Ethnomedicinal Plants: Evaluation of in vitro Antibacterial properties of *Verbascum thapsus* against *Bacillus subtilis* ATCC No. 21332

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**Abstract**

Ethnomedicinal plants are considered common resources for yielding supplements that could use as alternatives to antibiotics in cure of antibiotic-resistant micro-organism. *Verbascum thapsus* (VT) is a native plant in west of Iran, which the has been applied as an antioxidant, anti-inflammatory, antiviral, antifungal, and antipyretic in Iran. The aim of the recent study was assessment of antibacterial properties of VT aqueous extract on common pathogen (*Bacillus subtilis* ATCC No. 21332 (BS)). The antibacterial effects of VT was evaluated by macro-dilution method in Mueller-Hinton broth medium and agar well and disk diffusion methods. The results indicated that the aqueous extract of VT have strong levels of antibacterial activities against BS. By augmenting the concentration of the extract, the inhibition zone in many of the samples increased. Partially, in agar disk diffusion the widest inhibition zone of 18 mm was observed at 0.083 g/ml VT with no inhibition with distilled water. In agar well diffusion, the widest inhibition zone of 13 mm occurred at 0.083 g/ml. Minimum inhibitory and bactericidal concentrations of VT were 0.01 g/ml. Our findings revealed that VT aqueous extract had a potential to be used as antibacterial supplement or drug.

**Keywords**

*Verbascum thapsus; aqueous extract; antibacterial effects; macro-dilution method; agar disk diffusion method; agar well diffusion method*
INTRODUCTION

Antibiotics are drugs that used in the treatment of infectious diseases. But, in recent times, there have been enhancements in antibiotic resistant strains of clinically substantial bacteria, which have caused the emergence of novel bacterial strains that are multi-resistant. The spread of drug resistant bacteria is one of the greatest threats to successful therapy of bacterial diseases. Ethnomedicinal plants have been consumed as alternative remedies for the treatment of different diseases. There is growing concern in correlating phytochemical constituents of plants with its pharmacological effects. Out of so many plants most are used to cure bacterial disease. The original benefit of natural factors is that they do not enhance the “antibiotic resistance”, an event usually encountered with the long-term use of synthetic antibiotics; because they have a striking role in the defense system to bacterial diseases due to their intrinsic antioxidative and anti-bacterial activities. A plant extract is effective substance from the tissue of a plant, to be used for a particular therapeutic purpose. Plant extracts have a major variety of phytochemicals such as phenolic acids, flavonoids, lignin tannins, and other small compounds. In herbal medicines, raw plant extracts in the form of decoction, tincture, and infusion are traditionally used by the population to cure diseases including infectious diseases. The antibacterial effects of extracts have been identified for many years, and their rudiment have found applications as naturally occurring antibacterial supplements in the field of pharmaceutical botany, phytopathology, pharmacology, medical clinical bacteriology, and food maintenance, etc. In Iranian medicine, plant extracts are used in different forms by the population for the prevention, control, and treatment of diseases such as bacterial diseases. In studies presented; Iranian traditional medicine plant extracts have strong antibacterial efficacy against negative and positive bacteria such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis. VT is the member of family Scrophulariaceae and tribe Scrophularieae. VT has long been consumed in Iranian traditional medicine as a medicinal plant. In traditional medicine, different 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 flavonoid, phenolic compounds. These components are drawn to the bacterial outer membrane proteins to deactivate the matrix metalloproteinase and prevent the growth of bacteria or destroyed them 18.

The aim of the recent study was assessment antibacterial properties of aqueous extract of VT against BS in west of Iran (in Kermanshah).

MATERIALS AND METHODS

Plant sample collection
In the experimental study, medicine plant collected from Kermanshah in west of Iran.

Preparation of aqueous extract
Plant was washed, air dried for 7-8 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 25°C for 3-4 h. Whatman filter paper No.1 was used to filter the extract. Pressure was decreased to evaporate and dry the filtrates (after drying, powder of the extract was obtained).

Culture media
Mueller-Hinton broth and agar (Müller-Hinton agar is a microbiological growth medium that is usually applied for antibiotic susceptibility testing), Tryptic Soy broth and Nutrient agar were accumulated according to the manufacturer’s instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to avouch sterility before use.

Source of microorganisms
Lyophilized Bacillus subtilis ATCC No. 21332 (BS) 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 10^8 cfu/ml in Muller Hinton broth.

Evaluation of antibacterial activities
Agar disk and agar well diffusion were used as screen tests to assess antibacterial effects of aqueous extract of VT based on standard protocol. The solution of the extract was yielded in 1g/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 applied 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) guidelines\(^1\).

**RESULTS**

*Agar disk diffusion test*

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

![Figure 1](image)

**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.

**DISCUSSION AND CONCLUSION**

Plants are important resources in pharmaceutical market\textsuperscript{20-22}. Because of their safety and low cost as well as their effects on a large number of bacteria, ethnomedicinal plants may have the potency to cure bacterial resistance to several types of antibiotics\textsuperscript{23-25}. The antimicrobial properties of plant have been assessed and reviewed\textsuperscript{26-28}, and the mechanisms that enable the natural components of plants and spices to resist bacteria have been
discussed\textsuperscript{29,30}. Since the antibacterial effectiveness of ethnomedicinal plants destabilized significantly depending on the phytochemical characteristics of plant families and subfamilies, it is not surprising to note the diversity in this efficacy even when using samples taken from the similar plant, but from two different regions\textsuperscript{31}. VT has long been used in Asian countries as a medicinal plant for the treatment of diseases; it has been applied for treating several inflammatory and bacterial diseases\textsuperscript{19}.

As the tables showed, VT aqueous extract have inhibited the growth of BS and eradicated it. Also, by increasing the concentration of VT, the inhibition zone augmented. The results determined that in tested bacterium, there was a substantial difference in terms of sensitivity to aqueous extract of VT. Also, the results indicated that VT with 0.01 g/ml concentration has prevented BS from the growth and in this concentration destroyed it. In agar disk diffusion test, the widest inhibition zone was seen in 0.083 g/ml concentration (The value of growth inhibition zone was 18 mm in this dilution, and the value of growth inhibition zone of Amoxicillin against BS was 41 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.

In a study, the ethanolic extract of \textit{Verbascum qulebrium} was subjected to phytochemical screening and it was also evaluated against six microorganisms (BS, \textit{Staphylococcus aureus}, \textit{Salmonella typhi}, \textit{Saccharomyces pastorianus}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}) in nutrient agar using disc agar method and demonstrated this plant have strong antibacterial properties against above bacteria\textsuperscript{32}. In similar studies it was indicated that methanolic extract of VT have strong antibacterial effects against \textit{E. coli}, \textit{Yersinia pestis}, \textit{Bacillus cereus}, \textit{P. aeruginosa}, \textit{Listeria monocytogenes} and \textit{S. aureus}\textsuperscript{33}. In other studies, it was demonstrated VT has potent antibacterial efficacy against BS, \textit{S. aureus}, \textit{S. typhi} and \textit{P. aeruginosa} \textsuperscript{34}. Our study, presented that aqueous extract of VT have strong antibacterial activities, and its activities could be attributed to qualitative and quantitative discrepancies in the chemical constituents of the individual extract. It can be used as antibacterial supplement.

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