Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(6): 68-72

Received: 13-09-2018
Accepted: 14-10-2018

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GC-MS analysis and antimicrobial activity of alkaloids of *Tecomella undulata*

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Abstract
Tecomella undulata (Sm.) Seem is a monotypic genus belonging to family Bignoniaceae. The plant holds tremendous potential of medicinal value and has been traditionally used in various ailments like syphilis, leukoderma, blood disorders to name a few. The plant has gained prominence due to presence of some prominent secondary metabolites. The present study focuses on the GC-MS analysis of extracts of all the plant parts of *T. undulata* which revealed the presence of certain bioactive compounds like stigmasterol, sitosterol, thiazoline, phytol, pthalic acid, methyl alpha ketopalmitate and so forth. A total of about 20 bioactive compounds were identified. Antimicrobial activity of the extracts was assayed against pathogenic bacteria and fungi. The alkaloids from leaf extracts showed antimicrobial activity against *E. coli* and *B. subtilis*. The study thus infers that the presence of bioactive components may be the principle behind the antimicrobial property of different plant parts and therefore *Tecomella* forms a potential plant for herbal drug formulation.

Keywords: *Tecomella undulata*, bioactive compounds, GC-MS, antimicrobial activity

Introduction
Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. A natural product is a chemical compound or substance produced by a living organism like microorganisms, marine organisms, animal sources, plant sources. The definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism. The importance of medicinal plants in traditional healthcare practices has provided clues to new areas of research and in biodiversity conservation is now well recognized (Uniyal, 2006) [1]. Many conventional drugs originate from plant sources (Vickers *et al*., 2001) [2]. Because of the absence of an efficient excretory system plant produced secondary metabolites (Fraenkel, 1959; Whiting, 2001; Theis and Lerdau, 2003; Wijngaard *et al* 2012) [3, 4, 5, 6]. The genus Tecomella undulata is a tree species produces quality timber. In Rajasthan it is mainly found in western parts distributed in Barmer, Jaisalmer, Jodhpur, Pali, Ajmer, Nagaur, Bikaner, Churu and Sikar districts. Tecomella undulata belongs to family Bignoniaceae (Jacaranda family). The Bignoniaceae family comprising of about 110 genera and 650 species is a family of flowering plants, commonly known as the Trumpet Creeper family, Jacaranda family, Bignonia family, or the Catalpa family. Alkaloids belong to the broad category of secondary metabolites. This class of molecule has historically been defined as naturally occurring substances that are not vital to the organism that produces them. alkaloids do have important ecochemical functions in the defense of the plant against pathogenic organisms and herbivores or, as in the case of pyrrolizidine alkaloids, as pro-toxins for insects, which further modifiy the alkaloids and then incorporate them into their own defense secretions (Kutchan, 1995) [7] Alkaloids in medicinal plants are reported for their antimicrobial and antimalarial activity (Hadi and Bremner, 2001, Ameyaw and Eshun, 2009) [8, 9]. Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and the favorable effect on the animal intestinal system (Ljubiša *et al*., 2009) [10]. Plant extracts are able to restrict the growth of bacteria due to the presence of active principles in it.

Materials and methods
Plant material
The different plant parts (roots, stems, leaves and bark) of *Tecomella undulata* were collected in month of October - December from University of Rajasthan campus.

It was washed with tap water, dried at room temperature and ground to fine powder. The species specimen was submitted to herbarium, Department of Botany, University of Rajasthan. Jaipur, Rajasthan, India and got the voucher specimen no. RUBL211300.

Chemicals
All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Tests for Alkaloids
Iodine Test: A few drops of dilute iodine solution were added into 3 ml test solution. Blue colour appeared; and disappeared on boiling and reappeared on cooling (Khandewal, 2008).

