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Antibacterial Activity of Different Extracts of Sundakai (*Solanum torvum*) Fruit Coat

M. Sivapriya, R. Dinesha, R. Harsha, S.S.T. Gowda and L. Srinivas
Adichunchanagiri Biotechnology and Cancer Research Institute, Balagangadharanatha Nagara-571 448, Mandya District, Karnataka, India

Corresponding Author: Leela Srinivas, Adichunchanagiri Biotechnology and Cancer Research Institute, Balagangadharanatha Nagara-571 448, Mandya District, Karnataka, India

ABSTRACT

The main aim of this study was to find a new natural, non toxic, effective antibiotic from plant extract. In the present study, *Solanum torvum* (Sundakai) was selected. The antibacterial activity of different extracts of *Solanum torvum* (Sundakai) fruit coat was studied *in vitro* against human pathogenic strains. The Water Extract (WE) and Ethanol Water Extract (EWE) was found to be effective against all bacterial strains and inhibition is comparable to that of commercial antibiotics, choramphenicol and streptomycin. Initial screening of antibacterial activity was done by disc diffusion method in Nutrient agar medium. Minimum inhibition concentration was done serial dilution method. The MIC values of the Water Extract (WE), Ethanol Water Extract (EWE) and Ethanol Extract (EE) ranged from 9.6 to 19.2 μg mL^{-1}. It was observed that there was a correlation between the amounts of polyphenols and flavonoids content and effective antibacterial activities of the inhibited extracts. These results indicate that Sundakai coat may be yet an another source of natural antibiotic. Further, this study reaffirms the ethanomedicinal property of *S. torvum* plant.

Key words: *Solanum torvum*, sundakai, antibacterial, fruit coat, natural antibiotics

INTRODUCTION

In developing countries, Infectious diseases remain the main cause of high mortality rates recorded by WHO (1996). The treatment of infectious diseases is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to development of resistant strains (Shahidi Bonjar, 2004), mostly through the expression of resistance genes (Davis, 1994; Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions (Ahmad *et al*., 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources such as medicinal plants. Indigenous herbal remedies are widely used against many infectious diseases, but only few of them have been studied chemically and biologically in order to identify their active constituents (Otshudi *et al*., 2000). Medicinal plants are also known to be used as food preservative due to its antimicrobial activity (Sunilson *et al*., 2009). *Solanum torvum* (Solanaceae) commonly known as Sundakai is a small shrub, of which its edible fruits are used as essential ingredients in Thai and Indian cuisine (Iida *et al*., 2005). It has been used ethnomedically as a tonic and haematopoetic agent and for treatment of pain (Ambasta, 1992; Daziel, 1937; Watt and Breyer-Brandwijk, 1962). The Antiviral, anti-ulcerogenic property of leaf extract has been reported (Arthan *et al*., 2002; Nguelefack *et al*.,

2008). Leaf extract of *S. torvum* has been shown to process antibacterial activity (Valsaraj *et al.*, 1997; Wiart *et al.*, 2004). Sporadic alkaloids, saponins, sapogenins, flavonoids and glycosides have been reported from *S. torvum* (Arthan *et al.*, 2002; Chung *et al.*, 1998; Dopke *et al.*, 1975; Lu *et al.*, 2009). Nevertheless, there is no information about the antibacterial property of seed coat extract of *S. torvum* and its constituents.

Here we report the antibacterial property of Sundakai fruit coat extracts with various solvents or solvent mixture in order to ascertain the maximum antibiotic effect evoked by the fruit coat.

MATERIALS AND METHODS

Chemicals: Quercetin, β-carotene, α-tocopherol were purchased from Sigma Chemical Co., USA. Agar, beef extract, yeast extract, peptone were purchased from Himedia Private L., India in Jan 2006. All other chemicals and reagents were of analytical grade and solvents were distilled before use.

