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Original article

Synergistic antibacterial action of β -sitosterol-D-glucopyranoside isolated from *Desmostachya bipinnata* leaves with antibiotics against common human pathogens

Shankar Subramaniam, Manikandaraja Keerthiraja, Aravind Sivasubramanian*

Department of Chemistry, School of Chemical and Biotechnology, Sastra University, Thanjavur, India

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Desmostachya bipinnata (L.) Stapf, Poaceae, or Kusha in Sanskrit, is a sacred grass used extensively in Indian Vedic practices. It is well known for its medicinal value and is used in traditional Indian medicine to treat microbial infection in combination with other herbs. An effort has been made to isolate and characterize the bioactive compounds from the hydroalcoholic extract of *D. bipinnata* through bioassay guided fractionation, column chromatography. Their individual or combined antimicrobial properties were determined by the Resazurin Microtitre Assay, the checkerboard assay in combination with antibiotics, and by time kill curve analysis. β -Sitosterol-D-glucopyranoside was the bioactive compound identified to have the best antimicrobial activity (MIC 6-50 μ g/ml) and it works synergistically with most antibiotics, especially with ciprofloxacin. Time kill curves showed that BS kills most of the pathogens within 5-10 h. To our knowledge at its best, this is the first time report of antibacterial synergy of β -sitosterol-D-glucopyranoside from *D. bipinnata*.

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Introduction

Desmostachya bipinnata (L.) Stapf, Poaceae, is considered a highly sacred grass among Vedic scholars in India. It is known as 'Kusha' in Sanskrit. It constitutes a vital ingredient for almost all Vedic rituals and sacrifices. Its miraculous healing powers and medicinal value have been well documented in various Ayurvedic texts and preparations such as Tripanchamool, Kusablecha, and Kusadya-ghrita (Shrestha et al., 2000). Its medicinal main activities are as astringent, galactagogue, analgesic, antipyretic, aphrodisiac, anti-inflammatory, wound-healing, anti-asthmatic, anti-diuretic and as a sedative to

pregnant uterus. It is used along with other herbs to treat dysentery, diarrhea, jaundice, dysuria, nausea, menorrhagia and skin infections (Kirtikar and Basu, 1918; Joshi, 2003; Alikhan and Khanun, 2004).

The scarcity of fresh and novel therapeutic agents compound the severe, relentless global clinical problem: antibiotics 'resistance', and has also put on the spotlight, the generous use of already commercialized antibiotics (Boucher et al., 2009). This scenario thus signals a critical need for innovation, which is further exacerbated due to a global retreat on the development of new API (Active Pharmaceutical Ingredient) by the pharmacological sector.

* Corresponding author.

E-mail: arvi@biotech.sastra.edu, shankar@sastra.edu (A. Sivasubramanian).

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Combinations of various small bioactive molecules having particular individual or multiple targets have been suggested as potential antimicrobial agents, (Sharom et al., 2004; Lehar et al., 2007) evidenced by the effects observed in microbial models at various levels of gene expression, genetic communication and at the protein interface (Parsons et al., 2004; Costanzo et al., 2010). Molecule combinations are thus proposed to thwart microorganism resistance (Walsh, 2000). The repurpose of traditional drugs combined with new ones thus could lessen the costs for the development of new pharmaceutical leads into simple but modern day resistant diseases. It is also critically imperative that new drugs be evaluated along with other traditional drugs, for the interactions among them. Thus, the current trend in development of novel therapeutics lies in the exploration of them as combinations with other reputed drugs. We have attempted to track this trend with our bioactive molecule β -sitosterol-D-glucopyranoside (BS) isolated for the first time from *D. bipinnata*.

Our present study focuses on the bioassay guided isolation of β -sitosterol-D-glucopyranoside from leaves of *D. bipinnata* for the first time, and its ability to kill common bacterial pathogens. Emphasis has also been laid in finding out the actual time taken for antimicrobial action by BS on pathogens through time kill analysis.

