



IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 Issue: III Month of publication: March 2018 DOI: http://doi.org/10.22214/ijraset.2018.3375

www.ijraset.com

Call: 🛇 08813907089 🕴 E-mail ID: ijraset@gmail.com



Synergistic Effect of Essential Oils of Three Gymnosperms Against Vibrio Cholerae

Ravikant Singh¹, Rinki Singh², Preeti Singh³, Saket Jha⁴, Ashutosh Pathak⁵, Shashi Kant Shukla⁶, Anupam Dikshit⁷ Rohit K. Mishra⁹

^{1, 2, 3, 4}Research Scholar, ^{5,6}Assitant Professor, ⁷Professor and ⁸Associate Professor

^{1, 8}Dept. of Biotechnology, Swami Vivekanand University, Sagar-470228, M.P. India

^{2, 3}Center of Bioinformatics, IIDS, University of Allahabad, Allahabad-211002, U.P. India

^{4, 5, 6, 7}Biological Product Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, U.P. India

Abstract: The present investigation focused on the determination of antibacterial efficacy of essential oils obtained from leaves of three gymnosperms i.e. Pinus roxburghii Sarg., Taxodium distichum L. and Thuja occidentalis L. The oils were extracted from the leaves of aforementioned plant species using Clevenger type apparatus by hydro-distillation method. The antibacterial activity of the extracted essential oils was evaluated against Vibrio cholerae (MTCC No. 3906) using broth micro-dilution method recommended by Clinical Laboratory Standard Institute (CLSI). The Inhibition Concentration i.e. IC50 and Minimum Inhibition concentrations (MIC) using SpectramaxPlus384, of Molecular Devices Corporation, USA were recorded while Streptomycin as standard was taken. The IC50 value of P. roxburghii Sarg., T. distichum L. and T. occidentalis L., were showed 1.294, 0.914 and 1.277 mg/ml respectively. The P. roxburghii, was found most effective with their MIC 1.403 mg/ml while T. distichum L., showed least effective with their MIC 1.995 mg/ml against V. cholerae. Hence, essential oil from leaves of gymnosperms exhibit great potential for the development of eco-friendly, non-toxic, cost-effective anti-bacterial formulations. Key words: Gymnosperms, Essential oil, Synergistic effect, Broth Micro-dilution assay

I. INTRODUCTION

P. roxburghii Sarg. belongs to Pinaceae and commonly known as Chir pine. P. roxburghii is the native of Himalayas and distributed throughout India, Nepal, Bhutan and Pakistan. It is widely distributed from lower to midrange of Himalaya in India. P. roxburghii is a large tree attaining up to 55 m in height with a trunk diameter reaching up to 2 m (figure 1). The cones of P. roxburghii are ovoid, conic and usually open up to 20 cm to release the seeds (1). P. roxburghii oil has been traditionally used to treat blisters, cuts, boils and wounds (2). Phytochemical screening of Pinus needles revealed the abundant amounts of tannins, vitamin C and alkaloids while the stem has been primarily used as a source of turpentine oil (3-5). Some microbiological research suggests that the essential oil on P. roxburghii has shown significant anti-fungal activity (6) as well as antibacterial activity (7) while alcoholic extract of the needle, stem, and cones are reported to exhibit strong anti-bacterial activity. P. roxburghii grows in the region of forests of 1200-1850 m altitude at a temperature between $5-15^{\circ}C$ (8).

Taxodium distichum. (L.) belongs to Taxodiaceae and commonly referred as bald cypress. It is an unusual but interesting tree. It can grow over 25 m in height and over 3 m in diameter (figure 2). The leaves are small, green to yellow-green, 5–20 mm long and appeared in two-ranks. Young trees have a pyramid shape but eventually form an irregular flattened canopy. Cones are the fruits and are composed of scales forming a woody, brown sphere with rough surface. T. distichum has three extant taxa ranging from the eastern United States through Mexico to Guatemala (9). Heartwood of bald cypress is extremely rot and termite resistant (10). Leaves and cones are rich in essential oils and used traditionally to treat respiratory, skin, gastro-intestinal, inflammation, and infections (11, 12). Diterpenoids and Flavonoids are the main secondary metabolites (13). T. distichum trees can grow near lake margins, rivers, swamps, wet poorly drained habitats and are tolerant to various soil conditions and air pollution (14). These long-lived conifers have been widely used for landscape in many countries. The heartwood is used for building materials, and has been reported to resist the attacks of the subterranean termite (15).



