

Screening of Leaf Extracts of *Stevia rebaudiana* for Antibacterial Activity, Phytotoxic and Haemagglutination Activities

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Abstract: The crude methanolic extract and various fractions of leaves of *Stevia rebaudiana* were investigated for antibacterial, phytotoxic and haemagglutination activities. The biological investigation revealed that crude extract showed good activity against *E. coli* (69%), moderate activity against *B. subtilis* (51.8%) and *S. aureus* (50%). No considerable phytotoxic activity was shown by *S. rebaudiana* against *Lemnaminor*. *S. rebaudiana* showed no haemagglutination activity against all blood groups indicating that the herb lacks phytolectins. The test samples showed significant antifungal activity, negligible phytotoxic activity and no haemagglutination activity.

Key words: *Stevia rebaudiana* • Fractionation • Haemagglutination • Inhibition Zones

INTRODUCTION

The world health organization has estimated 80% of the world population to be relying on herbal medications [1]. The potential of plants to have therapeutic properties can be imagined from the fact that today almost 25% of pharmaceutical prescriptions in United States have one of the constituents drawn from plants [1]. One of the reasons of increasing inclination towards herbal medications is the upsurge in the antibiotic resistance which is one of the menacing issues of the medical world nowadays. Medicinal plants comprises of antimicrobial agents and are a source of many powerful potent and powerful drugs [2]. Prioritizing herbal medicine is attributed to its minimal side effects and stupendous potential against treatment of infectious diseases. Besides, meeting the food demands of rapidly increasing population is also a primary concern of the world food agencies. Tons of food spoils every year before reaching the consumers' hands. Various microbes that utilize food as carbon and energy source are involved in chemical reactions that cause nasty sensory changes in food that makes it unbecoming for human consumption. *Stevia rebaudiana*- commonly called as sweet leaf is a small herbaceous, perennial shrub belongs to the family asteraceae [3]. It is usually 1m high

but can be more in height [4]. Extensive root system is present in this plant. Their stems are brittle, leaves are small, sessile, elliptic and oppositely arranged. The surface of the leaves bear trichome structures. It consists of small white florets which are arranged in corymb of 2-6 florets. Small achenes are present which contain the seeds and the pollination is carried out by insects [5]. The plant is native to the northern region of South America and is growing as wild plant in Brazil and Paraguay. It is also grown commercially in many parts of Brazil, Paraguay, Israel and china etc. [6]. It traditionally used for making tea, was explored for its potential as therapeutic and preservation agent.

The aerial parts of *S. rebaudiana* are used for consumption particularly the desiccated and pulverized leaves. Diterpene glycosides, which are a complex mixture of natural sweet, are present in the leaves of *S. rebaudiana*. This mixture includes; 2-4 % rebaudiosides A, trace amounts of rebaudiosides B, 1-2% of rebaudiosides C, trace amounts of rebaudiosides D and E, 4-13% stevioside, trace amounts of steviolbioside and 0.4-0.7% dulcoside A. Some non sweet components have been recognized which are as follows: Sterols, flavonoids, labdanoid diterpene, triterpenes, volatile oil constituents, gums, pigments and inorganic matter [7]. Beside the sweet

nature of *S.rebaudiana*, it has also got plenty of uses. Many diseases can be treated using *S.rebaudiana* including obesity, hypertension [8], cavities, diabetes [9], fatigue, depression, cancer [10] and yeast infections. *Stevia rebaudiana* also possess some important properties like vasodilating, hypoglycemic, sweetening, taste improving, anti bacterial, anti-fungal, antiviral, antiinflammatory. It don't have any negative effects like non addictive, non toxic, non mutnicage, non carcinogenic, devoid of genotoxic effect and non teratogenic. It is safe for diabetic patients as it has no effect on blood sugar level [11]. The presence of steviosides plays a very important role in hypoglycemic action. According to Paraguayans, stevia nourishes the pancreas and thus helps in the restoration of normal pancreatic function so it is beneficial for hypoglycemia patients [12]. *Stevia* capsules and *Stevia* tea are publically permitted for sale in Brazil for the treatment of diabetes [13]. *S. rebaudiana* extracts are effective against a number of bacteria and fungi thus have got antibacterial and antifungal activities.