Materials and methods

Collection of plant material and extraction

Leaves of *Desmostachya bipinnata* (L.) Stapf, Poaceae, were obtained along the riverbeds of River Cauvery in and around Thanjavur, India. The samples were authenticated by Dr. Jayendran from the Department of Botany of Government Arts College, Ootacamund, India. A voucher specimen (JDB1422), after matching with authenticated herbarium sample RHT22739 (RAPINAT Herbarium, Trichy, India), was deposited in Government Arts College, Ootacamund, India. The leaves were shade dried and ground to a fine powder and used for extraction. Extracts were prepared by soaking 1 kg of plant material in *n*-hexane, ethyl acetate and 70% aqueous methanol successively at room temperature for 24 h and repeated thrice with the residue. The extract was filtered through Whatman n°1 filter paper, and then all the filtrates were pooled up successively and concentrated under vacuum by rotary evaporator (Buchi® Rotavap R-210).

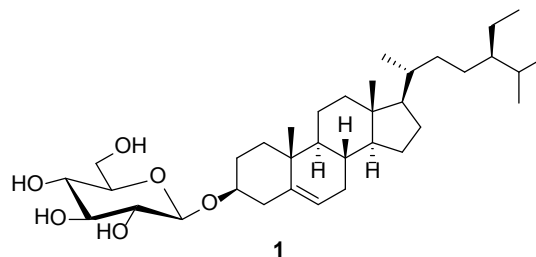
Chemicals and instrumentation

All reagents were purchased from Sigma-Aldrich. TLC was monitored with silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were visualized in the ultraviolet light chamber, Iodine chamber, 5% MeOH-H₂SO₄ mixture. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Thermo). All melting points were measured using Buchi-545. ¹H NMR and ¹³C NMR spectra were determined on a Bruker-400 NMR

spectrometer and chemical shifts were expressed as part per million against TMS as internal reference. Mass spectra were recorded on Agilent 1200 (Liquid Chromatography), Agilent 6320 (Quadrupole Mass Analyzer) spectrophotometer.

Bio-assay guided fractionation and compound isolation

The *D. bipinnata* extracts were subjected to antimicrobial assay and the hydroalcoholic extract showed the highest antimicrobial activity. Subsequently, hydroalcoholic extract (30 g) was taken for column chromatography using silica gel (60-120 mesh) (150 g) packed in a glass column of 4 × 45 cm with bed height of 30 cm. Elution was started with *n*-hexane, followed by increasing ethyl acetate (EA)-*n*-hexane combinations (5, 10, 20, 40 and 80% EA in *n*-hexane) and finally with EA followed by MeOH. The column elution was monitored by TLC and fractions were pooled based on similar TLC profiles. In total, seven fractions were collected and concentrated under reduced pressure in a rotary evaporator. Each fraction was determined for its antibacterial efficacy against various pathogens with Ciprofloxacin as control antibiotic. Fraction 6 (MF6) (3.5 g) and 7 (MF7) (3.1 g) were found to be most active which were then combined together and subjected to column chromatography with silica gel (100-200 mesh) and eluted with chloroform, chloroform-methanol (2, 4, 8, 10 and 20 %) and methanol successively. Two fractions that eluted at 4% methanol-chloroform were pooled together and precipitated to afford the bioactive compound β -sitosterol-D-glucopyranoside. (1)



Antimicrobial studies

Pathogens and antibiotics used

Staphylococcus aureus (MTCC 96, 3160), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (MTCC 723), *Vibrio cholera* (MTCC 3904), *Klebsiella pneumonia* (MTCC 432), *Proteus vulgaris* (MTCC 426), *Proteus mirabilis* (MTCC 425), *Shigella dysenteriae* (ATCC 23513), *Pseudomonas aeruginosa* (MTCC 741, 1688) were used for the antimicrobial studies. Commercial antibiotics were purchased from Sigma-aldrich India which included Amoxicillin (AMX), Ampicillin (AMP), Methicillin (MET), Ciprofloxacin (CIP), Gentamicin (GEN), Chloramphenicol (CHL), Azithromycin (AZM), Erythromycin (ERY), Tetracycline (TET), Polymyxin B (PMB). All pathogens were maintained on nutrient agar slant cultures at 4°C.