Extraction Procedure (Family: Bignoniaceae)
Powdered and weighed plant materials were taken in 100mL Erlenmeyer flasks containing distilled water (50mL/g) and 5mL of 0.05 N sulphuric acid was added to it. Mixture was macerated for 3-4 h and boiled gently for 25 minutes. Heavy magnesium oxide (2.5g/g) was added to the mixture and again boiled gently for 20 minutes. It was cooled at room temperature and an equal amount of distilled water was added to make up for loss of distilled water during boiling. Alcohol was added to remove the mucus. Mixture was filtered through Whatman filter paper (Kogan *et al*., 1953). Filtrate was evaporated to dryness in vacuo, reconstituted in distill water for further analysis.

GC-MS analysis of alkaloids
GC-MS conditions
GCMS-QP 2010 Plus was used for identification and quantification of phytoconstituents, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250 °C was used. The GC was equipped with a SE-30 capillary column a split injection piece (270 °C) and direct GC-MS coupling (280 °C). Helium (1.2 mL/min) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100 °C, maintained for 2 min, followed by a steady climb to 200 °C at a rate of 7 °C/min allowed to increase to 190 °C at a rate of 30 °C/min. This oven temperature was again maintained at 190 °C for 5 min and then allowed to increase to 300 °C at a rate of 7 °C/min. This oven temperature was maintained for 2 min and finally ramped to 300 °C at a rate of 10 °C/min and maintained for a further 22 min. Injection temperature was 270 °C and volume 250 °C and 1 µL, respectively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, Interference temperature of 260 °C, Ion source temperature of 250 °C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and Threshold -1000.

Identification
GC-MS is a valuable aid for identifying unknown peak as well as for confirming the identification of identified phytoconstituents. In some cases when no identical spectra were found, the structural type of the corresponding component was suggested only on the basis of its mass spectral fragmentation and retention data. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time.

Sources of test organisms
a. Fungi
The fungal strains *Aspergillus niger* (NCIM 0616), *Fusarium oxysporum* (NCIM 1228), Trichoderma reesei (NCIM 0992), *Penicillium funiculosum* (NCIM 1075), *Candida albicans* (NCIM- 3501), *Trichoderma viride* are procured from the National Institute for Complementary Medicine.

b. Bacteria
The bacterial strains *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* (MTCC 0087) (Gram +ve), *Pseudomonas aeruginosa* (MTCC 4646) (Gram +ve), Bacillus subtilis (MTCC 0121), *Klebsiella pneumoniae* (MTCC-0109) (Gram –ve) and *Streptomyces albudencus* (MTCC 1764), *Enterococcus faecalis* (ATCC- 29212) (gram +ve) were procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Culture of test microbes
For the cultivation of bacteria, Nutrient Broth Medium (NB) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 min. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH2PO4 + 7.23 g NaH2PO4 + 4.30 g, NaCl + 1 g peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37 °C for 24 hrs. However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27 °C for 48 hrs and the cultures were maintained on same medium by regular subculturings.

Fungicidal and Bactericidal Assay
For both, fungicidal and bactericidal assays agar well diffusion method was adopted (Bauer *et al*., 1996), because of reproducibility and precision. The different test organism were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around wells in solidified medium (5 mm in diameter), which were containing 2mg/ml and 4mg/ml of the test extracts, control solvent or steptomycin (1mg/ml) or ketokenazol (1mg/ml) as reference separately. These plates were initially placed at low temperature for 1 hr, so as to allow the maximum diffusion of the compounds from the wells into the plate and later, incubated at 37 °C for 24 hrs in case of bacteria and 48 hrs at 27 °C for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were then referred.