Plant material: Sundakai (*Solanum torvum*) was obtained from authentic sources of Ramanagara, Karnataka, India in the month of August to October 2007. The identification of the plant was confirmed by G.R. Shivamurthy, Taxonomist, University of Mysore, Mysore, Karnataka, India. The herbarium of the plant was deposited in the ABCRI against voucher no-ABCRI 7/2007.

Preparation of sundakai fruit coat extracts: The fruit coat extracts were prepared by suspending 10 g of coat powder into 100 mL of distilled water, ethanol, ethanol : water (1:1), hexane and acetone separately. The resultant solution was vortexed thoroughly for 15 min and kept overnight at 4°C. The resultant suspension was frozen and thawed, centrifuged for 20 min at 4°C and 10,000 rpm. The water extract was lyophilized at -37°C and referred to as WE (Water Extract of Sundakai coat). The Ethanol: Water (1: 1) extract was evaporated at 37°C till the alcohol gets evaporated and the remaining water was lyophilized at-37°C and referred to as EWE (Ethanol Water Extract (1:1) of Sundakai coat). Similarly, ethanol extract, hexane extract and acetone extract were concentrated separately by evaporating at 37°C to a brown residue are referred as EE (Ethanol Extract of Sundakai coat), HE (Hexane Extract of Sundakai coat) and AE (Acetone Extract of Sundakai coat).

Ten milligram of each concentrated dried extract was dissolved in 1.0 mL of corresponding solvent or solvent mixtures and mixed. The solution was filtered in 0.45 micron microbial filter and stored at -20°C for further studies. The standard antibiotics were used at the concentrations based on the normal dose given for the infection.

Antimicrobial activity

Microbial strains: Authentic pure clinical isolated cultures of human pathogenic bacteria, *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Streptococcus, Bacillus subtilis, Klebsiella pneumoniae, Salmonella cibrum, Salmonella typhimurium, Pseudomonas* sp., *Proteus vulgarigus* were obtained from Department of Microbiology, Adichunchanagiri Institute of Medical Sciences (AIMS), B.G. Nagara, Karnataka, India.

Agar well diffusion method: Antibacterial activity of various extracts of Sundakai coat was evaluated by the well diffusion method on nutrient agar medium (Forbes *et al.*, 1990). This was confirmed by the inhibitory effect on bacterial growth as reflected by the inhibited zone compared

to known antibiotics. The sterile nutrient agar medium (20 mL) in petridishes was uniformly smeared using sterile cotton swabs with test pure cultures of *Escherichia coli, Vibrio cholerae, Streptococcus, Staphylococcus aureus, Bacillus subtilis, Klebsiella, Salmonella typhimurium, Salmonella cibrum, Proteus vulgarigus* and *Pseudomonas*. The nutrient agar media was prepared by dissolving 0.3 beef extract, 0.3 yeast extract, 0.5 peptone, 0.5 NaCl and 1.5% agar in 1liter of distilled water. The wells of 5 mm diameter were made using sterile cork borer in each petriplates and the various extracts of Sundakai fruit coat were added, a blank well loaded without test compound was regarded as control. For each treatment 10 replicates were maintained. The plates were incubated at 37°C for 24 h and the resulting zone of inhibition was measured by comparing control and the standard antibiotic.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration of different extracts of Sundakai fruit coat were determined by serial dilution in the nutrient agar, with concentrations ranging from 5, 10, 20, 25, 50, 75 and 100 μg mL^{-1}. The inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24 h at 37°C. The MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth (Karou *et al*., 2005).

Proximate analysis: The proximate composition of different solvents extracts of Sundakai fruit coat were done such as, total protein (Bradford, 1976), total sugars (Dubois *et al*., 1956), total polyphenols (Kujala *et al*., 2000), total flavinoids (Woisky and Salatino, 1998), ascorbic acid (Sadasivam and Manickam, 1996) and α-tocopherol (Kivack and Mert, 2001) were determined according to the standard methods.

Statistical analysis: Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student's *t*-test. All results refer to Mean±SD. $p<0.05$ was considered as statistically significant as comparing to relevant controls.