Inoculum preparation

All determination procedures of antimicrobial activity were done and inoculum size was standardized according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1993). Mueller Hinton Broth (MHB; HiMedia, Mumbai, India) was used to prepare inoculum and grown in incubator orbital shaker at 37°C for 4-8 h until cultures attained turbidity of 0.5 McFarland Unit. Inoculum size was adjusted and standardized to 5×10^5 CFU ml⁻¹ throughout the experiments.

Determination of antibacterial efficacy

The minimum inhibitory concentrations (MIC) were determined for all *D. bipinnata* leaves extracts, fractions of column chromatography and isolated bioactive compound by broth dilution method. However, tests for purified isolated compounds were carried out in triplicate using modified Resazurin Microtitre Assay (REMA) (Martin et al., 2003). Stock solutions of samples at 500 µg/ml were prepared by dissolving the samples in 10% dimethyl sulphoxide (DMSO). The test samples were diluted in MH broth. The concentration range was 3.9-500 µg/ml. In 96-well microtiter plates, 100 µl of each of the compound dilutions was added to a mixture of 90 µl of MHB and 10 µl of bacterial inoculum. The negative control consisted of 100 µl of 10% DMSO, 90 µl of MHB and 10 µl of cell suspension; the positive control had ciprofloxacin added (3.9-500 µg/ml). Upon the incubation of the test plates at 30°C for 24 h, cell viability was determined by adding 15 µl of a 0.01% (w/v) Resazurin solution to each of the wells, following an extra incubation period of 2 h at 30°C. Viable microorganisms reduced the blue dye to a pink color, which was detected by fluorescence scanning using a microfluorimeter (FLX-800 fluorimeter, BioTek, Winooski, VT) set to an excitation/emission profile of 530 nm/590 nm.

The MIC plates after incubation were taken for determination of minimum bactericidal concentrations (MBC) which is the lowest concentration of agent that kills 99.9% of the test bacteria. This was done by plating out aliquots onto each appropriate agar plate. Ciprofloxacin was used as control.

Combination studies

To study the interaction of isolated bioactive compound with other antimicrobial agents, combinations of the compound with commercial antibiotics were assessed by the checkerboard test (Cha et al., 2007). Pure compound combined with antibiotics at concentrations ranging from $1/32 \times$ MIC to $4 \times$ MIC were prepared in MHB with standard inoculum size of 5×10^5 CFU ml⁻¹. Minimum bactericidal concentrations (MBC) were also found for the combinations prepared. The fractional inhibitory concentration index (FICI) was found as the sum of the FIC of each of the drugs. FIC is defined as the MIC of each drug used in combination, divided by the MIC of the drug when used alone. The interaction was defined as synergistic if the FIC index was less than or equal to 0.5; additive if the FIC index was greater than 0.5 and less than or equal to 1.0; indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0. All experiments were done in triplicates and data represented as arithmetic average.

Time kill curves

To assess the rate of growth inhibition of isolated bioactive compound against pathogens, bactericidal action was evaluated using time kill curves. Tubes containing isolated compound at MIC were inoculated with a suspension of pathogens yielding the final bacterial concentration in broth at $4.5-5.5 \times 10^5$ CFU. The tubes were thereafter incubated at 37°C and viable counts were performed at 0, 0.5, 1, 2, 3, 4, 5, 6, 12, 16, 20 and 24 h after compound addition. Aliquots of culture broth were taken, serially diluted and spread over agar plates and incubated for up to 48 h at 37°C. Repeated washing and centrifugation was done to minimize antibiotic carry over. Colony counts were done in triplicate and data represented as arithmetic mean.

Results

The compound (BS) was isolated as a white crystalline solid (1) and it responded positively to the Liebermann-Buchard test for steroids. On the basis of the spectral data, compound structure was elucidated as stigmast-5-en-3-O-β-D-glucopyranoside (β-sitosterol pyranoside). All the spectral data were in complete concurrence with the literature (Kojima et al., 1990).