Results and discussion:
Antifungal activity of alkaloid extract recorded against Fusarium was maximum by leaf and minimum by root and stem (leaf; IZ=18.66 ± 1.52 mm > bark; IZ= 16.00 ± 1.00 mm > root, stem; IZ=15.33 ± 2.08 mm). Against P. funiculosum, leaf showed maximum whereas stem showed minimum inhibition (leaf; IZ=18.66 ± 0.57 mm > root; IZ= 14.33 ± 0.57 mm > bark; IZ=13.00 ± 0.00 mm > stem; IZ=12.00 ± 2.00). Maximum activity against *C. albicans* was recorded by the

leaf extract while minimum by root (leaf; IZ=27.66 ± 1.52 mm > stem; IZ= 19.66 ± 1.52 mm > bark; IZ=14.33 ± 2.51 mm > root; IZ=9.33 ± 1.52 mm). Leaf extracts showed maximum activity against T. virdae whereas bark extract showed minimum inhibition (leaf; IZ=26.66 ± 1.05 mm > stem; IZ= 20.00 ± 2.64 mm > root; IZ=12.33 ± 2.51 mm > bark; IZ=12.00 ± 2.54 mm).

Antibacterial activity of alkaloids when tested against S. aureus leaf extracts showed higher activity as compared to stem extract which showed lower activity (leaf; IZ=17.10 ± 1.00 mm > root; IZ= 14.66 ± 3.05 mm > bark; IZ=13.33 ± 2.31 mm > stem; IZ=12.33 ± 2.30 mm). Stem showed maximum activity against E. coli and bark showed minimum activity (stem; IZ=31.66 ± 2.08 mm > leaf; IZ= 20.00 ± 1.00 mm > root; IZ=10.00 ± 1.00 mm > bark; IZ=9.33 ± 2.51 mm). Root, stem and leaf showed high activity against Enterococcus while bark showed lower activities (Root, stem and leaf; IZ=11.66 ± 2.08 mm > bark; IZ= 8.66 ± 1.52 mm). Against *B. subtilis* leaf exhibited maximum activity while stem exhibited minimum activity (leaf; IZ=21.66 ± 2.51 mm > bark; IZ= 14.66 ± 2.08 mm > root; IZ=11.66 ± 3.51 mm > stem; IZ=11.33 ± 2.08 mm). Against K. pneumonia, none of extracts showed any inhibitory activity (root, stem, bark, leaf; IZ=0.00 mm). Alkaloids from leaf extract show highest inhibition zone against both test fungi and bacteria this result may be due to presence of more kinds of alkaloids which shows higher activity against pathogenic bacteria and fungi tested. Also flavonoids extracted from leaves were potent against the test organisms then those from root, stem and bark.

GC-MS spectra of Alkaloids from roots, stem, bark and leaves are shown in Table 1, 2, 3, 4 and 5.

In *T. undulata,* Alkaloids extracted from leaves were highly active against *C. albicans* (27.66±1.52mm), Against bacterial strains alkaloids from stems showed maximum activity against *E. coli* (31.66±2.08mm).

Table 1: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in Stems of *Tecomella undulate*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol.wt
1	17.508	1.62	Tetradecanal	$C_{14}H_{28}O$	212
2	17.637	0.29	Cyclopropane, 1-methyl-2-(3-methylpentyl)-	$C_{10}H_{20}$	140
3	19.217	4.14	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
4	19.308	27.31	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
5	19.943	14.36	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
6	20.596	0.74	Phthalic acid, heptyl tridec-2-yn-1-yl ester	$C_{28}H_{42}O_4$	442
7	21.214	8.74	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
8	21.536	0.79	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
9	22.649	0.33	2-Tetradecynal, 4-hydroxy-	$C_{14}H_{24}O_2$	224
10	23.185	0.63	Phytol	$C_{20}H_{40}O$	296
11	23.585	3.53	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254
12	23.851	1.31	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282
13	25.652	0.62	5-Dodecen-1-al	$C_{12}H_{22}O$	182
14	25.759	3.27	cis-9-Hexadecenal	$C_{16}H_{30}O$	238
	26.024	4.87	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
15	28.843	17.05	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390
16	36.333	0.23	Cholesta-2,4-diene	$C_{27}H_{44}$	368
17	36.385	0.31	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.,22Z)-	$C_{31}H_{50}O_2$	454
18	36.565	0.3	10-12-Pentacosadiynoic acid	$C_{25}H_{42}O_2$	374
19	36.684	0.47	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
20	36.841	3.29	Cholest-5-en-3-ol (3.beta.)-, propanoate	$C_{30}H_{50}O_2$	442
21	38.13	1.14	Stigmasterol	$C_{29}H_{48}O$	412
22	38.598	1.61	.beta.-Sitosterol	$C_{29}H_{50}O$	414
23	38.715	0.7	1-Pentadecene, 2-methyl-	$C_{16}H_{32}$	224
24	39.905	0.18	2-Nonadecanone	$C_{19}H_{38}O$	282
25	40.906	1.41	Diisobutyl 2,2-dihydroxymalonate	$C_{11}H_{20}O_6$	248

