

## Antimicrobial potential of *Stevia rebaudiana* leaves extract against selected bacterial and fungal strains

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### Abstract

The antimicrobial potential of leaves extract of indigenous *Stevia rebaudiana* is investigated to treat infectious diseases. For this purpose, leaf extracts were prepared in two solvents, ethanol and water. The crude extract was tested against four bacterial and one fungal strain (*Aspergillus flavus*). Out of four bacterial strains, two were gram-positive, *Staphylococcus aureus* and *Lactobacillus acidophilus*, whereas *Salmonella typhi* and *Escherichia coli* were gram negatives. Clarithromycin and erythromycin were used as a positive control for bacterial growth. However, Terbinafine was used as a positive control against fungal strain, while DMSO was used as a negative control. Results showed that the ethanolic extract of *Stevia* showed higher antibacterial activity as compared to water extract against all the bacterial strains. The zone of inhibition against *E.coli* was 5.66mm. *Lactobacillus acidophilus* clear zone of inhibition in the ethanolic extract was 9.3mm. Similarly, the ethanolic extract of leaves showed inhibition against *Staphylococcus aureus* and *Salmonella typhi*, with an average zone of inhibition (7.33mm and 7.66mm). These findings suggested that leaves extracts have inhibitory activities against bacterial strains, although their antibacterial activities are lower than that of the standard that is Clarithromycin and Erythromycin. Leaves extract of *Stevia* showed no activity against *Aspergillus flavus*. It was concluded that *Stevia rebaudiana* ethanolic extract could be used as an antibacterial agent against selected pathogens due to its significant activity. In contrast, no significant activity against fungal pathogen was observed in both extracts.

**Keywords:** *Stevia rebaudiana*, infectious diseases, *Aspergillus flavus*, DMSO

### Highlight:

- ✓ treatment of infectious diseases through leaf extract of indigenous *Stevia rebaudiana*
- ✓ ethanolic extract of *Stevia* showed higher antibacterial activity as compared to water extract
- ✓ no significant activity against fungal pathogen

### 1. Introduction

With the advancement in urbanization and globalization, the rate of infectious diseases also increased. These infectious diseases became the primary cause of morbidity and mortality in developing countries. The discovery and effectiveness of antimicrobial agents have become a significant issue due to the emergence and dissemination of multidrug-resistant strains (Giamarellou, 2010; Shaik *et al.*, 2014). Most bacterial strains possess the genetic ability to gain and transfer-resistant genes against antimicrobial agents. Bacteria possess several resistant mechanisms like the presence of efflux pumps, site/surface modification, and metabolic inactivation (Hooper, 2001; Yala *et al.*, 2001). The primary reason for developing resistance among the bacterial strains is incorrect and excessive antibiotic usage; therefore, developing alternative drugs or antimicrobial agents against multidrug-resistant pathogens is in dire need (Elisha *et al.*, 2017). There is a need to develop antimicrobial drugs from medicinal plants to treat several diseases (Clark, 1996; Binutu, and Cordell, 2000)..

Using medicinal plants to treat infectious diseases caused by microorganisms is an old practice, and various phytotherapeutic products are also used to treat many infectious diseases. Sometimes an infection in a human causes a severe problem caused by pathogenic microorganisms such as bacteria and fungi. In recent years these infections in the immune-compromised individual have significantly increased, and these infections include many risk factors such as leucopenia, neutropenia, hepatotoxicity, and tissue lesions (Dolande *et al.*, 2002; Mottl and Wheat, 1994). Due to the random use of commercially available antimicrobial drugs to treat infectious diseases, multiple drug resistance (MDR) has been developed (Davis, 1994; Pitt. *et al.*, 1995). Owing to this problem, sometimes antibiotics show harmful effects on the host, such as immune suppression, hypersensitivity, and many allergic reactions (Ahmed *et al.*, 2014). Therefore researchers shifted their interest to low-cost, biocompatible alternatives with the most negligible side effects. Plant-based antimicrobial products can act as an alternative and effective treatment method for different infectious diseases.

Medicinal plants consider a valuable source in modern and traditional medicines (Dey *et al.*, 2013). According to the World Health Organization (WHO) 80% of the world population depends on herbal medication (Mukeshwar *et al.*, 2011). Plants naturally contain many phytochemicals and secondary metabolites such as glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins which act as natural/plant-derived antimicrobial substances (Danlami, and Elisha., 2017). Due to plants' natural potential, there is a need to explore different species that can combat diseases and have antimicrobial potential.