International Journal for Research in Applied Science & Engineering Technology (IJRASET) ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887 Volume 6 Issue III, March 2018- Available at www.ijraset.com



Fig1. P. roxburghii

Fig 2. T. distichum

Fig 3. T. occidentalis

Thuja occidentalis L. belongs to Cupressaceae and commonly known as White Cedar and Morpankhi in Hindi. It is native to Eastern Canada and other regions on United State; widely cultivated as an ornamental plant throughout the planet (figure 3). T. occidentalis has been used to treat uterine carcinomas, bronchial catarrh, psoriasis and rheumatism (16). The essential oil of the plant has been used for soft soaps, room sprays, disinfectants and insecticides. Cedar leaf oil can be obtained by hydro distillation of the foliage and is used for the production of perfumes, insecticides, soaps and deodorants (17, 18). The essential oil is an active ingredient in the production of antibacterial herbal formulations, cough suppressants, soap and perfumes, while many cultivars are grown for ornamental purposes (7, 19). The oil of eastern cedar leaves (T .occidentalis) has been independently investigated by Shaw (20) and Rudloff (20), who reported the thujone fraction as a mixture of Z-thujone and E-isothujone, while Keita et al. (21) in their analyses, reported twenty-two compounds including α -thujone (= Z-thujone) (49.64%), fenchone (14.06%) and β -thujone (= E-isothujone) (8.98%) as the most abundant compounds.

II. MATERIAL AND METHOD:

A. Extraction of essential oil

The plant materials of P. roxburghii Sarg., T. distichum L., and T. occidentalis L., were collected from Roxburgh Garden, Department of Botany, University of Allahabad. Plant were identified at Department of Botany, University of Allahabad(7). Leaves were crushed and loaded into a Clevenger type Apparatus for hydrodistilation for 4-5 hours at 45 degree Celsius (7). Essential oils of T. distichum (bald cypress) appears as dark yellow, T. occidentalis (white Cedar) as yellow in colour followed by P. roxburghii (chir pine) i.e., pale yellow. Oil content was stored at 4°C until analysis (7, 22).



Fig4 and 5. Clevenger type apparatus.

Fig6. Extracted oils



International Journal for Research in Applied Science & Engineering Technology (IJRASET) ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887 Volume 6 Issue III, March 2018- Available at www.ijraset.com

B. Preparation Of 0.5 Mcfarland Solution And Saline Media

On adding 1% of $BaCl_2$ to the freshly prepared solution of 200 ml of 1% H_2SO_4 , 0.5 McFarland solution is formed (7, 23). Now prepare saline media by dissolving 1 gm of NaCl into 100 ml of DDW (7). Take the O.D. of this saline media and McFarland solution.

C. Preparation of Mueller-Hinton broth (MHB) and Inocula

Take 1000 ml of DDW in a beaker. Add 21 gms of MHB powder. Shake well and boil up to 100 °C. Close the mouth with sterilized synthetic plug (7). Inocula were prepared by using saline media and bacteria (7).

D. Antibacterial Screening

Essential oils were screened for antibacterial activity against S. typhimurium. Minimum Inhibitory Concentrations (MIC) were determined using Broth Micro-dilution method recommended by Clinical Laboratory Standard Institute (CLSI). 96 well plate were used for broth microdilutions. Column-1 contains 190 μ L and 10 μ L of formaldehyde and known as negative control (7). Column-2 contains 200 μ L of MHB and known as broth control (7). Column-3 contains 180 μ L broth and 20 μ L drug in each row and known as drug control. Row A and B of column-3 contains 20 μ L of Streptomycin. Row C and D contains 20 μ L of chir oil. Row E and F contains bald cypress oil whereas row G and H contains cedar oil. Now add 100 μ L of broth from column-4 to column-12. In column-4, add 80 μ L broth and 20 μ L drugs in each row as mentioned previously. Now homogenize and dilute the drugs 1:1 in MHB horizontally from column-4 to column-11. Finally add 100 μ L inocula from column-4 to column-12 (7) (figure 7). Final volume of each well were 200 μ L. The solutions were incubated at 37 °C for 24 hours (7, 24). *Streptomycin* used as positive control. Formaldehyde was used as a negative control.