Keeping in view the medicinal potential of *S.rebaudiana* and the presence of complex mixture of natural sweet diterpene glycosides, the current study was aimed to screen the crudemethanolic extract and various fractions of leaves of *S.rebaudiana* to explore its potential to be used as a phytomedicine and preservative.

MATERIAL AND METHODS

Collection of Plant Material: The leaves of *S. rebaudiana* were purchased from PCSIR laboratories where the plant is grown through tissue culture techniques.

Extraction: Leaves of *S. rebaudiana* were shade dried and grounded into fine powder using an electric grinder. The powdered leaves (500 g) were then macerated in commercial grade methanol for 7 days at room temperature with sporadic shaking. The soaked plant material was then filtered. The filtrates were concentrated below 40 °C in rotary evaporator. A blackish crude methanolic extract of 134 g was obtained.

Fractionation: The crude methanolic extract of *S.rebaudiana* (114 g) was suspended in distilled water (300 ml) and partitioned with n-hexane (3°C 200ml) giving n-hexane (30 g) and aqueous fraction (34g). Before making fractions 20 g of crude methanolic extract of *S.rebaudiana* was set aside for biological screening. The n-hexane fraction and aqueous fraction will contain non polar and polar compounds respectively.

Antibacterial Activity: Antibacterial activity of crude methanolic extract and fractions of *S.rebaudiana* leaves were examined against *K.pneumonis*, *E.Coli*, *S. aureus*, *S.typhae* and *B.subtilus*.. Stock solution was made by dissolving 3 mg/mL of the test samples in sterile dimethyl sulfode (DMSO, Merck). The nutrient agar media was autoclaved, poured in sterile petriplates and were allowed to solidify. The plates were then inoculated with test microorganisms by taking small amount of inoculum from the nutrient broth containing the culture and transferred to plates (pour plate method). Wells were made with the help of 6mm borer in those nutrient agar plates. The test samples (100µl) were then introduced into the wells using micropipette. Amoxicillin was employed as positive control while less than 1% of DMSO was used as negative control. The plates were then incubated at 37°C for 24 hours. The results were taken by measuring the diameter of zone of inhibition produced by each sample and thus percent (%) inhibition was calculated.

Phytotoxic Activity: Phytotoxic activity of crude methanolic extract and fractions of *S.rebaudiana* leaves were examined against *Lemnaminor* available at the Department of Botany, University of Peshawar. The standard protocol of McLaughlin *et al.* [14] was followed for this activity. Stock solution is made by dissolving 20 mg/mL of the test samples in methanol. E-media was also prepared for the growth of *L.minor*. Three concentrations were prepared from stock solution i.e 10, 100, 1000µg/mL and were introduced into three separate flasks and left at room temperature till the methanol was evaporated. 20 mL of the E-medium and sixteen healthy plants with a rosette of three leaves were added to all the flasks and incubated at (28 ±1) °C for 7 days. Paraquat at a concentration 0.015 ig/ml was used as standard growth inhibitor. Results were taken by counting the number of damaged and healthy plantlets and percent (%) growth inhibition was calculated.

Haemagglutination Activity: Haemagglutination activity of crude methanolic extract and fractions of *S.rebaudiana* were screened against human erythrocyte for all blood groups. The protocol of Naqvi *et al.* [15] was followed. Fresh blood samples were collected from healthy volunteers, centrifuged at 6000 rpm for 10 mins and the erythrocytes (pellet) were separated. 2% erythrocyte suspension was prepared by dissolving 2 ml RBC in 98 ml phosphate buffer (or 0.5 ml RBC in 24.5 ml buffer pH 7.4). Stock solution is made by dissolving 1 mg/mL of the test

samples in sterile dimethyl sulfoxide (DMSO, Merck) and different dilutions (1:2, 1:4, 1:8, 1:16) were made from it. 1 ml erythrocyte suspension (2%) was added to each dilution and incubated at 37°C for 30 mins. Positive and negative results are indicated by rough granules and smooth button formation. Extent of deposition determined the intensity of positive result.