A bio assay guided fractionation of the hydroalcoholic extract was performed and seven fractions (MF1- MF7) were obtained (Fig. 1). Fractions MF1 and MF2 were inactive against all tested strains. Other fractions- MF3, MF4 and MF5 did not exhibit considerable activity against all pathogens (Table 1). Fractions MF6 and MF7 exhibited significant activity against all the pathogens, and hence they were taken for further studies. The phytochemical analysis of MF6 and MF7 led to the isolation of the bioactive compound (β-sitosterol-D-glucopyranoside). BS exhibited acceptable activity against most of the pathogens, and particularly against *E. faecalis*, *S. dysenteriae*, and *P. aeruginosa* with MIC values of 15, 12 and 10 µg/ml respectively (NCCLS, 2007). The MIC determined was relatively high for *S. aureus* (MTCC 3160) (50 µg/ml) and moderate against the other *S. aureus* strain (MTCC 96). The MIC for BS against *P. mirabilis* and *K. pneumonia* (24, 25 and 25 µg/ml respectively) were also, relatively moderate. The MBC values for BS were gratifying and were on par with the tested antibiotics (Tables 1 and 2).

A vital observation grasped was that, BS worked synergistically in many cases, especially with ciprofloxacin, against most of the pathogens (FICI < 0.5) and additively against the rest of the pathogens (FICI 0.5 - 1.0). In addition, BS + polymyxin B combination evidenced synergistic activity against *E. coli* (FICI - 0.72), *V. cholera* (FICI - 0.5), *K. pneumonia* (FICI-0.5), and *P. aeruginosa* (FICI-0.5 & 0.35). Chloramphenicol blended well with BS to yield synergy against *E. faecalis* (FICI-0.46), *E. coli* (FICI - 0.5), *K. pneumonia* (FICI-0.41), *P. mirabilis* (FICI-0.5), and *P. aeruginosa* (FICI - 0.5). Additive combinations of BS were also found to be plenty (especially with amoxicillin, methicillin; against most of pathogens), when compared to non-interactive and antagonistic combinations (Table 2). The rate of bacterial growth killing by BS, assessed by time-kill curves, showed that most of the pathogens die within 5-10 h from addition of BS in culture broth. However, much time was needed (20 h) for BS to kill Gram-positive *S. aureus* (MTCC 3160),

indicating the slightly resistive nature of the pathogen. Gram-negative organisms like *K. pneumonia* and *P. mirabilis* portrayed resistive patterns (for 24 h, respectively). Time-kill curves of

other bacteria showed considerable similarity in their kill-curve patterns which apparently explains the similar action and time taken by BS to kill the tested pathogens (Fig. 2).

Table 1

Minimum inhibitory concentration of different components of *Desmostachya bipinnata*.

Pathogen	Type	Antimicrobial Agents						
		DBME (MIC ₅₀)	MF3 (MIC)	MF4 (MIC)	MF5 (MIC)	MF6 (MIC)	MF7 (MIC)	BS (MIC/MBC)
<i>Staphylococcus aureus</i> (MTCC 96)	Gram Positive	400	NA	NA	120	42	48	24/40
<i>Staphylococcus aureus</i> (MTCC 3160)		NA	NA	NA	NA	100	88	50/75
<i>Bacillus subtilis</i> (MTCC 441)		200	120	NA	80	20	14	12.5/20
<i>Enterococcus faecalis</i> (MTCC 439)		400	120	NA	80	32	24	15/26
<i>Escherichia coli</i> (MTCC 723)	Gram Negative	460	NA	80	NA	46	46	12.5/25
<i>Vibrio cholera</i> (MTCC 3904)		250	NA	100	NA	24	14	6.2/15
<i>Klebsiella pneumonia</i> (MTCC 432)		320	NA	NA	NA	34	36	25/56
<i>Proteus vulgaris</i> (MTCC 426)		NA	NA	NA	NA	36	42	17/42
<i>Proteus mirabilis</i> (MTCC 425)		NA	NA	NA	NA	48	52	25/50
<i>Shigella dysenteriae</i> (ATCC 23513)		200	120	NA	100	20	18	12/30
<i>Pseudomonas aeruginosa</i> (MTCC 741)		NA	NA	NA	NA	38	38	10/16
<i>Pseudomonas aeruginosa</i> (MTCC 1688)		NA	NA	NA	NA	30	30	10/24

MIC of *D. bipinnata* hydro-methanolic extract (DBME), Methanol fraction 6 and 7 (MF6 & 7); and β -Sitosterol-D-glucopyranoside (BS). Other fractions not represented are considered not active against all of the pathogens. The experiment was done in triplicates and values represented are arithmetic average. NA, Not Active/ No inhibition found over that concentration of antibacterial agent.

