from minor skin infections to life-threatening diseases. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery(7).

The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved(1).

Methicillin-resistant S. aureus (MRSA):

Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to various antibiotic medications (Superbug). MRSA inquires significant risks of short-term morbidity and mortality. It has inevitably become a worldwide concern that colonizes and mainly infects patients who have been hospitalized or in other health care environments, this is specifically known as health care-associated MRSA (HA-MRSA). Another variation of MRSA infection occurs within a community that inhabits healthy individuals, this form is called community-associate MRSA. It is spread through skin-to-skin contact. Crowded areas are at higher risk. MRSA constantly evolves and rapidly resists the development of many new drugs. In fact, many public health experts are alarmed by the spread of tough strains of MRSA because it is extremely difficult to treat (13,14).

Methicillin-susceptible Staphylococcus aureus (MSSA):

Methicillin-susceptible Staphylococcus aureus (MSSA) is strain of staph bacteria that responds well to medicines used to treat staph infections. MSSA infections can cause toxic shock syndrome, staph food poisoning, most MSSA infections can be treated by washing the skin with antibacterial cleanser and applying an antibiotic ointment. Also it can be treated by draining the infection of pus or fluid (13, 14).

Propolis:

Antimicrobial Resistance (AMR) prevents effective treatment of a wide range of infectious agents that are caused by bacteria and viruses. AMR is gradually becoming a public health concern to countries and various sectors of the world, as it threatens the achievements and accomplishments of modern-day medicine. The resulting factors are increased illness, fatality, and rising health-care costs. Therefore, as a society, it is vital that we acquire novel antimicrobial agents (13, 14).

Propolis (bee glue), a flavonoid-rich product of honey comb, derives from the Greek pro “before” and polis “city”. The color of the Propolis ranges from yellow to dark brown depending on the origin of the resins. But even transparent Propolis has been reported. [3] Its composition is a mixture of various amounts of beeswax and resins collected by honeybees from living plants, particularly from flowers and leave buds. Whilst in the process of collecting resins, it is mixed with saliva and other secretions produced by the bees, such as wax and pollen. The utilization of propolis aids in constructing a protective shield at the entrance of the beehive, filling cracks in the hive, attaching corners/frames to the grooves in the hive, and polishing the cells of the honeycomb. Additionally, lizards, snakes, and mice that have attempted to invade the hive are killed and sealed against the walls with Propolis, which protects the colonies against unpleasant odor and bacterial flora (25).

Not only is Propolis used as building material but helps maintain low levels of fungal/bacterial concentrations in the hive. In various Propolis

samples, it has been identified that its composition includes more than 150 compounds such as polyphenols, phenolic aldehydes, quinones, coumarins, amino acids, steroids and inorganic compounds. Among the compounds reported to exist within these samples, phenolic acid and flavonoids are most important since many of propolis' alleged biological activities are attributed to these substances (17, 25).

Furthermore, Propolis exhibits antibacterial and anti-inflammatory properties which indicate that it can be an extremely powerful natural antibiotic and useful when fighting off upper respiratory infections, such as the common cold and influenza viruses (8, 12, 18). Propolis is extensively utilized in folk medicine, there are a number of investigations that have conveyed how Propolis possesses antibacterial, antiviral, and antifungal properties. The antioxidant, antimicrobial and antifungal activities of Propolis offer scope for application in food technology (20).

The aim of this study is to determine the antimicrobial activity of the propolis extract against Methicillin-susceptible *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus.*

II. METHODS AND MATERIAL

This was a descriptive cross-sectional conducted at Soba University Hospital from October to December 2017.

Study populations:

100 Clinical isolate of *Staphylococcus aureus* from different clinical samples, 50 isolates of MSSA and 50 isolates of MRSA were enrolled and Propolis extract from two areas in Sudan, Al-Gelly and Al-Fao were used.

Methodology:

Culture:

Bacteria was sub cultured on Manitol Salt Agar then followed by aerobic overnight incubation at 37°c. The isolates were re- identified based on colonial morphology, Gram's stain, and other biochemical tests according to the Gram Stain.

Detection of MRSA:

Oxacillin susceptibility testing was performed with 1µ oxacillin disks on Mueller Hinton Agar, incubated aerobic overnight at 37°C. The interpretation of the bacterial susceptibility results were recorded as "resistant," intermediate and "sensitive." The antibiotic susceptibility was determined according with methods of Clinical and Laboratory Standards Institute (CLSI) for the purpose of the study.

Sensitive	Intermediate	Resistant	Antibiotic Disc
≥13	11-12	≤10	Oxacillin

Propolis collection:

Perforated plastic plate's technique: the plastic plate's (6×6×1mm) were installed on the top of bee hives, specifically placed between combs and the bottom board of the hives. Monthly the plate was removed from traps and subsequently it was cooled for a few hours in a refrigerator or freezer.

Preparation of Propolis Ethanol Extract (EEP):

A small aliquot of Propolis was grounded into a fine powder, then in a small container 2 g of the Propolis powder were measured. To the powder, 10 ml of 95% ethanol were added to obtain 20% (w/v) of Propolis extract. At this point, each extraction mixture were stored at room temperature in the dark for about 7 days, and were mixed from time to time. After the last day of incubation, using centrifuge the extraction mixture were spun and then the supernatants were

removed which were designated as an ethanolic extract of propolis (EEP), from the ethanolic extract an aliquots were prepared at different concentration 0.5%, 1%, 5%, 10%, and 20% consecutively. In sterile tubes, each concentration mixture were taken and filtered using a 0.45 micrometer filter. each of the mixtures were stored in -10°C freezer.