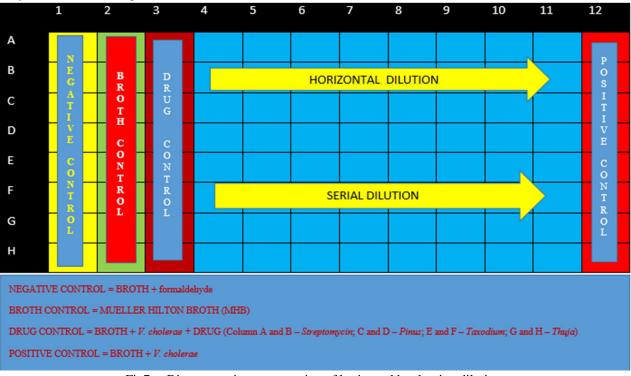


Fig7. - Diagrammatic representation of horizontal broth microdilution.

III. RESULTS

The results were recorded in terms oil Inhibition Concentrations (IC50) and Minimum Inhibition Concentrations (MICs) via SpectramaxPlus384, Molecular Devices Corporation, USA. IC50 value of P. roxburghii, T. distichum and T. occidentalis were showed 1.294, 0.914 and 1.277 mg/ml respectively (Figure 8, 9). The minimum inhibition concentrations (MIC) of P. roxburghii, T. distichum and Thuja occidentalis were recorded 1.403, 1.995 and 1.864 mg/ml respectively (Figure 8,9). P. roxburghii was found to be most effective with their MIC 1.403 mg/m1 whereas T. distichum was found to be least effective with their MIC 1.995 mg/m1 against V. cholerae (Figure 9).



International Journal for Research in Applied Science & Engineering Technology (IJRASET)

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887 Volume 6 Issue III, March 2018- Available at www.ijraset.com

SoftMax Pro - [Vc BMD 1.pda]

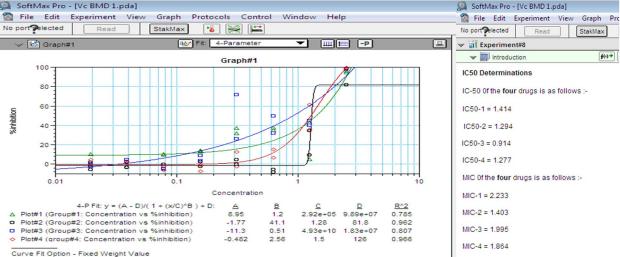


Fig8. Graph obtained for antibacterial activity of essential oils and values of IC50 and MIC.

