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RESEARCH ARTICLE

PHYTO-CHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF STEVIA REBAUDIANA

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ABSTRACT

Stevia rebaudiana is an herb with a very high medicinal value. It has more than 100 phytochemicals. It is used as sugar substitute. The extracts from leaves are used for the microbial assay and study the antimicrobial activity. The microbes used for the testing the activities are E.coli, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Aspergillus niger, Candida parapsilosis, Trichophyton rubrum.* With different concentrations of the extracts starting from 2.5µg/ml, 5.0µg/ml, 7.5µg/ml, 1.0µg/ml and the highest activity is shown by *Staphylococcus aureus* and *Candida parapsilosis.*

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INTRODUCTION

We use natural products as therapeutic activity to protect ourselves from many microbes which has the ability to cause infection (Brandle, 1998). The natural products extracted from plant have a long history were the main source of medicines we use now a day's. Screening of these compounds systematically has leaded the researchers to the discovery of the antimicrobial compounds/products. Generally plants have their own mechanism to protect themselves from predators and the infectious agents (Rajesh, 2010 and www.iiste.org). These antimicrobial compounds produced by plants are the best example. Treatment of infections with synthetic drugs is costly as well as they show side effects too. Therefore we have the need to search for such plants with medicinal value. Here is such a plant with more than 100 phyto-chemicals used to treat Diabetes, Candidacies, Blood Pressure and used for loss of weight (Réjeanne Gougeon 2004). And used as artificial sweetener it belongs to Asteraceae family Stevia rebaudiana known as Madhu patri in telugu. The bioactive compounds produced by the plants like flavanoids, alkaloids, phenolic compounds etc, have their effect on Human body. (Yin Wei Mak *et al*., 2012; Sunanda Singh *et al*., 2012; Mousumi Debnath, 2008 and Kuntal *et al*., 2009) Stevia rebaudiana rich in terpenes and flavanoids and the phytochemicals of it are austroinullin, b- carotene, rebaudi oxides, riboflavin, steviol etc,. The medicinal uses of s.rebaudiana are it is used as anti inflammatory, vasodilator and anesthetic (Manish, 2006 and Sathishkumar Jayaraman, 2008).

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Micro organisms

The test organisms Staphylococcus aureus, Bacillus subtilis, Escherichia coli, *Trichophyton rubrum,* Pseudomonas aeruginosa, *Salmonella typhi, Candida parapsilosis* and *Aspergillus niger* were cultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques. Stock cultures were maintained as Glycerol stocks at 4°C checking their viability time to time.

Evaluation of antimicrobial activity

The experiment was done with care and perfect handling in aseptic conditions. nutrient agar medium (25 ml) was taken in a sterile petridish and broth cultures of the test isolate (0.1 ml) containing 1.0 X 10^5 CFU/ml of organisms were added. The extracts were dissolved in Ethyl alcohol and used. The concentrations used for the test are 10, 20, 40 and 50 mg/ml. Ampicilin (10µ g/ml) was used as standard antibacterial agent and Griseofulvin was used as standard antifungal agent.

MATERIALS AND METHODS

Phytochemical Analysis

Plant material was collected from the college campus. Chemicals such as wagnes reagent, chloroform, 2% H2SO4, Concentrated sulphuric Acid, 10% Lead acetate, Benedict's reagent, 0.1% ferric chloride, Fehling's solution, dilute NaOH,2% HCL, 10% Ammonia, 10%HCL, distilled water, Ethyl Alcohol are provided by the management of the college.

Preparation of solutions

- **Fehling's solution:** A mixture of equal volume of copper sulphate, sodium potassium tartar ate and sodium hydroxide is prepared in a beaker.
- **Wagner's Reagent:** Mixing 2gm of Iodine, 6gm of potassium iodide in 100ml of water.

Collection of sample

Healthy leaves of Stevia rebaudiana were taken and washed under running water to remove the dust and other external pollutants. The plant leaves, roots and flowers were air dried for few days. Normally it takes 15 to 21days for drying.

Grinding the sample

The dried leaves are grinded to a fine powder in a mixer and the powder is collected in clean polythene bags.