Table 2: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in roots of *Tecomella undulate*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol.wt
1	16.946	4.86	Benzoic acid	$C_9H_{10}O_4$	182
2	17.533	1.79	Dodecanal	$C_{12}H_{24}O$	184
3	17.645	0.62	3-Undecene, 7-methyl-, (E)-	$C_{12}H_{24}$	168
4	19.311	30.54	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
5	19.398	4.86	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
6	19.678	16.26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	21.215	9.00	n-Hexadecanoic acid	$C_{16}H_{32}O$	256
8	21.536	1.31	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
9	23.592	2.38	1-Undecanol	$C_{11}H_{24}O$	172
10	23.775	0.24	Cyclohexanepropanol-	$C_{19}H_{18}O$	142
11	23.845	1.29	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282
12	24.16	0.47	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340
13	25.767	3.71	trans-2-Dodecen-1-ol, trifluoroacetate	$C_{14}H_{23}F_3O_2$	280
14	26.032	4.5	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
15	28.853	4.54	1,2-Benzenedicarboxylic acid, dioctyl ester	$C_{24}H_{38}O_4$	390

16	36.336	0.51	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306
17	36.385	0.53	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{31}H_{50}O_2$	454
18	36.84	5.62	Cholest-5-en-3-ol (3.beta.)-, propanoate	$C_{30}H_{50}O_2$	442
19	38.129	1.39	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412
20	38.601	2.05	.beta.-Sitosterol	$C_{29}H_{50}O$	414
21	38.713	0.82	1-Pentadecene, 2-methyl-	$C_{16}H_{32}$	224
22	39.787	0.64	Ethanone, 1-(1,2,3,4,7,7a-hexahydro-1,4,4,5-tetramethyl-1,3a-	$C_{17}H_{26}O$	246

Table 3: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in Bark of *Tecomella undulate*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. Wt
1	16.876	15.64	Benzoic acid, 3,4-dimethoxy-	$C_{19}H_{10}O_4$	182
2	17.467	1.88	.Delta.-(2)-dodecanol	$C_{12}H_{26}O$	186
3	17.616	0.69	Cyclopropane, 1-methyl-2-(3-methylpentyl)-	$C_{10}H_{20}$	140
4	19.193	1.5	1-Decene, 8-methyl-	$C_{11}H_{22}$	154
5	19.278	21.21	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
6	19.332	0.27	Z-4-Tridecen-1-yl acetate	$C_{15}H_{28}O_2$	240
7	19.912	15.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
8	21.228	6.14	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
9	21.54	1.82	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
10	23.792	0.27	1,3-Isobenzofurandione, hexahydro-	$C_{18}H_{10}O_3$	154
11	23.848	1.52	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282
12	26.045	2.32	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
13	28.857	7.06	1,2-Benzenedicarboxylic acid, dioctyl ester	$C_{24}H_{38}O_4$	390
14	33.505	0.96	Methyl alpha-ketopalmitate	$C_{17}H_{32}O_3$	284
15	36.569	1.35	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-	$C_{22}H_{34}O_3$	346
16	36.683	0.46	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436
17	36.841	12.62	.beta.-Sitosterol	$C_{29}H_{50}O$	414
18	38.132	1.82	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412
19	39.267	2.69	Urs-12-ene	$C_{30}H_{50}$	410
20	40.82	0.66	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298