-					pda]											
\$	File	Edit	Exper	iment	View	Grap	h Pro	otocol	s Cor	ntrol V	Vindov	~ н	elp			
lo	o port s	elected		Read		StakM	lax	1	#							
	-	Plate#	±1				Sett	tings	Temp1	late 🛛 🔽 R	eductio	n	Display		///	4
						Pla	te#1									
_	1	2	3	4	5	6	7	8	9	10	11	12				
Γ	-0.026	-0.011	-0.015	0.055	0.818	0.893	0.906	1.235	1.293	1.475	1.469	1.296	6 Endpo	oint		
ŀ	0.125	0.009	-0.015	0.078	1.371	0.936	0.984	1.253	1.298	1.475	1.294	1.478	Lm1	492		
┢								1.473						mix: Or	nce	
ŀ	-0.021	0.007	-0.008	0.263	0.946	1.525	1.377		1.503	1.409	1.525	1.454	Calibr	ate: O		
Ŀ	-0.001	0.014	-0.008	0.261	1.304	1.560	1.445	1.410	1.448	1.490	1.418	1.482				
Ŀ	-0.019	-0.003	0.014	0.021	0.864	0.724	1.069	1.310	1.504	1.375	1.473	1.444	4 Start F	Read: B AM	4/4 5 /	2001
	-0.019	0.033	0.021	0.034	0.797	0.985	0.407	1.387	1.518	1.415	1.436	1.334		Am	4/15/2	2001
	-0.020	0.006	0.013	0.009	0.930	1.343	1.476	1.541	1.533	1.398	1.445	1.446	в			
Ŀ	-0.018	-0.013	0.024	0.015	0.554	1.223	1.262	1.454	1.467	1.419	1.372	1.377	7			
	1.			roup#1 (m				adari ka				Group#3	(mg/ml)			
	Sample C BL1	concentration 2.60	0 A4	alues Me		0.016 24	.2 96	156	Sample C	Concentration 2.500	Wells V		an Values S		V96 9	%inhibition 98.52
1	Sample C BL1 BL2	oncentration 2.60 1.25	0 A4 84 0 A5	0.055 0.078 0.818	an Values S	0.391 35	.2 96 94 .7 43	166 566 206			Wells V/ E4 0 F4 0 E5 0	alues Me 0.021 0.034 0.884	an Values S 0.028	0.009 3		98.52 97.63 39.99
E	BL1	2.60	0 A4 B4 0 A5 B5 5 A6	alues Me 0.055 0.078 0.818 1.371 0.893	an Values 9 0.067	0.018 24	2 96 94 7 43 4 3 37	.156 .566 .206 .781 .962	BL1	2.500	Wells Vi E4 0 F4 0 E5 0 F5 0 E6 0	0.021 0.034 0.864 0.797	0.028 0.831	0.009 3	2.7	98.52 97.63 39.99 44.60 49.68
E	BL1 BL2 BL3	2.60	0 A4 B4 0 A5 B5 6 A6 B6	alues Me 0.055 0.078 0.818 1.371	an Values S 0.067 1.094	0.016 24 0.391 35 0.031 3	2 96 94 7 43 4 .3 37 34	156 566 206 781	BL1 BL2	2.500	Wells V/4 E4 0 F4 0 E5 0 F6 0 F6 0	alues Me 0.021 0.034 0.864 0.797	0.028 0.831 0.855	0.009 3 0.047 0.184 2	6.7	98.52 97.63 39.99 44.60 49.68 31.59
	BL1 BL2 BL3 BL4	2.50 1.25 0.62 0.31	0 44 84 0 45 85 5 46 86 3 47 87	slues Me 0.055 0.078 0.818 1.371 0.893 0.936 0.936 0.936	an Values S 0.067 1.094 0.916 0.945	0.016 24 0.391 35 0.031 3 0.055 5	2 96 94 7 43 4 3 37 34 8 37 34 8 37	.156 .566 .206 .781 .962 .961 .101 .683	BL1 BL2 BL3 BL4	2.500 1.250 0.625 0.313	Wells V/4 E4 0 F4 0 F5 0 F6 0 F7 0	alues Me 0.021 0.034 0.864 0.797 0.724 0.985 1.069 0.407	an Values S 0.028 0.831 0.855 0.738	0.009 3 0.047 0.184 2 0.468 6	5.7 5.7 1.6 3.4	98.52 97.63 39.99 44.60 49.68 31.59 25.74 71.69
8	BL1 BL2 BL3 BL4 BL5	2.50 1.25 0.62 0.31 0.15	Wells V 0 A4 B4 0 0 A5 B5 5 3 A7 B7 6 B8	alues Me 0.055 0.078 0.893 0.936 0.893 0.984 1.2353 1.253	an Values 9 0.067 1.094 0.915 0.945 1.244	0.016 24 0.391 36 0.021 3 0.055 5 0.013 1	.2 96 94 .7 43 .3 37 .3 37 .34 .8 37 .31 .1 14	156 566 206 781 962 961 .101 683 241 942	BL1 BL2 BL3 BL4 BL5	2.500 1.250 0.825 0.313 0.158	Wells Vields E4 C F5 C F6 C F7 C F8 F8	alues Me 0.021 0.034 0.797 0.724 0.985 1.069 0.407 1.310 1.387	an Values S 0.028 0.831 0.855 0.738 1.248	0.009 3 0.047 0.184 2 0.468 6 0.055	2.7 5.7 1.6 3.4 4.1	98.52 97.63 39.99 44.60 49.68 31.59 25.74 71.69 9.02 3.64
