

Ethnomedicinal Plants: *In vitro* Antibacterial Effects of Ethanolic Extract of *Stevia rebaudiana*

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Abstract

Appending microbial persistence to chemical drug and their probabilistic side activities cause popularity of medical herbs, so there is an instantaneous need for novel antimicrobial compounds from plants. *Stevia rebaudiana* (SR) is a vernacular plant and an ethnomedicinal plant in Iran. As per our knowledge, there is no documented proof on antibacterial effects of alcoholic extract of SR on *Staphylococcus aureus* (SA) in west of Iran. As a screen test to find antibacterial activities of the extract, agar disk and agar well diffusion methods were used. Macrobroth tube test was accomplished to specify MIC. The results of agar disk and agar well diffusion tests demonstrated that SR inhibits the growth of SA. Also in many of the samples by increasing the concentration of SR, the zone of inhibition was increased. The MIC and MBC values were 0.125 g/ml for SR. Thus, the present research indicates the antibacterial properties of the medical plant on SA, offering to use as an antibacterial supplement.

Keywords

Stevia rebaudiana; Alcoholic Extract; Antibacterial Effects; Macro-dilution method; Agar Disk Diffusion Method; Agar Well Diffusion Method



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INTRODUCTION

A plant extract is a substance or an active compound that is removed from the tissue of a plant with favourable activities, to be used for a peculiar goal. Herbal extracts have antibacterial effects on a wide number of Gram positive and Gram negative bacteria^{1, 2}. The compounds of plant extracts contain innumerable health-related properties such as antimutagenic, anticarcinogenic, antithrombotic, and antibacterial activities³. In Iranian medicine, plant extracts are consumed by the population for the remedy of diseases including bacterial diseases^{4, 5}.

In western states of Iran, a plant with the scientific name of SR has traditional medical usage. SR is a plant species in the genus *Stevia* of the *Asteraceae* family. The genus *Stevia* comprises about 154 species. The genus is concentrated in Asia, Europe and South America. *Stevia* has been widely used as a sweetener for decades. SR is one of the eatable plants which have produced a lot of interest throughout human history as a medicinal panacea⁶. The historical tradition of SR use in medicine is significant. SR is known to have advantageous properties on a large number of diseases, antioxidant, anti-inflammatory, antimalarial, antiviral, antifungal⁷.

Based on the knowledge of the authors, in comparison to many other pharmaceutical-industrial plants, there is very little data about antibacterial activities of alcoholic extract of SR collected from Kermanshah province, west of Iran. Hence, the aim of the new study was evaluation of antibacterial effects of the alcoholic extract of plant on common pathogen (SA) with broth macro-dilution and agar disk and agar well diffusion methods.

2. MATERIALS AND METHODS

2.1. Plant sample collection

In this empirical-experimental study, medicine plant was collected from Kermanshah. The sample was cleaned from any strange plants, dust or any other contaminants.

2.2. Preparation of ethanolic extract

Successive solvent extraction was performed for SR. Plants were washed and air dried for 7-8 days and ground into powder before they were placed into the flask of the Soxhlet apparatus for extraction using 100% ethanol with increasing order of polarity to extract the phytoconstituents separately at 20°C for 3-4 h (The ethanol used was HPLC grade obtained from Sigma-Aldrich, Germany). Whatman filter paper No.1 was

then applied to filter the extract. After that, reduced pressure was applied to evaporate and dry the filtrates which were stored at (-) 20°C in labelled, sterile, screw capped bottles.

2.3. Source of microorganisms

SA (ATCC No. 25923) was procured in lyophilized form, from Iranian Research Organization for Science and Technology. Bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 24 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24h; cell concentration acquired concentration of 10⁸cfu/ml using Muller Hinton broth.

2.4. Culture media

Mueller-Hinton Agar was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and distributed at 20 ml per plate in 12 x 12cm Petri dishes.

2.5. Evaluation of antimicrobial activities

Agar disk and agar well diffusion were used as screen tests to evaluate antibacterial property of SR based on standard protocol. The solution of the SR was produced in 1g/ml from which six fold serial dilutions (v/v) were accumulated. Each disk was splashed with 60 µl of dilution on and well in order. After a period of 24 h incubation, the diameters of growth inhibition zones

around the disks and wells were measured. Distilled water was used as negative control whereas Cefalexin was used as positive control in case of SA. The last can be demonstrated by pouring 60 µl of Minimum Inhibitory Concentration (MIC) tube and all dilutions before contents on agar plate. In this case, after incubation period, the lowest concentration which makes no growth is Minimum Bactericidal Concentration (MBC). For specification of MIC value, macrobroth dilution manner was used. Interpretation of the results was done due to national accepted letter from Clinical and laboratory standards institute⁸.

2.6. Statistical Analysis

Antibacterial effect was determined by one way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.01$.

RESULTS

3.1. Agar disk diffusion test

About SR, the widest zone was seen in 0.25 g/ml concentration (The value of growth inhibition zone was 11 mm in this dilution). There was no inhibition zone in SA due to 0.031, 0.015, and 0.007 g/ml concentrations. Growth inhibition zones due to different

dilutions are listed in figure 1. No inhibition zone was observed due to distilled water.