*Stevia* is a genus of approximately 200 species of shrubs and herbs. *Stevia rebaudiana* is a perennial shrub that belongs to the sunflower family (Asteraceae). It is referred to as the "sweet herb of Paraguay" because it was native to a specific region of South America like Brazil and Paraguay. The major sweet component of *Stevia rebaudiana* is present in its leaves which contain stevioside, and its taste is about 300 times sweeter than sucrose. Therefore it is a 100% natural source of flavor enhancers and non-fermentable things. It is used for sweeteners in soft drinks, pickles, skincare products, ice-creams, and soya sauce. There will be a high need for natural sweeteners in the food market (Gupta *et al.*, 2014) in the future. The leaves contain a mixture of eight sweet diterpene glycosides, including stevioside, steviolbioside, rebaudioside, and dulcoside A<sup>2</sup>. *Stevia* also contains bioactive compounds such as essential oil, flavonoids, and tannins. *Stevia rebaudiana* has been made health benefits both externally and internally due to the whole extract of its leaves (Abou-Arab, & Abu-Salem, 2010).

Previously antimicrobial activities of various herbs and spices in plants leaves, flowers, stems, roots, and fruits have been reported. Some studies investigated the antimicrobial activity of various extracts of *Stevia rebaudiana* (with chloroform, ethyl acetate, water, hexane, or acetone as solvent) against some selected microorganisms such as *Bacillus subtilis*, *Salmonella typhi*, *Vibrio cholera*, *Staphylococcus aureus*, and others have been examined (Debnath, 2008; Ghosh *et al.*, 2008; Jayaraman *et al.*, 2008; Tiwari, 2010 and Tadhani *et al.*, 2006). Many biomedical properties of *Stevia rebaudiana* have also been reported, including antibacterial, antioxidant, antifungal, anti-diabetic, hyperglycemic, hypertensive, antimicrobial, and anti-inflammatory (Goyal *et al.*, 2010; Akash *et al.*, 2015).

Pakistan has a large diversity of plant species containing many bioactive compounds which can be explored and used against different diseases. Due to the medicinal properties of *Stevia rebaudiana* leaves have very high demand in the world; regarding Pakistan, There is limited research on antimicrobial activity of *Stevia* leaves is reported; therefore, the current study was conducted to evaluate the antimicrobial activity of *Stevia* leaves.

The study aimed to evaluate the antimicrobial activity of *Stevia rebaudiana* leaves extract against Gram-positive, and Gram-negative bacterial strains (*Staphylococcus aureus*, *Salmonella typhi*, *Lactobacillus acidophilus*, *Escherichia coli*) and fungal strain (*Aspergillus flavus*), followed by a comparison of the antimicrobial efficacy of *stevia* leaves with commercial antibiotics.

## 2. Material and methods

The current research work was conducted in the Department of Microbiology, University of Haripur, Khyber-Pakhtunkhwa, Pakistan. Media used in the study were purchased from Merck, Muller Hinton agar (Merck, NutriSelect Plus) was used for antibacterial activity, while potato dextrose agar (Merck, NutriSelect Plus) was used for fungus growth.

Test pathogens including *Staphylococcus aureus*, *Salmonella typhi*, *Lactobacillus acidophilus*, *Escherichia coli*, and *Aspergillus flavus* were obtained from the microbiology laboratory of Quaid-e-Azam University, Islamabad. Pakistan.

### 2.1 Collection of Plant material

*Stevia rebaudiana* leaves were collected from National Agriculture Research Centre (NARC), Islamabad. Collected leaves were washed with running tap water, washed with sterile water, and then dried in an oven at 40 °C for 4 days. Then leaves were finely powdered using mortar, vortex, and stored in airtight bottles ((Fig. 1) while identification of the plant reported in Table 1.



**Figure. 1:** (a) Field photograph of *Stevia rebaudiana* (National Agriculture Research Institute, Islamabad). (b) Dried leaves of *Stevia rebaudiana* (c) *Stevia rebaudiana* leaves powder made by mortar and piston (d) Dried leaves powder of *Stevia rebaudiana*.

**Table: 1** Classification of *Stevia*

Kingdom	Plantae Plants
Subkingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae – Aster family
Genus	<i>Stevia</i> Cav. - candy leaf
Species	<i>Stevia rebaudiana</i> (Bertoni) Bertoni candyleaf

Source: *Stevia rebaudiana* (Bertoni) Bertoni, National Plant Data Center

## 2.2 Preparation of Plant extract (*Stevia rebaudiana*)

Dried powdered of *Stevia rebaudiana* were immersed in ethanol and distilled water separately in falcon tubes (conc. 100mg/mL). It was incubated at room temperature for 48 hours at 150 rpm in an orbital shaker. The prepared plant extract was filtered through filter paper.