Antimicrobial activity of Propolis:

Prepare suspensions of each type of the microorganisms MSSA and MRSA for a total of four suspensions for each isolates that was tested (see results below). Each suspension will contain 10^6 cells/ ml which were inoculated onto plate surfaces with sterile cotton swab. The test plates of 10 cm diameter were prepared with 20 ml of Mueller-Hinton agar (himedia laboratories pvt. ltd), and holes of 6mm in diameter were punched in the agar plates using cork borer. Each hole was filled with 50 µl of EEP. EEP were used at different concentrations for each type of propolis. The diameters of the growth inhibition zones around the holes were measured after incubation for 48 hours at 35°C. The interpretation depends on the measurement of the inhibition zone, if the inhibition zone of Propolis extract against the bacteria is greater than 6 mm then that was considered sensitive, otherwise if it is less than 6 mm it is considered resistant (23)

III. RESULTS AND DISCUSSION

RESULTS

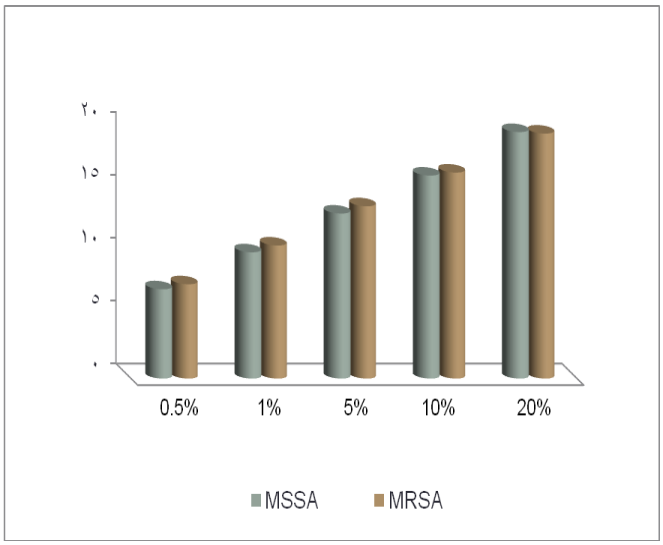
All of the study MSSA isolates (50/50; 100%) and MRSA (50/50; 100%) isolates which cultured with a different concentrations of Al-Gelly propolis extract were shown a resistant inhibition zone while (50/50; 100%) of the study MSSA isolates and (50/50; 100%) of the study MRSA isolates which cultured with a different concentrations of Al-Fao propolis extract were shown sensitive inhibition zone at different sensitivity levels (Table 1).

The sensitivity levels of both MSSA and MRSA to the Al-Fao propolis extract was significantly correlate with the concentration of the propolis extract *(P value 0.000, 0.000 respectively*).The greatest effect was a product of 20% concentration (Figure 1).

The mean sensitivity level of MSSA at 0.5% concentration was 7.10 ± 0.909 wherease the mean sensitivity level at 20% concentration was 19.6 ± 0.495. The mean sensitivity level of MRSA at 0.5% was 7.50 ± 0.886 wherease the mean sensitivity level at 20% was 19.48 ± 0.995 (Figure 2).

Table1 : Frequency of MSSA and MRSAs reaction to Al-Fao propolis extract and Al-Gelly propolis extract.

Propolis extract Area	MSSA		MRSA	
Al-Fao	Sensitive	Resistant	Sensitive	Resistant
	50/50 (100%)	0/50 (0%)	50/50 (100%)	0/50 (0%)
Al-Gelly	Sensitive	Resistant	Sensitive	Resistant
	0/50 (0%)	50/50 (100%)	0/50 (0%)	50/50 (100%)



MSSA P value 0.000 MRSA P value 0.000

Figure 1 : The mean sensitivity levels of MSSA and MRSA to different concentrations of Al- Fao propolis extract.

DISCUSSION:

In this study, 100% of the MSSA were found to be sensitive to Al-Fao propolis extract at different concentrations while were found resistant to Al-Gelly propolis extract and 100 % of the MRSA were found to be sensitive to Al-Fao propolis at different concentrations and resistant to Al-Gelly propolis extract. The sensitivity inhibition zones of both MSSA and MRSA were increased gradually with little level variations among the different concentrations, the most efficacies was refered to 20% concentration.(*P values 0.000, 0.000 respectively*). Those results is in agreement with the findings of Rahman 2010 and Rim 2017 whose found that all Propolis samples showed an inhibition in the growth of all examined bacteria but the inhibition varied according to the Propolis origin and concentrations (17,18). While it was disagreed with the results reported that the evaluation of antimicrobial activities of 13 propolis samples from different area revealed only minor variations in the antimicrobial activity according to the propolis origin (19).

IV.CONCLUSION

The MSSA and MRSA were sensitive to the Propolis that was extracted from the Al-Fao at different concentrations. the EEP prepared using Al-Fao Propolis is more effective on *Staphylococcus aureus* in different concentrations than the EEP extracted from Al-Gelly which is resistant.

V. ACKNOWLEDGMENTS

The authors would like to thank the Soba University Hospital, for allowing them to collect the sample. The authors also acknowledge Professor Mohammed Saeed Ali Al-Sarraj for giving me propolis.

Conflicts of interest

The authors wish to do research to find basic ingredients of two types of propolis and different between them and introducing effective ingredients to make treatment.

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Cite this article as :

Islam Abbas, Musa Abdulla Ali, "Antibacterial Activity of Sudanese Propolis Extract Against Methicillin-susceptible and Methicillin-resistant Staphylococcus aureus isolates in Khartoum State, Sudan", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 6 Issue 6, pp. 07-12, November-December 2019. Available at doi : https://doi.org/10.32628/IJSRSET19662

Journal URL : http://ijsrset.com/IJSRSET19662