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Methanol Extract of *Artemisia verlotiorum*: An Effective Inhibitor of *Staphylococcus aureus* Clinical Isolates

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Abstract: Due to the continuous use of antibiotics, many microorganisms have acquired resistance. One example includes *Staphylococcus aureus*. This Gram positive bacterium has been implicated in causing infections ranging from mild skin infections to severe and life threatening infections. Considering antibiotic resistance as a major problem in treating infections caused by this organism, scientists have been searching for new means to solve the problem. In this study aims, the antibacterial effect of *Artemisia verlotiorum*, a plant belonging to the family *Asteraceae*, on 18 clinical isolates of *Staphylococcus aureus*. Both the 5 methicillin-resistant strains of the organism (MRSA) and the 13 methicillin-sensitive strains (MSSA), were subjected to three different doses of the *Artemisia verlotiorum* methanol extract (in volumes of 100 µl, 200 µl and 300 µl) by the well agar diffusion technic and observing for the appearance of zones of inhibition of growth. The results clearly demonstrated that the extract had an antibacterial effect on both groups of *S. aureus*. This effect was dose dependent and was more prominent against the MRSA strains. It is suggested that further studies to determine the active antibacterial component of the methanol extract of *Artemisia verlotiorum*, may prove very important as one of the weapons to deal with this universal problem.

Key words: Antibacterial activity, Methicillin-resistant *S. aureus* (MRSA), Methicillin-sensitive *S. aureus* (MSSA), *Artemisia verlotiorum*, medicinal plants, antibiotic resistance.

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I. INTRODUCTION

Microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [1]. The emergence of antibiotic resistant bacteria is increasingly limiting the clinical use of antibiotics and, as resistant bacteria become more prevalent, there is an increasing concern that existing antibiotics will become ineffective against these pathogens [2]. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies [1]. Extracts of medicinal plants have shown to exhibit antibacterial activities against human, plant and fish pathogens [3]. Antimicrobial substances from natural sources like plants have been investigated to achieve higher levels of food safety and have been used because of their antimicrobial traits [4, 1]. Therefore, the development and spread of drug resistance and the appearance of undesirable side effects of certain antibiotics have led to the search for new antimicrobial agents, mainly from plant extracts, with new characteristics to overcome resistance [5].

In recent years, incidence of multidrug resistance in Gram Positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*), Gram Negative (*Escherichia coli*, *Shigella dysenteriae*, *Hemophilus influenzae*) and other bacteria like *Mycobacterium tuberculosis* has been reported from all over the world [6]. Among these organisms, *Staphylococcus aureus*, is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary and device related infections [7]. These different types of infections, particularly those caused by the methicillin resistant *S. aureus* (MRSA) strains, have increased the morbidity and mortality of patients due to the cumbersome treatment required for these patients [8].

Hundreds of plants worldwide are used in traditional medicine as treatment for bacterial infections [9]. Of these plants, the genus *Artemisia* of small herbs and shrubs, is one of the largest and most widely distributed genera of the *Asteraceae* family. Members of this genus have a characteristic scent or taste, have botanical and pharmaceutical interest, and are used in the liquor-making industry [10]. *Artemisia* species have been known in folk medicine, as they have been used as anti-inflammatory and diuretic agents and were reported to have been used for the treatment of epidemic hepatitis [11].

Artemisia verlotiorum, is an aromatic plant with a rhizomatous growth habit and ovoid-ellipsoid capitulum. The leaves have narrow primary lobes with few secondary lobes and serrations. In *A. verlotiorum*, the

branches are few and usually somewhat recurved and the inflorescence is conspicuously leafy almost to its apex [12].

The present paper deals with evaluating the potential antibacterial effect of *A. verlotiorum* plant extract on MRSA and MSSA.

II. MATERIAL AND METHODS

Source of the tested plant

Fresh samples of *Artemisia verlotiorum* were obtained from the Byblos region located in the Mount Lebanon Governorate of Lebanon.