8	BL1 BL2 BL3 BL4	2.50 1.25 0.62 0.31	n Wells ∨ 0 A4 0 A5 85 5 A6 86 3 A7 6 A8 8 A9	alues Me 0.055 0.078 0.818 1.371 0.935 0.935 0.906 0.984 1.235 1.253 1.293	an Values S 0.067 1.094 0.916 0.945	0.016 24 0.391 36 0.021 3 0.055 5 0.013 1	.2 90 94 .7 43 .3 37 .3 34 .8 37 .1 14 .3 10	156 566 206 791 962 961 101 693 241 942 206	BL1 BL2 BL3 BL4 BL6 BL6	2.500 1.250 0.825 0.313 0.156 0.078	Wells Vield E4 C F5 C F6 C F7 C F8 F8 F9 F9	alues Me 0.021 0.034 0.797 0.724 0.985 1.069 0.407 1.210 1.210 1.287 1.504 1.518	an Values S 0.028 0.831 0.855 0.738 1.248 1.511	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010	2.7 5.7 1.6 3.4 4.1 0.7	98.52 97.63 29.99 44.60 31.59 25.74 71.69 9.02 2.64 -4.45 -5.43
8 8 8 8	BL1 BL2 BL3 BL4 BL5	2.50 1.25 0.62 0.31 0.15	1 Wells ∨ 0 A4 B4 0 A5 B5 5 A6 B6 3 A7 B7 6 A8 B8 8 A9 B9 9 A10	alues Me 0.055 0.078 0.818 1.371 0.893 0.936 0.906 0.984 1.225 1.225 1.293 1.293 1.475 1.475	an Values 9 0.067 1.094 0.915 0.945 1.244	0.018 24 0.391 35 0.031 3 0.055 5 0.013 1 0.004 0	2 90 94 .7 43 4 3 37 34 .8 37 .31 .1 14 12 .3 10 9 0 -2	156 500 791 982 981 101 683 241 942 206 881 471	BL1 BL2 BL3 BL4 BL5	2.500 1.250 0.825 0.313 0.158	Wells Viet E4 C F4 C E5 C F6 C F7 C E8 F F8 F E9 F F9 F	alues Me 0.021 0.034 0.884 0.797 0.885 1.088 0.407 1.210 1.387 1.504 1.518 1.375	an Values S 0.028 0.831 0.855 0.738 1.248 1.511	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010	2.7 5.7 1.6 3.4 4.1	98.52 97.63 29.99 44.60 31.69 25.74 71.69 9.02 2.64 -4.45 -5.43 4.45
8 8 8 8 8 8	BL1 BL2 BL3 BL4 BL6 BL6	2.50 1.25 0.62 0.31 0.15 0.07	1 Wells ∨ 0 A4 B4 0 A5 B5 6 A6 B6 3 A7 6 A8 B7 6 A8 B9 9 A10 B10 0 A11	alues Me 0.055 0.078 0.818 1.371 0.893 0.936 0.906 996 1.235 1.235 1.293 1.293 1.475 1.475 1.475 1.469	an Values \$ 0.067 1.094 0.915 0.945 1.244 1.295	0.016 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0	2 90 94 .7 43 4 .3 37 .4 .3 37 .3 4 .3 .3 .1 14 .1 12 .3 10 .0 -2 .2 .0 -2	156 506 206 781 962 961 101 683 241 942 206 851 471 450 013	BL1 BL2 BL3 BL4 BL6 BL6	2.500 1.250 0.825 0.313 0.156 0.078	Wells Vi E4 C F4 C E5 C F6 C F7 C E9 F E9 F E10 F10 E11 F	alues Me 0.021 0.034 0.984 0.985 0.724 0.985 0.407 1.310 1.327 1.504 1.518 1.375 1.473	an Values S 0.028 0.831 0.855 0.738 1.248 1.511 1.395	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010 0.028	2.7 5.7 1.6 3.4 4.1 0.7	86 52 97.63 39.99 44.68 31.59 28.74 71.69 9.02 3.84 -4.45 -5.43 4.45 4.48 1.79 -2.30
	BL1 BL2 BL3 BL4 BL5 BL6 BL7 BL8 C50 = 1.41	2.80 1.25 0.82 0.31 0.15 0.07 0.03 0.02	1 Wells ∨ 0 A4 B4 0 A5 5 A6 B7 6 A8 B8 8 A9 B9 A10 B10	slues Me. 0.055 0.078 0.818 1.371 0.893 0.936 0.934 1.225 1.253 1.293 1.298 1.475	an Values \$ 0.067 1.094 0.915 0.945 1.244 1.295 1.475	0.016 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0	2 90 94 .7 43 4 .3 37 .4 .3 37 .3 4 .3 .3 .1 14 .1 12 .3 10 .0 -2 .2 .0 -2	156 500 791 962 961 101 683 241 942 200 851 471 450	BL1 BL2 BL3 BL4 BL6 BL6 BL7	2.500 1.250 0.625 0.313 0.156 0.078 0.029 0.020	Wells Vi E4 C F4 C E5 C F6 C F7 C E9 F E9 F E10 F10 E11 F	alues Me 0.021 0.864 0.864 0.866 0.797 0.724 0.866 0.407 1.210 1.287 1.518 1.375 1.415	an Values S 0.028 0.831 0.855 0.738 1.248 1.511 1.395	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010 0.028	2.7 5.7 11.6 3.4 4.1 0.7 2.0	96 52 97 63 39 99 44 60 49 68 21 59 28 74 71 69 9 02 3 84 4 45 4 45 4 48 4 79 2 30