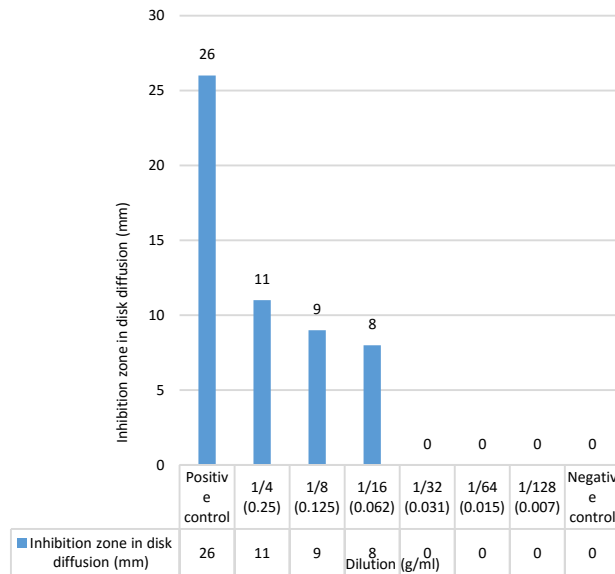


Figure 1 The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of SR

3.2. Agar well diffusion test

In regard to SR, the widest zone was seen in 0.25 g/ml concentration (The diameter of growth inhibition zone was 9 mm in this dilution). No inhibition zone was observed due to distilled water. The data are discoverable in figure 2.

3.3. MIC and MBC determination

The values for MIC and MBC were 0.125 g/ml.

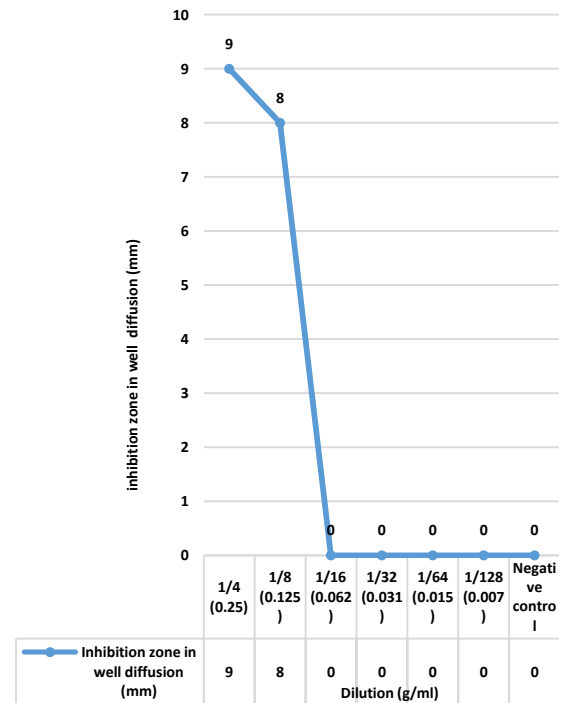


Figure 2 The diameters of growth inhibition zones in agar well diffusion test in different dilutions of SR.

4. DISCUSSION

SA, a gram positive bacteria, has been the primary cause of critical illnesses recently. This bacterium is becoming resistance to cephalexin. Cephalexin or cephalosporin, is an antibiotic that can treat a large number of bacterial infections. It destroys gram-positive and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. But this antibiotic has several common side effects like stomach upset, diarrhea and allergic. It has become a great concern for finding a desirable substitution (such as herbs) for curing them⁹.

Herbs have been screened for their potential uses as other orders for the remedy of different microbial diseases¹⁰⁻¹⁸. The antibacterial effects of plant extracts from a wide numerous of plants have been appraised and reviewed, and these reports have been indicated very strong antibacterial effect of them¹⁻⁵. Many SR plants have long been used in Asian countries as an ethnomedicinal plant for the treatment of diseases; it has been applied for treating various inflammatory and bacterial diseases^{6,7}.

As evident from the above figure the zone of inhibition in many of samples was increased when the amount of extract was increased. The results demonstrated that in tested bacterium, there was a substantial variation in terms of sensitivity to SR. In agar disk diffusion test, the widest inhibition zone was seen in 0.25 g/ml concentration (The value of growth inhibition zone was 11 mm in this dilution, and the value of growth inhibition zone of Cefalexin was 26 mm). In agar well diffusion test, the widest zone was seen in 0.25 g/ml concentration (9 mm). SR with 0.125 g/ml concentration inhibited the growth of SA and killed it. Thus, the research shows the antibacterial effects of the medical herb on SA.

There are correspondences between this result and the similar studies. The antibacterial properties of the alcoholic extract of SR on *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus megaterium*, SA was studied and it was concluded that extract have notable antibacterial effects on SA¹⁹. In other study indicated moderate antibacterial activities of alcoholic extract of SR against *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, SA and indicated that gram negative bacteria were more sensitive than gram positive bacteria in the selected plant extract²⁰.

5. CONCLUSION

The results demonstrated that ethanolic extract of SR has antibacterial effects. In fact SR prevented the growth of SA and destroyed it. Also in many of samples by increasing the concentration of the extract, the inhibition zone was increased. Therefore, it can be used as antibacterial supplement in the developing countries towards the development of recent remedial agent. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of the plant as an

antibacterial agent in topical or oral applications.

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Authors' Contribution

The core idea of this work came from Mohammad Mahdi Zangeneh and Akram Zangeneh. The experiments, evaluation and Statistical Analysis of antimicrobial activities done by Mohammad Mahdi Zangeneh, Fariba Najafi, Reza Tahvilian, Saman Salmani, Lida Haghazari, Akram Zangeneh, and Rohalah Moradi.

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