Source of bacterial isolates used in the study

The 18 bacterial isolates of *Staphylococcus aureus* used in this study, were clinical isolates provided by the microbiology laboratory of the Lebanese American University medical center-Rizk hospital (LAUMC-RH)

Definitive identification of the isolates and detection of their resistance to methicillin

The identity of the isolates was confirmed by the standard coagulase test. To detect whether the organisms were methicillin sensitive or resistant, the standard method introduced by the CLSI was performed by the disk-agar diffusion method using cefoxitin discs [13].

Preparation of the methanol extract

Fifty grams of the leaves and flowers of *Artemisia verlotiorum* were chopped and blended using a blender. The crude powder was mixed well in 187.5 ml of 80% methanol. The extract was then transferred to a flask and incubated in a shaking incubator (80 rpm at 42 ℃) for 1 week. The extract was then filtered using a Millipore filter.

Preparation of the agar plates with the test organisms

Muller-Hinton agar (MHA) was used and prepared as recommended. The thickness of the agar in each plate was 4 mm. The surface of each of the plates was then heavily seeded with the test organism (suspended in saline to a turbidity equivalent to a 0.5 McFarland standard).

The well agar diffusion assay

Using a cork borer, wells in the middle of each of the MHA plates were introduced. For each of the tested organisms, plates containing different volumes of the extract (100µl, 200µl and 300µl) were prepared. The plates were then incubated at 35 ℃ for 24 hours, after which the diameter of any zone of inhibition around the wells (if present), was measured from different directions and the average was reported. For each isolate, wells containing only methanol (no extract), were used as negative controls

III. RESULT

Five of the *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA), while the remaining 13 were methicillin sensitive (MSSA). Tables 1 and 2 show the antibacterial effect of the *Artemisia verlotiorum* methanol extracts, using 3 different volumes (100 µl, 200 µl and 300 µl), by the well agar diffusion assay, on the MRSA and MSSA isolates respectively. The results are reported as averages of the diameters of the zones of inhibition of growth observed after incubation (as seen in Figure 1). Using methanol as a negative control, the results showed that the extract exhibited a concentration dependent growth inhibitory effect against all 18 isolates, thus indicating its potential antibacterial activity. Using 100 µl of the extract, the diameters of the zones of inhibition of growth ranged between 16.7 mm and 22 mm for the MRSA isolates and 0 mm - 19.3 mm in the MSSA isolates. The diameters of the zones of inhibition of growth of the isolates increased when 200 µl of the extract was used; the diameters of the zones then ranged between 19 mm and 23.3 mm for the MRSA isolates and 18.0 mm - 21.7 mm for the MSSA isolates. Further increase in the antibacterial effect was also observed when a volume of 300 µl of the methanol extract was used; the zones of inhibition of growth then ranged between 20.0 mm and 26.7 mm for the MRSA isolates and 19.7 mm – 23 mm for the MSSA isolates.

Table 1: Antimicrobial activity of methanol extract of *Artemisia verlotiorum* on methicillin resistant *Staphylococcus aureus* (MRSA), reported as averages of the diameters of the zones of inhibition of growth of the tested strains.

	C	100 µl	200 µl	300 µl
MRSA isolate no.1	0	17.3	21.0	21.3
MRSA isolate no.2	0	18.0	19.0	20.0

MRSA isolate no.3	0	22.0	23.3	26.7
MRSA isolate no.4	0	16.7	20.3	21.0
MRSA isolate no.5	0	17.3	21.3	22.0

C: Control (only methanol); zones of inhibition in (mm); diameter of the well (12mm)

Table 2: Antimicrobial activity of methanol extract of *Artemisia verlotiorum* on methicillin sensitive *Staphylococcus aureus* (MSSA), reported as averages of the diameters of the zones of inhibition of growth of the tested strains.

	C	100 µl	200 µl	300 µl
MSSA isolate no.1	0	18.7	21.7	23.0
MSSA isolate no.2	0	16.7	20.7	22.3
MSSA isolate no.3	0	17.7	20.7	22.0
MSSA isolate no.4	0	17.3	19.7	20.7
MSSA isolate no.5	0	15.3	18.0	21.3
MSSA isolate no.6	0	17.0	20.7	20.7
MSSA isolate no.7	0	0	18.7	20.7
MSSA isolate no.8	0	0	18.3	22.0
MSSA isolate no.9	0	16.3	19.0	19.7
MSSA isolate no.10	0	17.7	19.7	21.3
MSSA isolate no.11	0	19.3	20.0	22.3
MSSA isolate no. 12	0	17.7	19.3	21.7
MSSA isolate no.13	0	0	19.0	20.3

C: Control (only methanol); zones of inhibition in (mm); diameter of well (12mm)

Figure 1: The zones of inhibition of growth of the tested *Staphylococcus aureus* isolates, as seen using the well agar diffusion technic.

