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STUDIES ON ANTIBACTERIAL ACTIVITY OF STEVIA REBAUDIANA AGAINST WOUND INFECTION CAUSING BACTERIA

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ABSTRACT

Stevia rebaudiana belonging to the family Asteraceae is thought to inhibit the growth of certain bacteria. Antibacterial activities of crude extracts were examined by well diffusion method. Each plant extracts was dissolved in respective solvents such as Acetone and Chloroform, Ethanol, Hexane, and Petroleum ether. Among the five types of extract, the highest inhibition zone was observed in Ethanol extract and second most acetone extract followed by chloroform, petroleum ether and hexane. In our current studies *S. rebaudiana* was highly suppressed to *Staphylococcus aureus*, second most *Klebsiella pneumonia* followed by *Escherichia coli* and *Pseudomonas aeruginosa.* Our current results plant 2 (GKVK university Bangalore) sample were exhibited high antibacterial activity against wound infected pathogens second most plant 4 (Yercaud) followed by plant 1 (kolli hills) and plant 3 (Jaipur).

INTRODUCTION

Stevia is a genus of about 200 species of herbs and shrubs in the family of Asteraceae. It grows up to 1 m tall and has leaves 2-3 cm long. The plant is indigenous to northern region of South America and is still found growing in wild in Brazil and Paraguay, Uruguay, Central America, Israel, Thailand, and China. The leave extracts of *stevia*, 300 times the sweetness of sugar has documented of antibacterial, antifungal, ntiinflammatory, antimicrobial, antiviral, antiyeast, cardiotonic, diuretic, hypoglycemina, and hence a boon to diabetic people hypotensive tone and vasodilator (Curi, 1986). *Stevia* is completely natural non-synthetic product Stvioside (the sweetener) contain absolutely no calories (Pankajkishore *et al.*, 2010). The leaves can be used in their natural state. It has enormous sweetening power only small quantity needs to be used. The antibacterial activity of the acetone extract of *stevia* leaves was higher than that of other extracts. The acetone extract showed greater activity against Gram positive organism than against Gram negative organism. The higher antibacterial activity of the acetone and ethyl alcohol extracts may be due to the greater solubility of the in the organic solvents (De Boer *et al.*, 2005). The selection of crude plant extract for screening program has the potential of being more successful in initial steps than the screening of pure compounds isolates from natural products (shah *et al.*, 2005). Emergence of multi-drug resistance human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin (Iqbal ahamed and Arina Beg 2001). The objectives of the study include *in vitro* organogenesis of *S. rebaudiana* and comparison of antimicrobial activity of dried leaf extract (*in vitro and in vivo*) in different solvent system. The aim of the present study has been evaluate the effects of *S. rebaudiana* on wound infection causing bacterial isolates.

MATERIALS AND METHODS

Purulent materials were collected aseptically with aid of sterile swab sticks from forty (40) patients with different wounds infection *Escherichia coli, Pseudomonas aerougenosa, Staphylococcus aureus* and *klebsiella pneumonia* at Namakkal dist in TamilNadu surrounding hospitals. Culture plates of Eosin Mthylene Blue Agar, Mac Conkey Agar, Cetramide agar, Blood Agar and Mannitol Salt Agar were used. The swab sticks used for the collection of the samples were streaked directly on the labelled agar plates and incubated at 37^{o} C for 24 hour. After incubation, the cultures were examined for significant growth by Cown (1985), Fawole (1988) and Cheesbrough (2004) methods.

Collection of plant material

The fresh plant *S. rebaudiana* was collected from 4 different areas in India such as plant 1 kolli hills, plant 2 GKVK Bangalore, plant 3 Jaipur and plant 4 Yercaud.

Preparation of plant material

The fresh leaf was harvested, rinsed with tap water and air dried under shade for 14 days and reduced to coarse powder using grinded to fine powder. The powder was stored in an airtight bottle until needed for use.

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Preparation of Extracts

Dried plant materials (100g) were extracted with 500 ml of Acetone and Chloroform, Ethanol, Hexane, and Petroleum ether in a soxhlet apparatus. Aqueous extract was prepared by hot maceration. The extraction process was completed 72 cycles of 8 hours per day from 9 days. When the solvent was driend colourless, the extract was stopped.

The solvent was completely removed by using rotary flash evaporator or water bath to obtain semisolid mass except water extract which was obtained as dried powder. These extracts were resuspended in Acetone, Chloroform, Ethanol, Hexane and Petroleum ether to yield 100 mg residue 100 ml solvent (Gugulothu *et al.,* 2011).

Antibacterial activity of plant extract against wound infecting bacteria.

Antibacterial activities of crude plant leaf extracts were examined by the well diffusion method. Each plant extract was dissolved in respective solvents such as Acetone, Chloroform, Ethanol, Hexane, and Petroleum ether tested they were evaluated for each bacterium. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zone.

