

Research Article

In Vitro Antimicrobial Activity of Stevia Rebaudiana Bertoni Leaves

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Abstract

Purpose: The aim of the present study was to investigate antimicrobial activity of the various extracts of *Stevia rebaudiana* leaves.

Method: *Stevia rebaudiana* Bertoni leaves were extracted in water, methanol, ethyl acetate and hexane and their antimicrobial activities were examined against few selected microorganisms including *B. subtilis*, *S. aureus*, *M. luteus*, *S. marcescens*, *P. aeruginosa*, *B. megaterium*, *E. coli*, *P. vulgaris*, Yeast, *A. niger* and *R. oligoporus* using cup plate method.

Results: Water extract of *Stevia* leaf showed activity against *B. subtilis* and *S. aureus* only. Methanol extract gave the highest zone of inhibition against *P. aeruginosa* whereas minimum zone of inhibition was found against *S. aureus* and yeast. *B. megaterium* and yeast were found to be highly susceptible towards ethyl acetate and hexane extracts, respectively whereas *A. niger* and *B. subtilis* were found to be least susceptible against ethyl acetate and hexane extracts, respectively. Hexane extract showed the highest activity against yeast among the tested microorganisms.

Conclusion: The study confirms the possible antimicrobial potentiality of the leaf extract of *Stevia rebaudiana*.

Keywords: *Stevia rebaudiana*, antimicrobial activity, leaf extracts

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INTRODUCTION

The use of natural products with therapeutic properties has a long history, plant, animal, and mineral products were the main source of medicines¹. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds². Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration³. In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects⁴. In recent years, concern over pathogenic and spoilage microorganisms in foods has increased due to the increase in outbreaks of food borne disease⁵. There are growing interests in using natural antimicrobial compounds, especially extracted from plants, for the preservation of foods. In addition, there are more consumers who tend to question the safety of synthetic additives and would prefer natural foodstuffs⁶⁻⁷. There is therefore the need to search for plants of medicinal value. The plant used in the present study was *Stevia rebaudiana* (Asteraceae), which is used traditionally for the source of natural sweetener. The dry extract from the leaves of stevia (*Stevia rebaudiana* Bertoni) contains sweet diterpene glycosides, flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids (caffeic, chlorogenic, etc.), neutral water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils, and trace elements⁸. To the best of our knowledge, nobody has investigated the potential antimicrobial effect of *Stevia rebaudiana* leaves in India until now. Therefore, the presents study was planned to study the antimicrobial activity of water, methanolic, ethyl acetate and hexane extracts of *Stevia rebaudiana* leaves against the selected microorganisms.

MATERIALS AND METHODS

Plant Material and Microorganisms: Dried stevia leaves were supplied by Growmore Biotech Ltd., Hosur, Tamilnadu, India. Leaves were packed in Polyethylene bags and stored at -18° C until used. The bacterial strains used were obtained from the stock culture of the Department of Microbiology, V.P. Science College, Vallabh Vidyanagar, India. The organisms included in the present study were *B. subtilis*, *S. aureus*, *M. luteus*, *S. marcescens*, *P. aeruginosa*, *B. megaterium*, *E. coli*, *P. vulgaris*, Yeast, *A. niger* and *R. oligoporus*. All the bacterial strains used for the experimental purpose were grown and maintained on nutrient agar medium. Yeast was isolated from curd sample on Sabouraud agar medium in the laboratory and maintained on the same medium. *R. oligosporus* and *A. niger* were grown and maintained on potato dextrose agar medium.

Preparation of extracts: Dry powdered stevia leaves were extracted separately with water, methanol, ethyl acetate and hexane (all solvents were of HPLC grade) using environmental shaker (New Brunswick, USA) at 250 rpm for 24 hours. Then, the extracts were filtered using Whatman No. 1 filter paper and the filtrates were then evaporated to dryness under reduced pressure. Residues were stored in labeled sterile screw-capped bottles at -20°C.