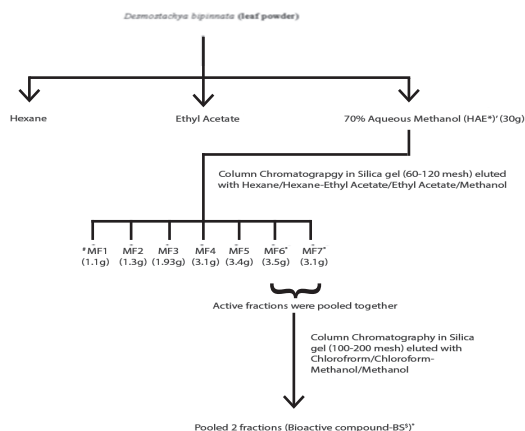


Figure 1 - Isolation of bioactive compound through bioassay guided fractionation.

†Antimicrobial active fraction/compound; *Hydroalcoholic extract; # Methanol fraction; \$ β -Sitosterol-D-glucopyranoside.

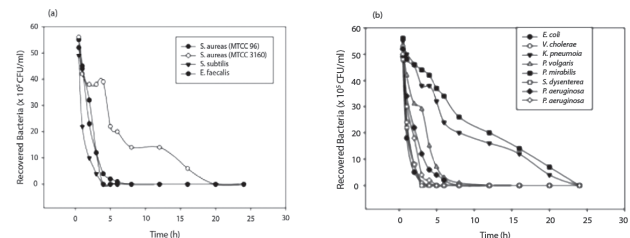


Figure 2 - Rate of action of β -sitosterol-D-glucopyranoside (BS) on various pathogens (Time kill curves). Bactericidal activity of β -sitosterol-D-glucopyranoside at MBC against (a) Gram positive bacteria, (b) Gram negative bacteria. Most of the pathogens are killed within 5-8 h for Gram positive except *Staphylococcus aureus* (MTCC 3160) which exhibits some kind of resistive patterns and takes longer duration to die. Gram negative organisms are mostly killed within 5-10 h except *Klebsiella pneumonia* and *Proteus mirabilis* showing resistive configurations followed by *Proteus vulgaris* slightly showing late death.

Table 2Combination effects of β -sitosterol-D-glucopyranoside with commercial antibiotics.