RESULTS AND DISCUSSION

The fruits of Sundakai (*Solanum torvum*) have been reported to be widely used in traditional medicine as tonics for diarrhea, stomachache and for treating cough and pain. In the present study, we have observed that Sundakai fruit coat exhibit significant antibacterial activity against pure strains of pathogenic bacteria.

The results summarized in Table 1, indicate that among all the tested extracts, only the Water Extract (WE) and Ethanol Water Extract (EWE) exhibited effective antibacterial activity against *E. coli, V. cholerae, S. aurenus, B. subtilis, S. cibrum, S. tryphimurium* and *Pseudomonas* sp. Inhibition zone diameters between 20 and 26 mm). Water extract and Ethanol Water extract were as potent as standard antibiotics, chloramphenicol and streptomycin (Table 1). In general all the pure strains of microorganism were not susceptible to the hexane and acetone extract of Sundakai fruit coat.

Water extract ethanol water and ethanol extracts when tested in agar dilution assays for determining Minimum Inhibitory Concentration (MIC), it was observed that they all posses antibacterial growth activities with MIC values ranging from 9.6 to 19.2 μg mL^{-1}. The tested extracts showed comparable MIC values with standard antibiotics which ranged from 11.7-20 μg mL^{-1} (Table 2). Thus the extracts were as potent as standard antibiotics in inhibiting the growth of bacterial strains.

Table 1: Antibacterial activity of various solvent or solvent mixture extracts of Sundakai fruit coat in comparison with commercial antibiotics

	Diameter of inhibition zone (mm)					Antibiotics		
Bacterial species	WE	EWE	EE	AE	HE	G	Cp	Sm
Escherichia coli	21±1	23±1	14±2	14±3	11±2	26±2	18±1	17±2
Vibrio cholerae	21±2	22±1	12±2	-	-	23±1	16±1	18±1
Staphylococcus aureus	21±1	23±2	15±1	-	-	26±1	18±1	21±1
Streptococcus	19±2	21±1	16±2	-	-	24±2	18±2	20±1
Bacillus subtilis	24±2	26±1	17±1	12±2	12±3	30±1	18±1	17±1
Klebsiella pneumoniae	16±1	19±2	16±2	-	-	21±1	16±1	18±1
Salmonella cibrum	21±2	24±1	16±1	-	-	26±1	16±1	18±2
Salmonella typhimurium	20±1	22±2	14±2	-	-	24±2	16±1	18±1
Pseudomonas	19±2	23±1	15±1	-	-	24±1	17±1	16±2
Proteus vulgarigus	15±1	17±1	12±2	-	-	20±1	15±1	14±1

The results are Mean±SD (n = 10). WE: Water extract, EWE: Ethanol water (1:1) extract, EE: Ethanol extract, HE: Hexane extract, AE: Acetone extract, G:Gentamycin, Cp: Chloramphenicol, Sm: Streptomycin

Table 2: Minimum inhibitory concentration (MIC) of solvent or solvent mixture extracts of Sundakai fruit coat in serial dilution method

	MIC (μg mL^{-1})					Antibiotics		
Bacterial species	WE	EWE	EE	AE	HE	G	Cp	Sm
Escherichia coli	16.8±0.1	18.4±0.2	11.2±0.3	11.2±0.8	8.8±0.9	20.8±0.2	14.4±0.3	13.6±0.4
Vibrio cholerae	16.8±0.2	17.3±0.5	9.6±0.5	-	-	18.4±0.3	12.8±0.2	14.4±0.2
Staphylococcus aureus	16.8±0.7	18.4±0.3	12.0±0.6	-	-	20.8±0.4	14.4±0.4	16.8±0.3
Bacillus subtilis	19.2±0.5	20.8±0.7	21.2±0.4	9.6±0.7	9.6±0.4	24.0±0.3	14.4±0.2	16.0±0.2
Klebsiella pneumoniae	12.8±0.7	15.2±0.2	12.8±0.2	-	-	16.8±0.4	12.8±0.4	13.6±0.1
Salmonella cibrum	16.8±0.3	19.2±0.4	12.8±0.1	-	-	20.8±0.4	12.8±0.2	14.4±0.2
Salmonella typhimurium	15.6±0.2	17.6±0.7	11.2±0.3	-	-	19.2±0.2	12.8±0.4	14.4±0.4
Pseudomonas	15.2±0.6	18.4±0.2	12.0±0.3	-	-	19.2±0.4	13.6±0.2	12.8±0.2
Proteus vulgarigus	15.0±0.2	13.6±0.5	9.6±0.2	-	-	16.0±0.2	11.2±0.2	12.3±0.4