## 2.3 Antibacterial Activity of *Stevia rebaudiana*

This bioassay was employed for testing the antibacterial activity of leaves of *Stevia rebaudiana*. This assay was performed by the disc diffusion method in Muller Hinton agar plates. After this, the pure bacterial strains were spread on solid agar plates with a sterile swab. With the help of sterile forceps, the sterile filter paper disc of ethanol and water extract of *Stevia* was laid down on the surface of agar plates containing a bacterial culture. Then, the positive control (clarithromycin and erythromycin) and negative control (DMSO) discs were placed on the agar plates. The plates were incubated at 37 °C for 24 hours. After this, the zone of inhibition was measured in mm, around the disc to check the antibacterial activity.

## 2.4 Antifungal activity of *Stevia rebaudiana*

In this bioassay, Potato dextrose agar was used. The media was prepared and poured into Petri plates. After solidification, the pure fungal strain was spread on Petri plates, and then with the help of sterile forceps, the disc of ethanol and water extract of *Stevia* was also placed on the plates. In contrast, Terbinafine was used as a positive control, and DMSO was used as a negative control. The plates were incubated at 25 °C for 4 days. The antifungal activity was determined by measuring the zone of inhibition (Rajeh *et al.*, 2010).

## 2.5 Statistical analysis

The zone of inhibition was measured with the help of scale in cm then the centimeters were converted into millimeters (1cm = 10mm). Statistical analysis was done with MS Excel, and experimental values were represented as mean  $\pm$  SEM.

## 3. Results

The ethanolic and water extracts of *Stevia rebaudiana* leaves were used for the current study against four bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus acidophilus*, *Salmonella typhi*) and one fungal strain (*Aspergillus flavus*). DMSO was used as a negative control. Erythromycin and clarithromycin were used as a standard control for bacterial strains. While terbinafine, the antifungal disc was also used as a standard control for fungal strain.

### 3.1 Antibacterial Activity of *Stevia rebaudiana*

After 24 hours, *Stevia* showed its activity against *E. coli*. It was observed that ethanol extract of *Stevia* showed a zone of inhibition against *E. coli* which was 5.66mm; the zone of inhibition observed was 3.00mm. Antibiotics clarithromycin and erythromycin, used as the positive control, showed the highest activity against *E.coli* with a zone

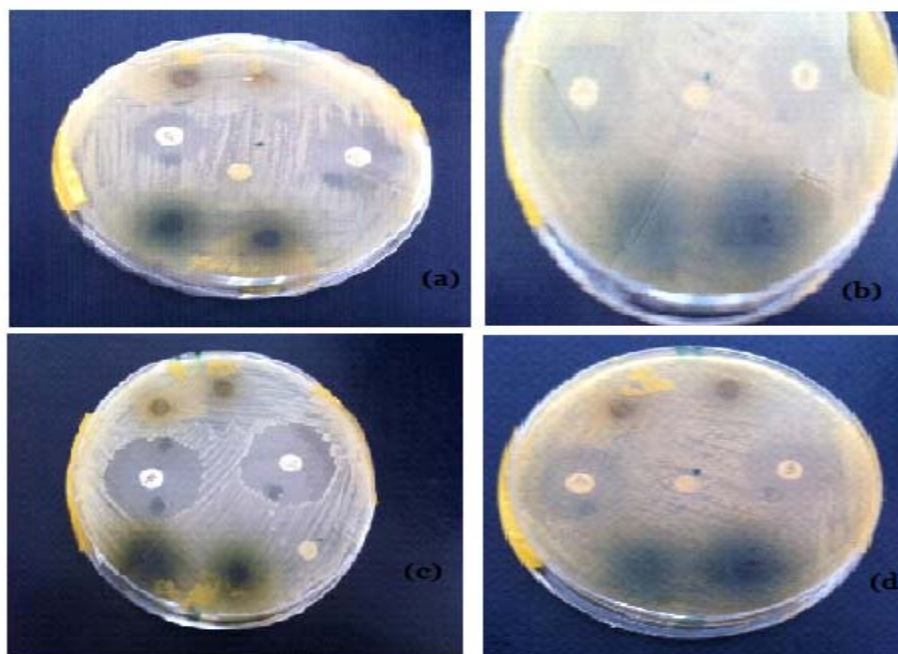
of inhibition recorded at 9.66mm and 5.33mm, respectively (Table 2). *Clarithromycin* showed higher activity against *E. coli*, ethanol extract of *Stevia* and erythromycin showed moderate activity, and water extract of *Stevia* showed lower activity. DMSO showed no activity, which was used as a negative control (Fig. 2).

