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EVALUATION OF THE IN VITRO ANTIBACTERIAL ACTIVITIES OF ALCOHOLIC EXTRACT OF *STEVIA REBAUDIANA* AGAINST *ESCHERICHIA COLI* O157: H7 (ATCC No. 25922)

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ABSTRACT

There is a steady need for novel antibacterial compounds from plants. *Stevia rebaudiana* (SR) is an indigenous plant in Iran, which the plant has been used as an antioxidant, antifungal, antiviral, and antiflammatory agent in Iran. As we know, there is no documented proof on antibacterial activities of SR alcoholic extract against *Escherichia coli* O157: H7 (EC) in west of Iran. As a screen test to discover antibacterial properties of the extract, agar disk and agar well diffusion methods were employed. Macro broth tube test was performed to specify MIC. The results of agar disk and agar well diffusion tests represents SR have prevented the growth of EC. Also in many of samples by increasing the concentration of SR, the inhibition zone increased. The MIC and MBC values were 0.031 g/ml for SR. Thus, the research showed the antibacterial effects of the ethnomedicinal herb on EC. We believe that the article provide support to the antibacterial activities of the extract.

KEYWORDS

Stevia rebaudiana, Alcoholic extract and Antibacterial activities.

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INTRODUCTION

Plants contain medicinal effects which make them potent to treat or inhibit bacterial diseases[1-4]. Some ethnomedicinal plants used in traditional Iranian medicine are effectual in treating various ailments caused by bacteria[5-9]. A plant extract is a substance or an active with eligible properties that is removed from the tissue of a plant, to be used for a particular

goal. Extracts are used in many industries including pharmacy and medicine[10]. They are expected to form recent sources of antibacterial drugs especially against bacteria[11]. The antibacterial activities of extracts has been divided into a good, medium or bad[12]. In Iranian medicine, plant extracts in the form of infusion, tincture or herbal extract are consumed by the population for the treatment of diseases including bacterial diseases[13,14].

In Iran, a plant with the scientific name of SR has traditional medical usage. The genus *Stevia* of the family *Asteraceae* comprises about 154 species. SR is concentrated in Asia, Europe and South America[15]. The historical tradition of SR use in medicine is substantial. SR is known to have beneficial effects on a large confine of diseases, nutritional, anti-cholesterol, gastro protective, antidiabetic, protective, anti-inflammatory, antioxidant, antimalarial, antiviral, and antifungal[16]. Based on knowledge of authors, there is a very little data about antibacterial properties of alcoholic extract of SR collected from Kermanshah province, west of Iran. Therefore, the aim of the recent study was assessment of antibacterial activities of the alcoholic extract of plant against EC with broth macro-dilution and agar disk and agar well diffusion methods.

MATERIAL AND METHODS

Source of microorganisms

Bacterial specie namely EC (ATCC No. 25922) was procured from Iranian Research Organization for Science and Technology as lyophilized. Bacterial strain was activated on Tryptic Soy broth, constant 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 10^8cfu/ml using Muller Hinton broth.

Culture media

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

Plant sample collection

In this empirical-experimental study, medicine plant collected from Kermanshah.

Preparation of alcoholic extract

Successive solvent extraction was performed for SR. Plant powders were placed into the flask of the Soxhlet apparatus for extraction using 100% ethanol with increasing order of polarity to extract the plant compounds separately at 20ºC for 4-5 h (The ethanol used was HPLC grade obtained from Sigma-Aldrich, Germany). Whitman filter papers No.1 were then applied to filter the extract. After that, reduced pressure was applied to evaporate and dry the filtrates which were stored at -20ºC in labeled, sterile, screw capped bottles.

Evaluation of antimicrobial activities

Agar disk and agar well diffusion were accomplished as diagnosis tests to assessment antibacterial property of SR based on standard protocol. The solution of the SR was yielded in 1g/ml from which six fold serial dilutions (v/v) were accumulated. 60 µl of each dilution was splashed 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. Distillated water was used as negative control whereas Kanamycin was used as positive control in case of EC. 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 shows Minimum bactericidal concentration (MBC). For specification of MIC value, macro broth dilution manner was used. Interpretation of the results was done due to national accepted letter[17].

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

Agar disk diffusion test

About SR, the widest zone was seen in 0.25 g/ml concentration (The value of growth inhibition zone was 16 mm in this dilution). There was no inhibition zone in EC due to 0.015 and 0.007 g/ml concentrations. Growth inhibition zones due to different dilutions are listed in Figure No.1. No inhibition zone was observed due to distillated water.