Preparation of plant extract with Ethyl Alcohol

Taken 10gms of leaf powder and added 50ml of ethyl alcohol stirred it constantly for 30 minutes and the solution was kept at room temperature for 24 hours (minimum) and then filtered. The filtered solution is again filtered with whatman filter paper No.3 and then it was stored at 4 degrees centigrade (in a freezer) until use.

Antimicrobial activity

Leaves and Roots Collection

The leaves, flowers and roots for the present study from the plant Stevia rebaudiana were collected from University College for Women, Koti, Hyderabad. And were allowed to dry under shade and made into a fine powder. The powder (100grams) was Soxhlet extracted with methanol and dried under rotavapor at 40-50°C for 3-4 hours. This measure was taken in order to evaluate the antimicrobial activity.

Equipment preparation

To conduct the experiment, the nutrient agar media was prepared by dissolving 28g of nutrient agar in 1000ml distilled water. It was sterilized in autoclave along with the petridishes, forceps, spreader, cotton balls and 25ml conical flasks. The sterilized agar was then transferred into the petridishes and was allowed to solidify. Thereafter, the procedure was executed in laminar air flow to ensure proper aseptic conditions

Preparation of Paper Discs

The mode of anti microbial activity of the above medicinal plant leaves were performed using the whatman no.1 paper. The fine round paper discs were obtained and were sterilized.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activity of leaf and root extracts were examined against Gram positive and Gram-negative bacteria and fungal strains by measuring zone of inhibition. The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 2.5, 5.0, 7.0, 10µg/ml respectively. Ampicillin (antibacterial), Itraconazole (antifungal) as the standard drug at a concentration of 200µg/ml. LB Agar was used as the culture media for antibacterial and potassium dextrose agar was used as culture media for the antifungal activity. The results of the antimicrobial activity are shown in figures and tables.

Stevia rebaudiana *leaves*

Organism/conc^n of extract	2.5µg/ml	5µg/ml	7.5µg/ml	10µg/ml
E.coli	1.0cm	1.1 cm	1.3 cm	1.5 cm
Staphylococcus aureus	1.1 cm	1.3cm	1.3 cm	1.5 cm
Bacillus subtilis	0.7 cm	0.9 cm	1.1 cm	1.2 cm
Salmonella typhi	0.8 cm	1.2 cm	1.3 cm	1.6 cm
Aspergillusniger	0.6cm	0.8 cm	0.8cm	1.0 cm
Candida parapsilosis	1.0 cm	1.2 cm	1.3 cm	1.5 cm
Trichophyton rubrum	0.9 cm	1.0 cm	1.2 cm	1.1 cm

The Stevia rebaudiana leaf extract showed high activity against *Staphylococcus aureus* at very low concentration (2.5µg/ml) compared to *E.coli*, *Bacillus subtilis,* leaf extract showed high activity against *Candida parapsilosis* at a very low concentration (2.5µg/ml) compared to *Aspergillus niger.* The zone of inhibition is calculated in cm.

Stevia rebaudiana *roots*

Organism/conc^n of extract	2.5µg/ml	5µg/ml	7.5µg/ml	10µg/ml
E.coli	1.0cm	1.1cm	1.1cm	1.2 cm
Staphylococcus aureus	0.9 cm	0.9cm	1.0cm	1.2cm
Bacillus subtilis	1.0 cm	1.2 cm	1.4 cm	1.4 cm
Salmonella typhi	0.6 cm	0.8cm	0.9 cm	0.8 cm
Aspergillusniger	0.8cm	1.0 cm	1.1cm	1.1 cm
Candida parapsilosis	0.8cm	1.0cm	1.2cm	1.3cm
Trichophyton rubrum	0.7cm	0.8 cm	1.0 cm	0.9 cm

The *Stevia rebaudiana* root extract showed high activity against the *E.coli and Bacillus subtilis* at very low concentration (2.5µg/ml) when compared and root extract showed high activity against *Candida parapsilosis* and *Aspergillus niger* at a very low concentration (2.5µg/ml) compared to *Trichophyton rubrum.* The zone of inhibition is calculated in cm.

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