Antimicrobial Assay: The yield of water, methanol, ethyl acetate and hexane extracts of the leaf were found to be 22.44, 30.20, 10.31 and 1.74g %, respectively, on dry weight basis. Respective solvents were used to prepare a final concentration of 50 mg ml⁻¹ and sterilized by filtration through a 0.45 µm nylon membrane filter. Various extracts of Stevia leaves were subjected to antimicrobial assay using the cup-plate method⁹. Nutrient agar plates were prepared by pouring 20 ml of nutrient agar in sterile Petri dishes for antibacterial assay. Similarly, potato dextrose agar plates were prepared for antifungal assay. These were allowed to solidify. The bacterial cultures used for assay were 24 hours old whereas fungus cultures were 4 to 5 days old. Concentration of these organisms was prepared

Table 1: Antimicrobial activity of water, methanol, ethyl acetate and hexane extracts of *Stevia rebaudiana* leaves (zone of inhibition in mm)

Microorganisms	Standard	Water extract	Methanol extract	Ethyl acetate extract	Hexane extract	F values
<i>B. subtilis</i>	33.67 ^b ± 0.88	8.33 ^a ± 0.33	9.00 ^a ± 0.58	9.00 ^a ± 0.58	8.67 ^a ± 0.33	372.73*
<i>S. aureus</i>	19.33 ^d ± 0.67	9.33 ^a ± 0.33	8.33 ^a ± 0.33	12.33 ^b ± 0.33	15.33 ^c ± 0.33	114.19*
<i>M. luteus</i>	12.33 ^b ± 0.33	-	8.67 ^a ± 0.33	8.67 ^a ± 0.33	13.67 ^c ± 0.33	319.38*
<i>S. marcescens</i>	33.67 ^c ± 0.88	-	10.00 ^a ± 0.58	11.67 ^a ± 0.33	20.67 ^b ± 0.88	400.92*
<i>P. aeruginosa</i>	27.00 ^c ± 0.58	-	11.00 ^a ± 0.58	12.00 ^a ± 0.58	19.67 ^b ± 0.88	288.34*
<i>B. megaterium</i>	35.00 ^c ± 0.58	-	10.67 ^a ± 0.33	13.00 ^b ± 0.58	16.33 ^c ± 0.33	913.44*
<i>E. coli</i>	23.33 ^d ± 0.33	-	8.67 ^a ± 0.33	10.33 ^b ± 0.33	16.67 ^c ± 0.33	866.00*
<i>P. vulgaris</i>	23.67 ^c ± 0.88	-	9.33 ^a ± 0.33	10.67 ^{ab} ± 0.33	12.00 ^b ± 0.58	267.42*
Yeast	14.33 ^c ± 0.33	-	8.33 ^a ± 0.33	10.00 ^b ± 0.58	35.33 ^d ± 0.88	654.87*
<i>R. oligosporus</i>	15.00 ^c ± 0.58	-	8.67 ^a ± 0.33	11.00 ^b ± 0.58	22.00 ^d ± 0.58	295.75*
<i>A. niger</i>	14.00 ^c ± 0.58	-	10.00 ^a ± 0.58	8.33 ^b ± 0.33	16.33 ^d ± 0.33	222.94*

Values are the mean of three tests ± SEM

^{a-d} Mean values with the same superscript within a row do not differ significantly (p>0.05).

* Indicate significant difference (p<0.05).

to contain approximately 1×10^6 cfu/ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40-42° C. The tubes were then inoculated with 0.1 ml of the appropriate culture suspension of bacterium or fungus, mixed gently and poured onto previously solidified nutrient agar or potato dextrose agar plates, respectively. After setting, a cup borer (6 mm diameter) was properly sterilized by flaming and used to make four uniform cups in each Petri dish. The cups were then filled with different *Stevia* leaf extracts and allowed to diffuse for 45 minutes. The solvents used for extraction were analyzed similarly as control. Ciprofloxacin (10 µg/disc) and amphotericin-B (100 units/disc) were used as standards for bacteria and fungi, respectively. The plates were incubated at 37° C for 24 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm using a scale. The experiment was carried out in triplicates.

Statistical analysis: Analysis of variance (ANOVA) and Duncan's test were carried out using SPSS package for statistical analysis.