Pathogen		Antimicrobial Agents (MIC/MBC) ($\mu\text{g/ml}$)								
		BS ^b	AMX ^c	AMP	MET	CIP	GEN	CHL	AZM	ERY
<i>Staphylococcus aureus</i>	I ^a	24/40	26/48	45/60	5/8	8/16	4/8	22/52	16/30	5/8
	C		10/18	18/28	4/4	6/12	6/8	12/18	18/32	5/8
	F		0.78/0.83	1.12/1.16	0.96/0.96	1.0/1.0	1.72/1.2	1.0/0.79	1.84/1.86	1.2/1.2
	R		A ^d	N	A	A	N	A	N	N
<i>Staphylococcus aureus</i>	I	50/75	60/75	60/72	8/20	12/18	14/30	30/52	22/46	8/16
	C		20/40	34/52	6/12	6/10	20/42	20/34	30/42	12/18
	F		0.73/1.0	1.24/1.41	0.87/0.76	0.6/0.68	1.84/1.96	1.0/1.0	1.96/1.47	1.74/1.36
	R		A	N	A	A	N	A	N	N
<i>Bacillus subtilis</i>	I	12.5/20	12/26	20/32	16/36	6/10	4/10	12/18	16/40	12/20
	C		8/16	8/16	6/10	2/4	3/6	12/18	6/12	5/10
	F		1.3/1.41	1.1/1.3	0.85/0.7	0.5/0.5	1.0/0.9	1.96/1.9	0.85/0.9	0.81/1.0
	R		N	N	A	S	A	N	A	A
<i>Enterococcus faecalis</i>	I	15/26	18/30	18/32	24/34	6/12	20/42	6/12	14/22	18/30
	C		12/20	10/22	8/12	2/4	14/30	2/4	10/18	10/24
	F		1.46/1.43	1.2/1.53	0.86/0.81	0.46/0.48	10.63/1.86	00.46/0.4	1.38/1.51	1.22/1.72
	R		N	N	A	S	N	S	N	N
<i>Escherichia coli</i>	I	12.5/25	12/24	16/30	12/20	6/10	6/12	6/10	12/20	12/18
	C		4/8	12/20	10/18	2/4	2/4	2/4	4/8	10/16
	F		0.65/0.65	1.71/1.46	1.63/1.62	0.5/0.5	0.5/0.49	0.5/0.5	0.65/0.72	1.63/1.52
	R		A	N	N	S	S	S	A	N
<i>Vibrio cholerae</i>	I	6.2/15	15/30	8/14	24/32	4/10	3/8	4/8	20/38	40/62
	C		4/10	6/10	10/20	1/3	1/2	2/3	8/18	10/22
	F		0.91/0.8	1.71/1.38	2.0/1.95	0.41/0.5	0.5/0.38	0.82/0.6	1.69/1.67	1.86/1.82
	R		A	N	N	S	S	A	N	N
<i>Klebsiella pneumonia</i>	I	25/56	40/72	24/46	20/40	12/24	6/12	6/14	8/16	12/24
	C		12/20	8/16	10/18	4/6	3/6	2/4	2/4	12/26
	F		0.78/0.63	0.65/0.63	0.9/0.77	0.5/0.36	0.62/0.61	0.41/0.36	0.33/0.32	1.48/1.55
	R		A	A	A	S	A	S	S	N
<i>Proteus vulgaris</i>	I	17/42	25/38	40/72	30/52	16/36	12/20	12/18	12/26	40/78
	C		12/22	18/30	16/24	6/14	4/6	4/6	6/12	12/20
	F		1.19/1.1	1.5/1.13	1.47/1.03	0.73/0.72	0.5/0.44	0.5/0.48	0.85/0.75	1.0/0.73
	R		N	N	N	A	S	S	A	A
<i>Proteus mirabilis</i>	I	25/50	32/48	36/66	30/42	14/30	10/22	18/32	16/32	46/78
	C		14/24	28/50	25/46	8/18	6/16	14/22	20/36	32/60
	F		1.0/0.98	1.9/1.76	1.83/2.0	0.89/0.96	0.84/1.0	1.34/1.13	2.0/1.85	1.98/1.97
	R		A	N	N	A	A	N	N	N

Table 2 cont.

Pathogen	Antimicrobial Agents (MIC/MBC) ($\mu\text{g/ml}$)									
	BSb	AMXc	AMP	MET	CIP	GEN	CHL	AZM	ERY	
<i>Shigella dysenteriae</i>	I	12/30	16/32	20/40	18/32	4/6	4/8	22/42	10/20	20/36
	C		6/12	12/20	14/20	2/4	4/10	14/32	4/12	8/16
	F		0.88/0.78	1.6/1.17	1.94/1.29	0.67/0.8	1.33/1.58	1.8/1.83	0.73/1.0	1.0/0.75
	R		A	N	N	A	N	N	A	A
<i>Pseudomonas aeruginosa</i>	I	10/16	30/56	18/38	12/26	6/12	4/6	6/14	12/24	20/42
	C		6/12	4/8	2/4	2/4	1/2	2/4	4/6	12/20
	F		0.8/0.96	0.6/0.71	0.37/0.4	0.5/0.5	0.35/0.46	0.5/0.5	0.73/0.63	1.8/1.73
	R		A	A	S	S	S	S	A	N
<i>Pseudomonas aeruginosa</i>	I	10/24	28/42	12/24	12/24	8/14	4/8	8/18	8/18	24/42
	C		14/24	8/18	4/10	4/8	2/4	3/8	6/12	14/24
	F		1.9/1.57	1.47/1.5	0.73/0.83	0.9/0.9	0.7/0.67	0.68/0.78	1.35/1.17	1.98/1.57
	R		N	N	A	A	A	A	N	N

