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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY IN MEDICINAL PLANT (*STEVIA REBAUDIANA*) USING TWOSOLVENTS

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ABSTRACT

The present study was to investigate the antibacterial activity obtained from *Stevia rebaudiana*against *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiellapneumoniae, Proteus vulgaris* by disc diffusion method. The ethanol extract showed maximum activity against*Stevia rebaudiana* root in *E.coli* and the minimum zone of inhibition was observed in *Proteus vulgaris, E.coli* using ethanol extractin leaf.

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend in part on plants for the production of pharmaceutical compounds (Chand *et al*.,1997).

WHO estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. The developed nations are also looking for eco-friendly treatment of various diseases through plant based source.

In addition, many valuable herbal drugs have been discovered by knowing that particular plant was used by the ancient folk healers for the treatment of some kind of ailment (Ekka and Dixit, 2007). Microbiological investigations of stevia plant extract against pathogenic species using an array of solvents has neither been attempted earlier by many workers seriously nor reported much (Takai, 1985; Tomita, 1997; Tadhani *et al.,* 2006). *Stevia rebaudiana* leaf extracts demonstrated antibacterial, antifungal, antiyeast and antitumor activity. To the best of our knowledge, there is no previous reported work on the antimicrobial and antitumor activity of *Stevia rebaudiana* except that of (Tadhani and Subash, 2006).The present study was undertaken to further analyze the antibacterial activity of *Stevia rebaudiana*.

MATERIALS AND METHODS

Sample collection and solvent extraction

The materials selected for the present study were *Stevia rebaudiana* .The plants were collected from Cheruvarakkonam, Kanyakumari District, Tamilnadu, India. Plants were dried under shade condition for one month and cut into small pieces, pulverized in a grinder and stored in sterile containers for further use. A Soxhlet extractor apparatus was used for extraction, with ethanol and methanol solvents.

Bacterial Strains

In the present study five human pathogens were used, namely *E.coli, Klebsiellapneumoniae, Proteusvulgaris, Pseudomonas aeruginosa, Staphylococcusaureus*obtained from Inbiotics Institute of Biology of clinical Research, Nagercoil. Stock cultures were maintained in nutrient agar medium at 40^0C,then subcultured in nutrient broth at 37^0C prior to each microbial test.

Disc Diffusion Method

Disc Diffusion method was used to screen the antibacterial activity (Bauer *et al.,* 1966).The sensitivity test of the chloroform and ethanol extracts were determined using agar–

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disc diffusion method. Media were prepared using Muller-Hinton Agar poured in petridishes and inoculated with test organisms from the broth using cotton swabs. Disc impregnated with the plant extract were placed on the swabbed plate. The plates were incubated overnight at 37^0C for 24 hours. Amikacin was used as positive reference standard. After incubation, the clear zone around the discs were measured and expressed in mm as a measure of their antibacterial activity.

Table.1 Antibacterial Activity In *Stevia Rebaudiana*using A Disc Diffusion Method (Zone Of Inhibition In Mm)

S.no	Name of the bacteria	solvent	Stem	Root	Callus	Leaf
1	*E.coli*	Ethanol	9.00±0.50	12.16±0.30	10.33±0.05	7.03±0.15
		Methanol	8.25±0.25	-	8.0±0.26	8.16±0.35
2	*K.pneumoniae*	Ethanol	8.00±0.40	-	-	11.23±0.25
		Methanol	9.57±0.36	10.13±0.35	8.0±0.17	-
3	*P.aeruginosa*	Ethanol	11.00±0.10	8.00±0.26	8.03±0.20	10.16±0.15
		Methanol	9.00±0.10	-	-	-
4	*S.aureus*	Ethanol	10.00±0.20	8.00±0.26	-	-
		Methanol	9.00±0.10	8.01±0.32	-	-
5	*P.vulgaris*	Ethanol	12.13±0.15	8.06±0.28	10.0±0.20	7.03±0.15
		Methanol	-	-	8.0±0.10	-

RESULTS AND DISCUSSION

The results of antibacterial activity in two extract of medicinal plant like *Stevia rebaudiana* against human pathogens were shown in Table 1.The present investigation showed that the tested plant extract possess potential antibacterial activity against *E.coli, Klebsiellapneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus*.

The Ethanol extract of *Stevia rebaudiana*stem showed the antibacterial activity against five pathogens with the inhibition zones of 9.00,8.00,11.00,10.00 and 12.13mm, respectively. The methanol extract of *Steviarebaudiana* stem showed the inhibition zone of 8.25,9.57,9.0 and 9.0 respectively. The Ethanol extract of *Stevia rebaudiana* root showed the antibacterial activity against five pathogens with the inhibition zones of 12.16,8.00,and 8.01mm, respectively. The methanol extract of *Stevia rebaudiana* root showed the inhibition zone of 10.13,8.0and 8.06mm respectively.

The Ethanol extract of *Stevia rebaudiana* callus showed the antibacterial activity against five pathogens with the inhibition zones of 10.33,8.03 and10.0 respectively. The methanol extract of *Stevia rebaudiana*callus showed the inhibition zone of 8.0,8.0and 8.0mm respectively. The Ethanol extract of *Stevia rebaudiana* leaf showed the antibacterial activity against five pathogens with the inhibition zones of 7.03,11.23,10.16 and7.03mm respectively. The methanol extract of *Stevia rebaudiana* leaf showed the inhibition zone 8.16mm respectively. The maximum zone of inhibition was observed in *Stevia rebaudiana*rootagainst ethanol extract in *E.coli*(12.16mm). The minimum zone of inhibition was observed in *Stevia rebaudiana* leaf against ethanol extract in *E.coli*(7.03mm).In the present study, it was also observed that gram negative bacteria were more sensitive than gram positive bacteria in the selected plant extract.

Manish *et al.*, 2006 reported that hexane extract of *S. rebaudiana* leaf showed higher activity compared to methanol ethyl acetate extract aganist microorganism tested. Tomita *et al.*,1997 reported that the methanol extract was found to be the best solvent to result in good antibacterial activity but they did not examine other extract aganist selected bacteria.

CONCLUSION

Stevia is a natural sweetener plant having commercial importance and it is used all over the world.

From the above results, it is concluded that *S. rebaudiana* showed antibacterial activity and also used as a medicinal plant.

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