Table 4: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in Leaves of *Tecomella undulate*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol.Wt
1	14.238	0.17	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206
2	17.477	0.7	Octadecanal	$C_{18}H_{36}O$	268
3	19.213	0.4	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
4	19.309	10.95	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
6	19.938	6.26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	20.568	2.43	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278
8	21.236	6.42	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334
9	22.541	0.8	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334
10	22.79	0.29	Phthalic acid, cycloheptyl isohexyl ester	$C_{21}H_{30}O_4$	346
11	23.176	0.3	Phytol	$C_{20}H_{40}O$	296
12	23.425	0.22	Phthalic acid, pentyl tridec-2-yn-1-yl ester	$C_{26}H_{34}O_2$	282
13	23.857	0.25	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282
14	24.057	0.87	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	312
15	24.122	0.77	1-Docosene	$C_{22}H_{44}O_2$	312
16	25.754	1.13	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266
17	26.02	1.74	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
18	26.491	0.8	Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$	340
19	26.548	0.57	1-Docosanol	$C_{22}H_{46}O$	326
20	28.946	55.62	1,2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278
21	29.426	0.86	Heptadecyl heptafluorobutyrate	$C_{21}H_{35}F_7O_2$	452
22	33.446	0.52	Heptadecyl trifluoroacetate	$C_{19}H_{35}F_3O_2$	352
23	35.784	0.35	1-Heptacosanol	$C_{27}H_{56}O$	396
24	36.68	0.14	Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{48}O_2$	488
25	36.839	0.66	Cholest-5-en-3-ol (3.beta.)-, propanoate	$C_{30}H_{50}O_2$	442
26	37.288	0.22	1-Heptacosanol	$C_{27}H_{56}O$	396
27	38.122	0.36	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412
28	38.596	0.39	.beta.-Sitosterol	$C_{29}H_{50}O$	414
29	38.714	0.49	2-Undecene, 6-methyl-, (E)-	$C_{12}H_{24}$	168
30	39.815	0.31	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5, 6,6a, 6b,7,8,8a,9,	$C_{30}H_{48}O$	424
31	40.914	1.58	2-Tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydrox	$C_{40}H_{58}O_3$	586
32	43.032	0.72	Olean-12-ene	$C_{30}H_{50}$	410

Table 5: Bactericidal and fungicidal efficacy of alkaloids crude extracts of *Tecomella undulate*

Microorganisms		Root	Stem	Bark	Leaf
F. oxysporum NCIM 1228	IZ	15.33 +2.52	15.33 +2.08	16.00 +1	18.66 +1.52
	AI	0.64	0.64	0.67	0.78
P. funiculosum NCIM 1075	IZ	14.33 +0.57	12.00 +2	13.00 +0	18.66+0.57
	AI	0.68	0.57	0.62	0.88
C. albicans NCIM 3501	IZ	9.33 +1.52	19.66 +1.52	14.33 +2.51	27.66 +1.52
	AI	0.55	1.15	0.84	1.62
T. viride NCIM	IZ	12.33 +2.51	20.00 +2.64	12.00 +2.44	26.66+1.05
	AI	0.46	0.74	0.44	0.99
Bacteria					
S. aureus MTCC 0087	IZ	14.66±3.05	12.33±2.30	13.33±2.31	17.00±1
	AI	0.49	0.41	0.44	0.57
E. coli MTCC 1652	IZ	10.00±1	31.66±2.08	9.33±2.51	20.00±1
	AI	0.55	1.76	0.52	1.11
E. faecalis ATCC 29212	IZ	11.66±0.57	11.66±2.08	8.66±1.52	11.66±2.08
	AI	0.58	0.58	0.44	0.58
B. subtilis MTCC 0121	IZ	1.66±3.51	11.33±2.08	14.66±2.08	21.66±2.51
	AI	0.58	0.57	0.73	1.08
K. pneumonia MTCC 0109	IZ	0.00	0.00	0.00	0.00
	AI	0.00	0.00	0.00	0.00

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