



EVALUATION THE IN VITRO ANTIBACTERIAL EFFECTS OF AQUEOUS EXTRACT OF *VERBASCUM THAPSUS* AGAINST *STAPHYLOCOCCUS AUREUS* ATCC NO. 25923 IN WEST OF IRAN

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ABSTRACT

Plants are common remedies applied by a majority of people. Ethnomedicinal plants are considered new resources for yielding components that could act as alternatives to antibiotics in cure of antibiotic-resistant bacteria. *Verbascum thapsus* (VT) is a vernacular plant in Iran, which the plant has been used as an antioxidant, anti-inflammatory, tonic, indigestion, antipyretic, antiviral, and antifungal. In this study, authors describe properties of aqueous extract of VT against common pathogen (*Staphylococcus aureus* ATCC No. 25923 (SA)) with broth macro-dilution and agar well and disk diffusion methods. The antibacterial activities of the plant was assessed by macro-dilution method in Mueller-Hinton broth medium and agar well and disk diffusion methods. The results revealed that the aqueous extract of VT exhibited strong levels of antibacterial activities against SA. By increasing the concentration of the extract, the inhibition zone in many of samples augmented. Partially, in agar disk diffusion the widest inhibition zone of 15 mm occurred in 0.083 g/ml VT with no inhibition with distilled water. In agar well diffusion, the widest inhibition zone of 13 mm observed in 0.083 g/ml. MIC and MBC of VT were 0.01 and 0.02 g/ml, respectively. Find discrepancies in SA sensitivity to VT with greater inhibition in disk diffusion tests. Our findings indicated that VT aqueous extract had a potential to be applied as antibacterial agent.

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INTRODUCTION

Antibiotic drugs used in the cure and inhibition of bacterial infections. But a number of antibiotics have side effect¹. By consuming of medicinal plants can limited antibiotic resistance of bacteria^{2, 3}. Down the ages plants have evoked interest as sources of innate products⁴. They have been consumed as alternative remedies for the treatment of several bacterial diseases⁵. There is growing concern in correlating phytochemical constituents of plant with its pharmacological activities^{6, 7}. The original benefit of natural factors is that they do not increase the antibiotic resistance, because they have a significant role in the defense system of the plant to microbial diseases due to their intrinsic anti-oxidative and anti-microbial properties⁸⁻¹⁰.

A plant extract is effective substance from the tissue of a plant, to be used for a particular therapeutic purpose¹¹. Plant extracts have a major variety of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds^{12, 13}. In herbal medicines, raw plant extracts in the form of infusion, decoction, and tincture are traditionally consumed by the population for the treatment of diseases including infectious diseases¹⁴⁻¹⁶. The antibacterial properties of extracts have been identified for many years, and their rudiment have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food maintenance, etc¹⁷. There are reports of the active principles of extracts from different plants with antifungal or antibacterial effect^{18, 19}. In Iranian medicine, plant extracts in the several form are consumed by the population for the prevention,

control, and treatment of diseases such as bacterial diseases^{20, 21}.

VT is the member of plants family called *Scrophulariaceae*. It is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. VT have long been used in Iranian traditional medicine as a medicinal plant. The plant of different origins produced 7-10% aqueous extract. In traditional medicine, several extracts of this plant are traditionally used in treating different inflammatory, fungal, viral, parasitic, and bacterial diseases. Likely, the antibacterial effects of the plant are related to its phenolic, flavonoid, and flavones compounds. These components are adjoin to the bacterial outer membrane proteins, deactivate the matrix metalloproteinase and inhibit growth of bacteria or destroyed bacteria²². The aim of the new study was evaluation antibacterial activities of aqueous extract of VT against SA in west of Iran (in Kermanshah).

MATERIAL AND METHODS

Plant sample collection and Preparation of aqueous extract

In the empirical-experimental study, medicine plant collected from Kermanshah. Plant was washed, air dried for 5-6 days, and ground into powder before being placed into a Soxhlet apparatus for extraction with distilled water with increasing polarity to extract phyto-constituents separately at 20°C for 3-4 h. Whitman filter papers No.1 were used to filter the extract. Pressure was reduced to evaporate and dry the filtrates (after drying, powder of aqueous extract are obtained).

Culture media and Source of microorganisms

Mueller-Hinton broth and agar, Tryptic Soy broth and Nutrient agar were prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 10 x 10 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use. Lyophilized *Staphylococcus aureus* ATCC No. 25923 (SA) provided by The Iranian Research Organization for Science and Technology was activated on Tryptic Soy broth at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10⁸ cfu/ml in Muller Hinton broth.

Evaluation of antibacterial activities

Agar disk and agar well diffusion were used as screen tests to assess antibacterial properties of aqueous extract of VT based on standard protocol. The solution of the extract was yielded in 0.083 g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. Distilled water was used as negative control whereas Amoxicillin was used as positive control. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) were specified by macrobroth dilution assay based on Clinical Laboratory Standard Institute (CLSI) guidelines²³.

Statistical analysis

Descriptive statistics including the mean, standard error, median, minimum and maximum were calculated for all variables. The one-way ANOVA followed by Turkey post hoc test were used for comparison of different parameters. The data were analyzed by SPSS software, version 22.0 (SPSS

Inc., Chicago, IL, USA) and P<0.05 was accepted as statistically significant.