RESULTS

The *in vitro* antimicrobial activity of water, methanol, ethyl acetate and hexane extracts of dried *Stevia* leaves are shown in Table 1. The solvents used to prepare extracts showed no activity. Water extract showed activity against *B. subtilis* (8.33 mm) and *S. aureus* (9.33 mm) only whereas other three extracts showed activity against all tested microorganisms. All the four extracts did not show significantly different activity against *B. subtilis*. Ethyl acetate (12.33 mm) and hexane extracts (15.33 mm) showed significantly (p<0.05) higher activity compared to the water (9.33 mm) and methanol (8.33 mm) extracts against *S. aureus*. Activity of methanol, ethyl acetate and hexane extracts against *M. luteus*, *S. marcescens* and *P. aeruginosa* were found to be 8.67, 8.67 and 13.67; 10.00, 11.67 and 20.67; 11.00, 12.00 and 19.67 mm, respectively. In the case of *M. luteus*, *S. marcescens* and *P. aeruginosa*, methanol and ethyl acetate extracts activity did not differ significantly but these were found to be significantly lower (p<0.05) compared to activity of hexane extract. Methanol, ethyl acetate and hexane extracts zones of inhibition for *B. megaterium* and *E. coli* were 10.67, 13.00 and 16.33; 8.67, 10.33 and 16.67 mm, respectively,

which were significantly ($p < 0.05$) different for each other. *P. vulgaris*, ethyl acetate extract (10.67 mm) activity was not significantly different compared to methanol extract (9.33 mm) and hexane extract (12.00 mm) but hexane extract gave significantly higher activity compared to methanol extract. In the case of bacterial species, standard ciprofloxacin (10 μ l/disc) showed significantly higher ($p < 0.05$) activity compared to all the extracts except hexane extract against *M. luteus*. Zones of inhibition was found to be 8.33, 8.67 and 10.00 mm for methanol extract, 10.00, 11.00 and 8.33 mm for ethyl acetate extract and 35.33, 22.00 and 16.33 mm for hexane extract against yeast, *R. oligosporus* and *A. niger*, respectively. Hexane extract showed a significantly higher ($p < 0.05$) activity compared to methanol and ethyl acetate extract as well as standard amphotericin B (100 units per disc) against yeast and fungi.

DISCUSSION

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers^{3-6,10}. It is worthwhile to note that there are no data in the literature to indicate previous investigation of the antimicrobial activity of *Stevia rebaudiana* leaves for comparison. Water extract showed activity against only two microorganisms among the selected microorganisms. Methanol and ethyl acetate extracts have more or less similar activities but hexane extract showed significantly higher activity compared to other extracts against most microorganisms tested. The results of present study indicate that the stevia leaf extracts have inhibitory activities against microorganisms, although their antibacterial activities are lower than that of the standard (ciprofloxacin). However, in the case of fungi, hexane extract had significantly higher activity than the standard (amphotericin-B). The results of the present work indicate that stevia leaf extracts may be an ideal candidate for further research into their uses for food preservation as well as pharmaceutical and natural plant-based products.

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References

1. Kao MJ. *Encyclopedia of Chinese Material Medica*; Shin Wen Feng Press: Taipei, China, 1980.
2. Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekaju I, Totshiyuki T, Tetsuro I, Fujio A, Iriya I, Tsutomu N, Kazuhito W. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *J. Health Sci.*, 2002; 48: 273–276.
3. Cowan MM. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999; 12: 564-582.
4. Shariff ZU. *Modern Herbal Therapy for Common Ailments*. Nature Pharmacy Series (Volume 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom, 2001; pp 9-84.
5. Tauxe RV. Emerging food borne diseases: An evolving public health challenge. *Dairy Food Environ. Sanit.* 1997; 17: 788-795.
6. Nychas GJ. Natural antimicrobials from plants. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie Academic and Professional: London, United Kingdom, 1995; pp 58-59.
7. Smid EJ, Gorris LGM. Natural antimicrobials for food preservation. In *Handbook of Food Preservation*; Rahman, M. S., Ed.; Marcel Dekker: New York, 1999; pp 285-308.
8. Komissarenko NF, Derkach AI, Kovalyov IP, Bublik NP. Diterpene glycosides and phenylpropanoids of *Stevia rebaudiana* Bertoni (Asteraceae). *Rast. Res.* 1994; 1-2: 53-64.
9. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover HR. *Manual of Clinical microbiology*, 6th Ed. ASM Press, Washington DC, 1995; pp 15-18.
10. Mau JL, Chen CP, Hsieh PC. Antimicrobial effect of extracts from Chinese chive, cinnamon, and corni fructus. *J. Agric. Food Chem.* 2001; 49: 183-188.