The results are Mean±SD (n = 10). WE: Water extract, EWE: Ethanol water (1:1) extract, EE: Ethanol extract, HE: Hexane extract, AE: Acetone extract, G:Gentamycin, Cp: Chloramphenicol, Sm: Streptomycin

Table 3: Proximate analysis of various solvent or solvent mixture extracts of Sundakai coat

Extracts	Total polyphenols	Flavinoids	Total protein	Total sugars	Ascorbic acid	A-tocopherol
WE	56.3±2.3	32.3±2.5	8.23±0.7	7.04±0.9	3.30±0.40	19.2±1.0
EWE	67.8±3.1	42.0±2.8	8.0±0.60	1.0±0.10	0.50±0.01	27.8±2.1
EE	59.4±3.0	29.7±1.0	3.8±0.50	2.1±0.04	0.10±0.01	21.2±1.7
HE	22.6±1.1	24.0±1.2	0.5±0.07	0.8±0.02	0.04±0.01	28.4±1.3
AE	59.6±3.8	36.8±2.9	0.4±0.02	0.4±0.01	0.05±0.01	18.6±1.4

The results are Mean±SD (n = 10). Concentration expressed as mg/g. WE: Water extract, EWE: Ethanol water (1:1) extract, EE: Ethanol extract, HE: Hexane extract, AE: Acetone extract

In order to ascertain the components responsible for antibacterial activity, proximate analysis of various solvent extracts of Sundakai fruit coat was carried out. In Table 3, it appeared that Water Extract (WE) and Ethanol Water Extract (EWE) (1:1) of coat had the highest content of polyphenols and flavonoids, in comparison to that of acetone and hexane extract. Considerable amount of α-tocopherol was present in all the extracts where as protein, sugars and ascorbic acid

was in negligible amounts. It was interesting to observe that there is a correlation between the high amount of polyphenols and flavonoids content and effective antibacterial activities of Water extract and Ethanol water extract. Therefore it can be concluded that the antibacterial activity may be due to the presence of high concentration of polyphenols and flavonoids of the inhibiting extract.

Polyphenols and flavonoids are reported to be important antimicrobial components (Chung *et al*., 1998; Karou *et al*., 2005). There are also enough documented date, which suggests that there is a positive correlation between total phenolic content and antimicrobial activity (Shan *et al*., 2007; Wu *et al*., 2006; Kudo *et al*., 2004). Flavonoids isolated from the leaves of *Pluchea carolinensis* has shown antibacterial activity (Cordova *et al*., 2006). The flavonoids like kaempferol, kaempferol-3-O-galactoside, rutin (quercetin-3-Orutinosid), genistin (genistein-7-O-glucoside) and orientin (luteolin-8-C-glucoside) were isolated from methanolic extract of *Linum capitatum* Flower are also known to have antibacterial activity (Ilic *et al*., 2004). The antibacterial activity of flavonoids and polyphenolic compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

In conclusion, we have shown that extracts of Sundakai fruit coat exhibit significant antibacterial activity against pathogenic bacteria when compared to standard antibiotics. The inhibited extracts showed high polyphenols and flavonoids content. Therefore this Sundakai coat may be yet another source of natural antibiotic. This study rein firms the ethanomedicinal property of *S. torvum*.

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