RESULTS

Agar disk diffusion test

The widest zone was formed due to 0.083 g/ml of the aqueous extract of VT in SA culture. No inhibition zone was observed due to distilled water. Growth inhibition zones due to different dilutions are listed in figure 1.

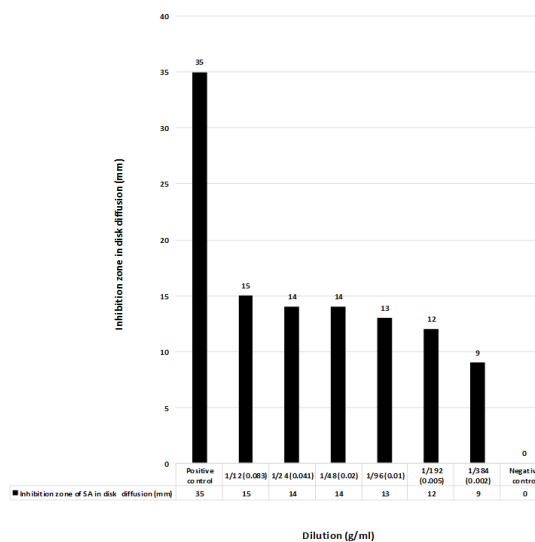


Figure 1 The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of aqueous extract of VT.

Agar well diffusion test

In regard to this plant, the widest zone was seen in 0.083 g/ml, due to SA (13 mm). No inhibition zone was observed due to negative control. The data are discoverable in figure 2.

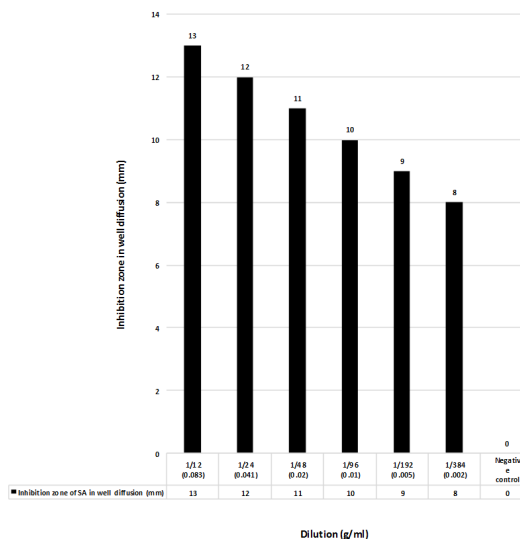


Figure 2 The diameters of growth inhibition zones in agar well diffusion test in different dilutions of aqueous extract of VT.

MIC determination and MBC ascertaining

In the examined bacterium, MIC and MBC values were 0.01 and 0.02 g/ml concentration, respectively.

DISCUSSION

Bacterial infections are responsible for many deaths each year. Antibiotics make the primary base for the prevention and treatment of bacterial disease. However excessive use of

antibiotics has become the major agent for the multi-drug resistant strains of different groups of bacteria¹. A way to limited antibiotic resistance of bacteria pathogenic species is by using of plants^{2,3}. They as a rich source of ethno medicinal compounds have continued to play a distinguished role in the maintenance of human health against several diseases²⁴⁻²⁷. Plants have been used as other orders for the remedy of different microbial diseases^{28, 29}. The antibacterial activities of plant aqueous extract from a wide numerous of plants have been appraised and reviewed, and these reports have been indicated very strong antibacterial effect of them¹⁴⁻¹⁶. Many VT plants have long been used in Asian countries as an ethno medicinal plant for the treatment of diseases; it has been applied for treating various inflammatory and bacterial diseases²².

As the tables showed, VT aqueous extract have prevented the growth of SA and destroyed it. Also, by increasing the concentration of extract, the inhibition zone increased. The results determined that in tested bacterium, there was a significant difference in terms of sensitivity to aqueous extract of VT. In agar disk diffusion test, the widest inhibition zone was seen in 0.083 g/ml concentration (The value of growth inhibition zone was 15 mm in this dilution, and the value of growth inhibition zone of Amoxicillin against SA was 35 mm) and no inhibition zone was observed due to distilled water. In agar well diffusion test, the widest zone was seen in 0.083 g/ml concentration (13 mm) and no inhibition zone was observed due to negative control. Also, the results indicated that VT with 0.01 g/ml concentration has prevented SA from the growth and in 0.02 g/ml concentration eradicated it.

In a study, the ethanolic extract of *Verbascum qulebrium* was subjected to phytochemical screening and it was also evaluated against six microorganisms (SA, *Salmonella typhi*, *Saccharomyces pastorianus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) in nutrient agar using disc agar method and demonstrated this plant have strong antibacterial properties against above bacteria³⁰. In similar study indicated that VT methanolic extract have strong antibacterial effects against *E. coli*, *Yersinia pestis*, *Bacillus cereus*, *P. aeruginosa*, *Listeria monocytogenes* and SA³¹. In other study presented that MIC of VT ethanolic extract against SA was 0.125 g/ml³².

CONCLUSION

VT is a medicinal plant with antibacterial effects toward SA. The growth of SA were inhibited and destroyed by the aqueous extract tested. The present research shows the antibacterial effects of the medical plant on SA, suggesting to use as an antibacterial agent. Extraction of active molecules will be the future work to peruse.

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