Experimental procedure

Well diffusion method (zone of inhibition)

The plant materials extracts were tested for antimicrobial activity by the well diffusion method (Chung *et al.,* 1990). This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish to an extract such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts (Cote and Ghemal 1994). Using micropipette 0.5 ml of each of the leaf broth containing 10-5-10-6 Efu/ml test organisms were incubated on the four plates of solidified agar and spreaded uniformly with a glass spreader. Then four well were cut out in the agar layer of each plate with an aluminium bore of 5 mm diameter to contain 0.5 ml extract, standard drug and DMSO and Methanol. All the work was carried out in freeze for one day. After addition to allow diffusion of the solution in to the medium and then incubated for 37º C for 24 hours for antibacterial activity .After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured. Tetracycline was used as standard drug for antibacterial activity.

RESULT AND DISCUSSION

A total number of 40 patients with different types of wounds during the study period. Among from those patients 40 types of bacterial species were isolated by selective culture medium and standard biochemical test. There were 40 swabs examined for our study, the swabs yielded growth of 33 (82.5%) isolates (Table-1). This means that some samples yielded more than one organism. There as a distribution of isolates from the different types of wound infections. Among the 40 swabs highest occurrence were *S. aureus* and *P. aeruginosa* (25%), second most *K. pneumonia* (20%) and lowest occurrence were *E. coli* (12.5%). Antibacterial activities of crude extracts were examined by well diffusion method. Each plant extracts was dissolved in respective solvents such as Acetone and Chloroform, Ethanol, Hexane, and Petroleum ether. Among the five types of extract the highest inhibition zone was observed in Ethanol extract and second most acetone extract followed by as chloroform, petroleum ether and hexane (Table 2-5).

Table 1 Isolation of wound infecting bacteria

S.NO	Samples	Number of samples	Isolates				%of Occurance
			S. aureus	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	
1	Wound samples	40	10(25%)	10(25%)	8(20%)	5(12.5%)	82.5%

Table 2 Antibacterial activity of *Stevia*- kolli hills (plant-1)

Organism Extract	Zone of inhibition (mm in diameter)			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Acetone	-	20	-	14
Chloroform	-	19	-	13
Ethanol	-	21	-	15
Hexane	-	12	-	-
Petroleum ether	-	-	-	-
Tetracyclin	40	34	39	39
Control	-	-	-	-

Table 3 Antibacterial Activity of *Stevia*- banglore (plant-2)

Organism Extract	Zone of inhibition (mm in diameter)			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Acetone	-	12	10	16
Chloroform	-	-	-	-
Ethanol	14	14	13	20
Hexane	-	-	-	11
Petroleum ether	-	12	-	14
Tetracyclin	40	34	39	39
Control	-	-	-	-

In our current studies *S. rebaudiana* was highly suppressed to *S. aureus*, second most *K. pneumonia* followed by E. coli and *P. aeruginosa*. Our current results plant 2 GKVK university Bangalore sample were exhibited high antibacterial activity against wound infected pathogens second most plant 4 Yercaud followed by plant 1 kolli hills and plant 3 Jaipur (Table 2-5).

Table 4 Antibacterial activity of *Stevia*- jaipur (plant-3)

Organism Extract	Zone of inhibition (mm in diameter)			
	E. coli	***K. pneumoniae***	***P. aeruginosa***	***S. aureus***
Acetone	-	-	-	14
Chloroform	-	-	-	19
Ethanol	17	-	-	13
Hexane	-	-	-	12
Petroleum ether	16	-	12	-
Tetracyclin	40	34	39	39
Control	-	-	-	-

Table 5 Antibacterial activity of *Stevia*- yercaud (plant-4)

Organism Extract	Zone of inhibition (mm in diameter)			
	E. coli	***K. pneumoniae***	***P. aeruginosa***	***S. aureus***
Acetone	12	-	-	15
Chloroform	11	-	11	13
Ethanol	13	14	13	19
Hexane	-	-	-	-
Petroleum ether	-	-	12	13
Tetracyclin	40	34	39	39
Control	-	-	-	-

CONCLUSION

The present investigation endow with the basic information about new non-antibiotic drug molecule of plant origin, especially Ethanol extract of *stevia* leaves which is found to be potent enough in exhibiting substantial antimicrobial activity against wound isolates. In our current study S. *rebaudiana* was highly suppressed to *S. Aureus*, second most *K. pneumonia* followed by *E. coli* and *P. aeruginosa*. There fore the results of the present work indicate that *Stevia* leaf extract may be an ideal source for pharmaceutical and natural plant based product. The large number of antimicrobial may explain why this plant showed very good anti microbial effect. The wide diversity of polarity of the antimicrobial compound may provide clinically useful leads.

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