**Table 2:** Antibacterial activity of *Stevia rebaudiana* against *Bacterial pathogens*

Bacteria strain	Ethanol extract	Water extract	Clarithromycin	Erythromycin	DMSO
<i>E. coli</i>	5.66±0.33	3.00±0.00	9.66±0.33	5.33±0.33	0.00±0.00
<i>L. acidophilus</i>	9.33±0.54	2.66±0.27	9.33±0.27	8.33±0.27	0.00±0.00
<i>S. aureus</i>	7.33±0.33	3.66±0.33	12.33±0.33	11.00±0.57	0.00±0.00
<i>S. typhi</i>	7.66±0.66	1.66±0.33	6.33±0.33	6.66±0.33	0.00±0.00

\*Shows mean zone of inhibition in mm±SEM

It was further observed that both ethanol and water extract of *Stevia* showed inhibitory activity against *Lactobacillus acidophilus*. Table 2 showed the mean diameter of the clear zone of inhibition of ethanol extract (9.3mm) and water extract was 2.6mm. The antibiotic disc that was used as a standard control also showed a zone of inhibition against *Lactobacillus acidophilus*. Table 2 showed that the mean diameter of the clear zone of clarithromycin inhibition was 9.3mm, and erythromycin was 8.3mm. Consequently, it was observed that ethanol extract and antibiotics disc (Clarithromycin and Erythromycin) showed a higher zone of inhibition while water extract showed significantly lower activity against *Lactobacillus acidophilus* (Figure 2).



**Figure. 2:** (a) Plate showing zone of inhibition against *E. coli*. (b) Plate showing zone of inhibition against *Lactobacillus acidophilus*. (c) Plate showing zone of inhibition against *S. aureus* (d) Plate showing zone of inhibition against *S. typhi*

Significant antibacterial activity of *Stevia rebaudiana* was observed against *Staphylococcus aureus*. After 24 hours, ethanol extract of *Stevia* showed a zone of inhibition of 7.33mm, and water extract of the plant showed a zone of inhibition of 3.66mm, while the positive control Clarithromycin and Erythromycin showed zone of inhibition of 12.33mm and 11mm (Table 2). In (Fig. 2), the antibiotic disc showed higher activity against *S. aureus* while ethanol and water extract showed lower activity against *S. aureus* than the antibiotic disc. Further, it was observed that after 24 hours, ethanolic and water extracts of *Stevia* exhibited antimicrobial activity and the zone of inhibition observed were 7.6mm and 1.66mm, respectively. While, an antibiotic disc of Clarithromycin and Erythromycin showed a zone of inhibition of 6.33mm and 6.66mm (Table 2, Figure 2). In (Table 2), the ethanol extract showed higher activity against *Salmonella typhi* and positive control antibiotic disc of Clarithromycin and Erythromycin showed moderate activity, and water extract of *Stevia* showed lower activity against *S. typhi*.

### 3.2 Antifungal Activity of *Stevia rebaudiana*

The antifungal activity *Stevia rebaudiana* showed that both ethanolic and water extracts exhibit no activity against *Aspergillus flavus*. Similarly, Terbinafine also showed no activity against *Aspergillus flavus* (Table 3, Fig. 3).

**Table 3:** Antifungal activity of ethanol and water extract of *Stevia rebaudiana* against *Aspergillus flavus*

Fungal strain	Ethanol extract	Water extract	Terbinafine	DMSO
<i>Aspergillus flavus</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

+ SD



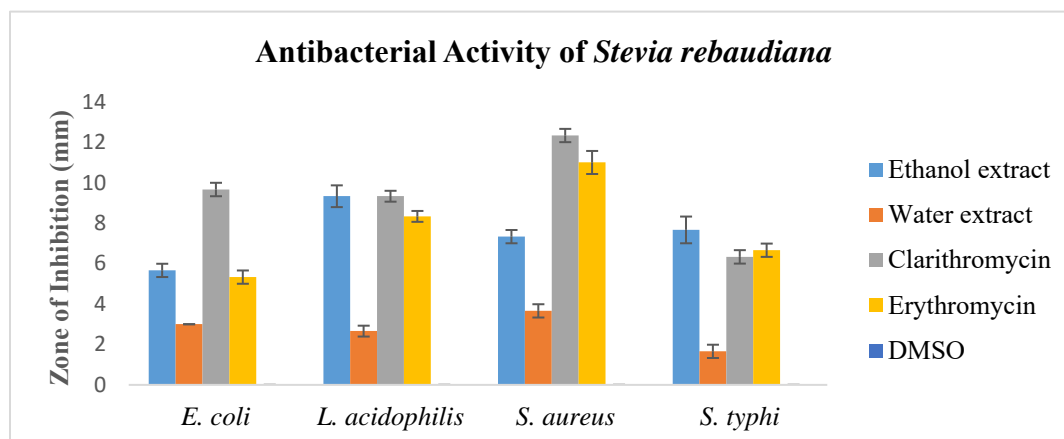
**Figure 3:** Plate showing activity of *Stevia* extract against *Aspergillus flavus*