RESULT AND DISCUSSION

Antibacterial Activity: Crude methanolic extract and various fractions of leaves of *S.rebaudiana* were screened against the test pathogens for possible antibacterial activity. The results are summarized in figure 1. The crude methanolic extract showed good activity against *E. Coli* (69%) while moderate activity was shown against *B. subtilus*(51.8%) and *S. aureus* (50%). No activity was shown against *K. pneumonia* and *S. typhae*. The n-hexane fraction showed low activity against *K. pneumonia* (33.3%), *E.coli* (30.4%), *S. aureus* (34.6%) and *B. subtilus* (33.3%) but no activity was shown against *S.typhi*. The aqueous extract showed a very low activity against *S. typhae* (29.6%) and *B. subtilus* (25.9%) but no activity against the rest of the microorganisms, this result are in agreement with pankaj *et al.* [6].

Phytotoxic Activity: *Lemna minor* is a small aquatic monocot. It is used as a model system to detect phytotoxic compounds because of their sensitivity to bioactive compounds [16]. The crude methanolic extract showed growth regulation of 18.75%, 18.75% and 6.25% at 1000, 100 and 10µg/ml respectively. The percent growth regulation shown by n-hexane extract was 0% at all the three concentrations. The percent growth regulation of the aqueous fraction was 6.25%, 18.75% and 6.25% respectively for 1000, 100 and 10µg/ml. The results are shown in figure 2. These results show that no considerable phytotoxic activity was shown by *S.rebaudiana* against *L. minor*.

Haemagglutination Activity: Phytolectins are proteins or glycoproteins that are derived from plants and are having the ability to bind specifically to sugar moieties in cell walls and plasma membranes. Plant lectins are also involved in agglutination reaction by changing the physiology of erythrocytes plasma membrane. The results of the haemagglutination activity of the test samples were determined against human RBCs of all blood groups. All of the test samples were inactive against RBCs of all blood groups. They don't show any agglutination which means that *S.rebaudiana* lacks phytolectins.

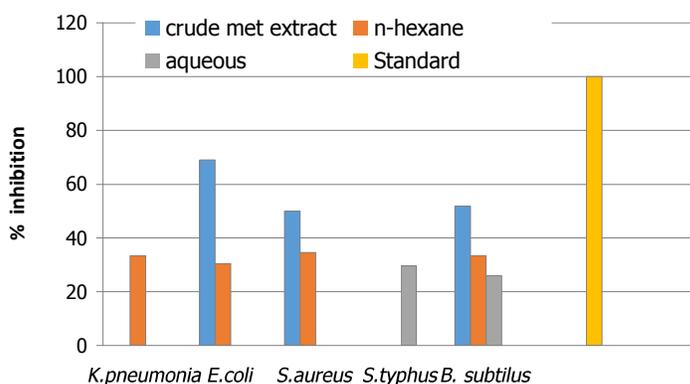


Fig. 1: Antibacterial activity of Crude methanolic extract and various fractions of *Stevia rebaudiana*

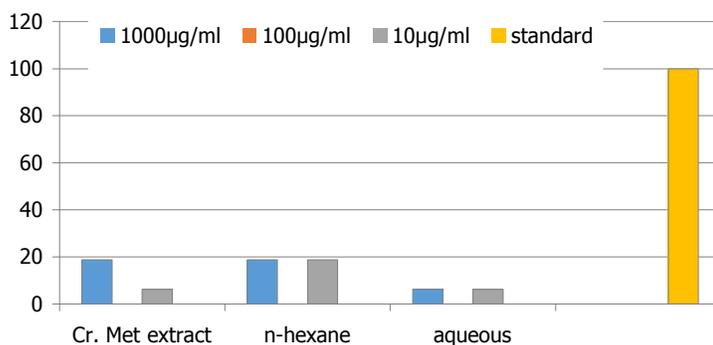


Fig. 2: Phytotoxic activity of crude methanolic extract and other fractions against *Lemna minor*

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