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 distillated water. The data are discoverable in Figure No.2.

MIC and MBC determination

The values for MIC and MBC were 0.031 g/ml.

DISCUSSION

EC is a gram negative bacterium has been the major cause of critical illnesses recently. This bacterium is becoming resistance to certain type of antibiotics, so it has become a great concern for finding a favorable substitution (such as plants) for curing them. The antibacterial effects of plant extracts from a large numerous of plants have been appraised and reviewed, these studies have been demonstrated very strong antibacterial activities of them[10-14].

As the figures indicated, the inhabitation zone in many of samples have been augmented when the extract amount has increased. The results defined that in tested bacterium, there was a notable difference 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 16 mm in this dilution, and the value of growth inhibition zone of Kanamycin was 26 mm). In agar well diffusion test, the widest zone was seen in 0.25 g/ml concentration (9 mm). SR with 0.031 g/ml concentration has inhibited the growth of EC and has destroyed it. Thus, the research shows the antibacterial properties of the ethno medical herb on EC. There are correspondences between this result and the similar studies. In a study represented moderate antibacterial effects of alcoholic extract of SR against EC, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus* and demonstrated that gram negative bacteria were more sensitive than gram positive bacteria in the selected plant extract[18]. In other study indicated alcoholic extract of SR have strong activities against a number of Gram-positive (*Staphylococcus albus, Bacillus subtilis*) and Gram-negative (EC, *Klebsiella aerogenes, Enterobacter aerogenes*) bacteria[19]. The antibacterial properties of the alcoholic extract of SR on EC, *K. pneumoniae, P. vulgaris, Micrococcus luteus, P.aeruginosa, B.subtilis, Bacillus megaterium, S. aureus* was studied and it was concluded that extract have significant antibacterial effects on EC[20].