### 4. Discussion

The plant has been a valuable natural product source for maintaining human health for natural therapies. Plant extracts and phytochemicals have a significant role in therapeutic treatment due to their antimicrobial properties (Santos *et al.*, 1995). According to the world health organization (WHO), the primary source of various drugs is a medicinal plant. 80% of individuals use traditional medicines (Ellof, 1998) in a developed country.

A previous study reported that *Stevia* extracts possess antibacterial, anti-yeast, antifungal, and antitumor activity (Tadhani *et al.*, 2006). The aim of the study was to evaluate the antimicrobial activity of ethanolic and water extract of indigenous *Stevia rebaudiana* against four bacterial strains and one fungal strain. In this study, the antibacterial activity of ethanol and water extract of *Stevia* was compared with two standard antibiotics (Clarithromycin and Erythromycin). In contrast, the antifungal activity of both extracts was compared with the antifungal drug (Terbinafine). The ethanol extract of *Stevia* shows the highest activity against four bacterial strains (*S. aureus*, *Lactobacillus acidophilus*, *S. typhi* and *E. coli*), but water extract of *Stevia* showed the lowest activity against these strains.

In contrast, the *Stevia* extracts and Terbinafine showed no activity against fungal strain. The ethanol extract of *Stevia* showed a zone of inhibition against *E. coli*, *Lactobacillus acidophilus*, *S. aureus*, and *S. typhi* was 5.66±0.33, 9.33±0.54, 7.33±0.33, and 7.66±0.66. But, the water extract of *Stevia* showed the lowest zone of inhibition which was 3.00±0.00, 2.66±0.27, 3.66±0.33, and 1.66±0.33 (Fig. 4).



**Fig. 4:** Comparative antimicrobial activity of *Stevia rebaudiana* ethanolic extract, water extract, and antibiotics.

The present study is in accordance with the reports of Tadhani *et al.*, (2006), who observed less antibacterial activity of water-based extract of *Stevia* (Tadhani *et al.*, 2006). Further, many researchers have also reported that the

water extract did not show higher activity against pathogenic bacteria (Vlietinck *et al.*, 1995). Debnath (2008) also reported that water extract does not show activity against bacterial strain.

The result of ethanol extract of *Stevia* against *Lactobacillus acidophilus* was also in accordance with a previous study reported by Gamboa, & Chaves, (2012). The ethanolic extract of *Stevia* leaves against *E. coli* and *S. aureus* showed almost the same results compared with the study of Ghosh *et al.*, (2008); the results of Ghosh *et al.*, (2008) against *E. coli* and *S. aureus* was 5mm, but the present study showed zone of inhibition of 5.66mm and 9.33mm. According to Malik *et al.*, (2011) the higher activity of ethanol extract is due to the more excellent solubility of extract in the organic solvent, or this could be due to the concentration of the active substances. These findings suggested that leaves extracts of *Stevia* exhibited a significant amount of antibacterial agents. Therefore it is suggested that these leaves extracts could be used for remedial purposes against selected bacterial pathogens. Further, a dose-dependent study of crude extract of *Stevia* leaves and in vitro evaluation of antimicrobial activity against different pathogens is required.

## 5. Conclusion

The present research was conducted to evaluate the antimicrobial activity of ethanol and water extract of *Stevia rebaudiana* against four bacterial strains (*Staphylococcus aureus*, *Salmonella typhi*, *Lactobacillus acidophilus*, and *Escherichia coli*) and one fungal strain (*Aspergillus flavus*). From this study, it was concluded that *Stevia* has antibacterial activity. The ethanol extract is more effective than the water extract of *Stevia*. The overall results of the present study showed that ethanol extract of the plant showed higher results against bacterial strains than antibiotics, but water extract of the plant showed shallow activity, whereas *Stevia* showed no activity against fungal strains (*Aspergillus flavus*). The medicinal plant could be the best source of new antimicrobial compounds; therefore, more study is required to evaluate and purify the new active antibacterial and antifungal constituent.

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## Conflict of interest

The authors reported no potential conflict of interest

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