AMP, Ampicillin; MET, Methicillin; CIP, Ciprofloxacin; GEN, Gentamycin; CHL, Chloramphenicol; AZM, Azithromycin; ERY, Erythromycin; TET, Tetracycline; PMB, Polymyxin-B.

^aI, Individual; C, Combination; F, Fractional inhibitory concentration index (FICI); R, Results.

^bBS, β -Sitosterol-D-glucopyranoside.

^cCommercial Antibiotics; AMX, Amoxicillin.

^dObservations: S, Synergistic; A, Additive; N, No Interaction; T, Antagonistic; FICI, < 0.5 (Synergy), 0.5 - 1.0 (Additive), 1.0 - 2.0 (No interaction), > 2.0 (Antagonistic).

Discussion

Desmostachya bipinnata (L.) Stapf, Poaceae, is noted for its medicinal properties against skin infections and digestive problems. In the present study, a glycosidic compound (β -sitosterol-D-glucopyranoside) was isolated and its antimicrobial synergism with other antibiotics against common pathogens is demonstrated for the first time, with further studies on time kill curve analysis. This sacred plant has always been used in combination with other medicines in preparations of Ayurvedic/Siddha Indian system (Kirtikar and Basu, 1918; Joshi, 2003). Much research has not been done in this plant and very few constituents have been isolated and characterized. One such compound isolated in this study is β -sitosterol-D-glucopyranoside. β -Sitosterol-D-glucopyranoside has been previously reported in *Triticum aestivum* (Rozenberg et al., 2003), and *Vitis vinifera* (Rivero-Cruz et al., 2008). The antibacterial activity of β -sitosterol-D-glucopyranoside has also been previously reported (Chattopadhyay et al., 2001), where fraction containing β -sitosterol-D-glucopyranoside, isolated from the leaf extracts of *Alstonia macrophylla* exhibited MIC at 1000-2000 $\mu\text{g/ml}$ against resistant bacteria and > 128 $\mu\text{g/ml}$ against fungal organisms.

The fact that *D. bipinnata* extracts increase the properties of other medicinal preparations (works synergistically) is well documented by traditional medics in India (Shrestha et al., 2000), and our work further substantiates this fact. Our study proves significant synergy and additive effects of BS-antibiotic combinations against various pathogens. To gain better insight about rate of action of our isolated compound, Time kill analysis was performed, which could be generally used to determine rapidity and duration of antibacterial action against particular pathogens (Devi et al., 2010). Our studies show that, BS completely kills Gram-positive and Gram-negative bacteria within 5-10 h, except *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* and *Proteus mirabilis* (Fig. 2). Further phytochemical pursuits for other similar glycosides or different bioactive molecules from this medicinally active plant will open up new prospects in this field of research. Also, combination of such anticipated molecules with other well established drugs could further solve the question of drug interactions and synergism as substantiated by traditional texts.

In conclusion, our study details the concepts concerned with the isolation of a bioactive compound called β -sitosterol-D-glucopyranoside and its structural elucidation. Antibacterial activity of BS and combinations of bioactive compound with antibiotics yielded patterns of synergism and addition being few the observations of non-interaction and antagonism, all this conforming the unique result of this study. The duration

of bactericidal action exhibited by BS was determined and it was inferred that 5-10 h timescale was required for BS to exhibit complete cidal activity on most of the pathogens.

Authors' contributions

SS contributed in plant material collection, extraction, chromatography and microbiology. MK contributed to chromatography and assisted in microbiology. AS and SS designed the study and AS supervised the laboratory work, spectroscopic analysis and to the critical reading of the manuscript.

Conflicts of interest

The authors declare no conflicts interest.

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