IV. DISCUSSION

Emergence and spread of antibiotic resistance among pathogenic bacteria represents a major obstacle in the treatment of infectious diseases. Antimicrobial resistance (AMR) is a global health problem associated with increased morbidity and mortality [14]. Due to the frequent use of antibiotics, *S. aureus* strains have evolved resistance against the most abundantly used therapeutics and the treatment of methicillin-resistant *S. aureus* (MRSA), infections is complicated by the fact that these strains are susceptible to only few antimicrobials if any [15].

To face the unprecedented spread of bacterial resistance to many antimicrobials, one of the effective alternatives was the use of natural plants. Plants have always played a central role in traditional systems of medicine for the prevention and treatment of disease around the world [16]. They produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria [17].

Being considered as one of the diverse genera of the *Asteraceae* family, Artemisia is rich in many important medicinally valuable essential oils and secondary metabolites [18]. Artemisia plants are used as spices and in folk remedies as antiseptics. For example, powdered leaves of *A. absinthium*, *A. biennis*, *A. frigida*, and *A. ludoviciana* have been applied externally in salves and washes by native North Americans to treat sores and wounds and internally to treat chest infections [19]. This information suggested the importance of studying representatives of this family of plants to better understand their antimicrobial activity. A previous study, examining the effect of six organic solvents of *Artemisia nilagirica* against fifteen phytopathogens and clinically important standard reference bacterial strains showed that all the extracts had inhibitory activity for Gram-positive and Gram-negative bacteria except for *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Staphylococcus aureus* indicating the resistance of these organisms to *A. nilagirica* extracts [18].

Our study demonstrated the effective antibacterial activity of *Artemisia verlotiorum* methanol extract against 18 *S. aureus* isolates including methicillin resistant strains. This antibacterial effect against the *S. aureus* isolates was assessed by the presence of zones of inhibition of growth around the agar wells containing different volumes of the extract. As clear from Tables 1 and 2, our results showed that the extract was effective against both groups of *S. aureus*. The antibacterial activity is also shown, in the same tables, to be proportional to the concentration of the extract used, since the diameters of the zones of inhibition of growth of the different isolates increased with an increase in the volume of the extract used. Moreover, the average diameters of the zones of inhibition of growth of all the MRSA isolates seemed to be higher than the average of those of the MSSA isolates, suggesting that, the antibacterial activity of the extract against the MRSA strains seemed to be more prominent than its activity against the MSSA strains. To support this it was noted that, three out of the thirteen MSSA isolates (7, 8 and 13) were not inhibited by the extract when a volume of 100 µl was used, in contrast to the MRSA isolates that all were inhibited at the same volume of the extract. This suggested that the methanol extract of *Artemisia verlotiorum* was more efficacious against multi drug resistant organisms than the drug sensitive ones.

Table 3: The average diameters of the zones of inhibition of growth of all the isolates of each of the 2 groups of *S. aureus* tested

	C	**100 µl**	**200 µl**	**300 µl**
MRSA 5 isolates	0	18.26	20.98	22.20
MSSA 13 isolates	0	13.36	19.65	21.38

C: Control (only methanol); zones of inhibition in (mm)

The results of this study prove without any doubt that the methanol extract of *Artemisia verlotiorum* has a prominent anti-staphylococcal activity.

V. CONCLUSION

The present study unveiled the potential anti-staphylococcal activity of *Artemisia verlotiorum* methanol extract. Due to the emergence of resistant strains of *Staphylococcus aureus*, we can now consider this plant as a possible source of antibacterial agents that can treat infections caused by this dangerous organism. The fact that the MRSA strains were more susceptible than the MSSA strains to the extract tested, makes the results more valid and useful for future follow up.

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