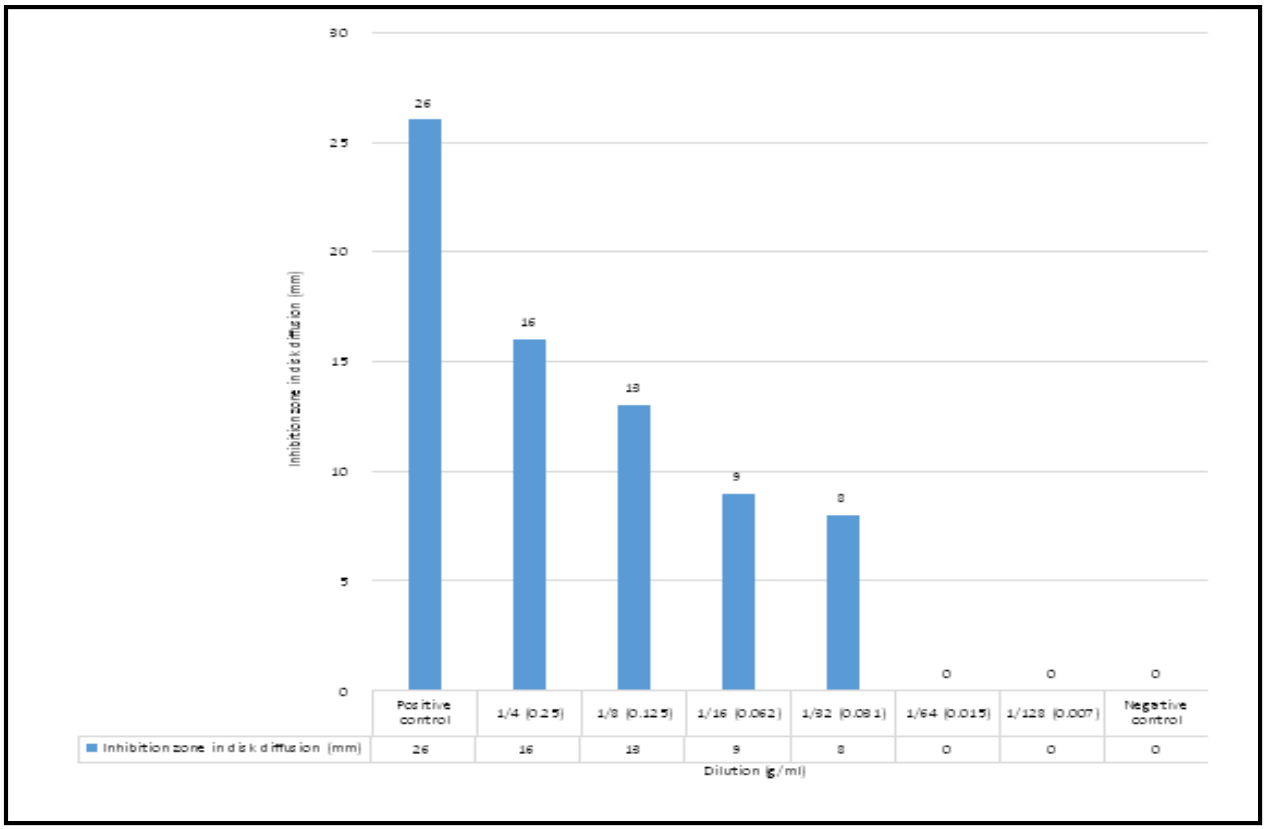

Figure No.1: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of SR

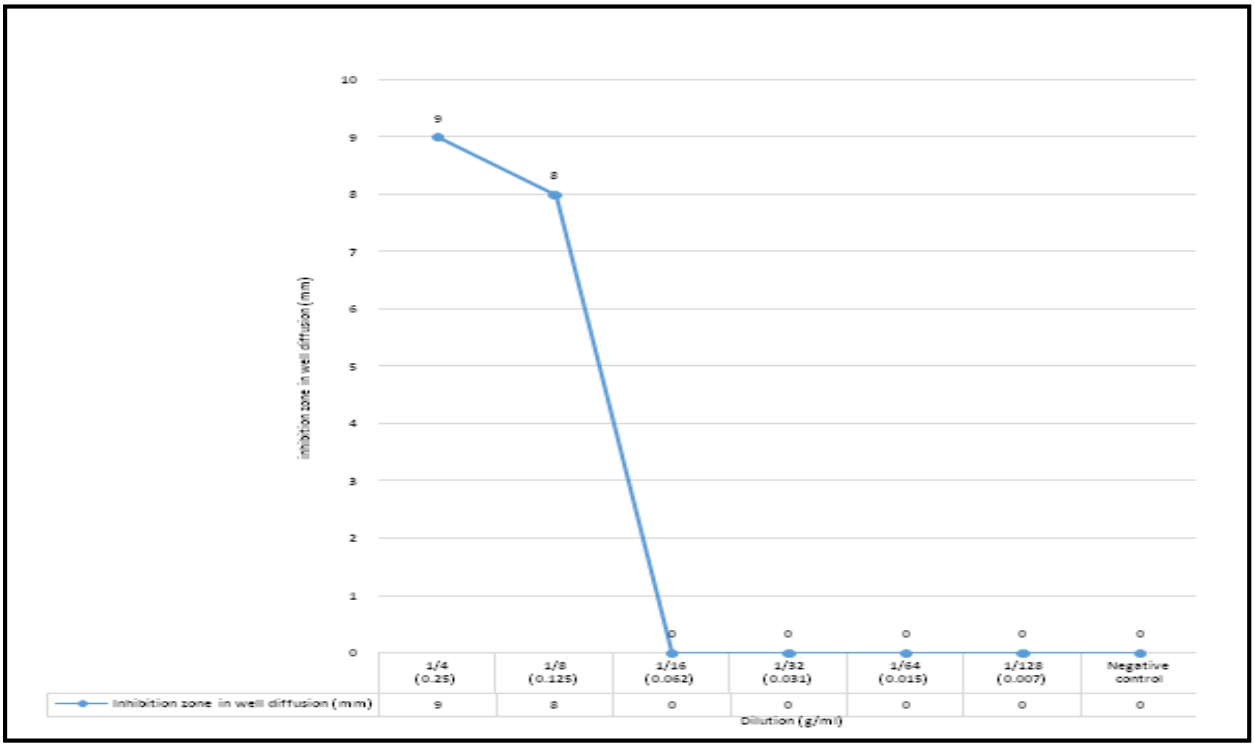

Figure No.2: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of SR

CONCLUSION

From the study it can be concluded that the alcoholic extract of SR possess antibacterial activities. In fact SR have inhibited the growth of EC and eradicated it. Also in many of samples by increasing the concentration of the extract, the inhibition zone increased. The results indicated that in tested bacterium, there was a notable discrepancy in terms of sensitivity to SR. In other words, the most sensitivity was observed in disk diffusion method. Our results support the use of the plant in traditional medicine and offer that alcoholic extract of SR possess good antibacterial effects. Thus, the present research demonstrates the antibacterial effects of the medical plant on EC, suggesting to use as antibacterial supplement. Also, further assessment is incumbent on potential of the plant as an antibacterial agent in topical or oral applications.

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AUTHOR CONTRIBUTION

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

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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