	BL1 BL2 BL3 BL4 BL5 BL5 BL5 BL7 BL8 C50 = 1.41 MIC = 2.233	2.60 1.25 0.82 0.31 0.15 0.07 0.03 0.02	1 Wells ∨ 0 A4 B4 0 A5 B5 6 A6 B6 3 A7 6 A8 B7 6 A8 B9 9 A10 B10 0 A11	Bitues Me 0 0.55 0 0.55 0 0.55 1 2.71 0 0.936 0 906 0 906 1 2.255 1 2.993 1 2.993 1 2.993 1 4.755 1 4.475 1 4.294	an Values 5 0.067 1.094 0.915 0.945 1.244 1.295 1.475 1.381	0.018 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9	2 90 94 .7 43 4 .3 37 .4 .3 37 .3 4 .3 .3 .1 14 .1 12 .3 10 .0 -2 .2 .0 -2	150 500 200 7781 982 981 101 882 241 842 241 842 200 8851 200 8851 200 851 200 851 200 851 150	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL8	2.500 1.250 0.625 0.313 0.156 0.075 0.035 0.020	Wells Vi E4 C F4 C E5 C F6 C F7 C E9 F E9 F E10 F10 E11 F	alues Me 0.021 0.034 0.984 0.985 0.724 0.985 0.407 1.310 1.327 1.504 1.518 1.375 1.473	an Values S 0.028 0.831 0.855 0.738 1.248 1.511 1.395	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010 0.028 0.026	2.7 5.7 11.6 3.4 4.1 0.7 2.0	96 52 97 63 39 99 44 60 49 68 21 59 28 74 71 69 9 02 3 84 4 45 4 45 4 48 4 79 2 30
	BL1 BL2 BL3 BL4 BL5 BL6 BL7 BL8 C50 = 1.41	2.60 1.25 0.82 0.31 0.15 0.07 0.03 0.02	n Wells ∨ 0 A4 84 85 86 86 86 86 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 85 86 86 86 86 86 86 86 86 86 86 86 86 86	Bitest Me 0 0.55 0 0.55 0 0.51 1 271 0 0.936 0 9.936 0 9.936 1 225 1 225 1 298 1 475 1 469 1 294	an Values 5 0.067 1.094 0.916 0.946 1.244 1.295 1.475 1.281 .475	0.018 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9	2 90 94 .7 43 4 .3 37 .4 .3 37 .3 4 .8 37 .3 4 .3 1 1 1 4 .3 10 .0 -2 .2 .0 -2	150 500 200 7781 982 981 101 882 241 842 241 842 200 8851 200 8851 200 851 200 851 200 851 150	BL1 BL2 BL3 BL4 BL5 BL5 BL7 BL3 IC50 = 0.9 MIC = 1.995	2.500 1.250 0.625 0.313 0.156 0.075 0.035 0.020	Wells Vi E4 C F4 C E5 C F6 C F7 C E9 F E9 F E10 F10 E11 F	Blues Me 0 021 0 024 0 924 0 924 0 924 0 924 0 924 0 926 0 926 1 0407 1 3210 1 3216 1 1472 1 426	on Values 5 0.028 0.831 0.855 0.738 1.248 1.511 1.355 1.454	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010 0.028 0.026	2.7 5.7 11.6 3.4 4.1 0.7 2.0	96 52 97 63 39 99 44 60 49 68 21 59 28 74 71 69 9 02 3 84 4 45 4 45 4 48 4 79 2 30
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL8 C50 = 1.41 MIC = 2.233 F Grou	2.50 1.25 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2	1 Wells ∨ 0 A4 B4 0 A5 B5 5 A6 B5 6 A6 B5 8 A9 9 A10 B10 0 A11 B11	Bit Less Me 0.055 0.075 0.055 0.075 0.051 1.371 0.832 0.832 0.936 1.253 1.253 1.293 1.475 1.475 1.294 1.294	an Values 3 0.067 1.094 0.915 0.945 1.244 1.296 1.475 1.281 * fe3* fe3*	0.018 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9	.2 96 94 .7 43 .3 37 .3 34 .8 37 .1 11 .3 110 .3 10 .0 22 .0 -2 .0 10	150 500 200 300 300 300 100 100 100 200 200 300 300 300 300 300 300 300 3	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL6 MIC = 0.9 MIC = 1.995 MIC = 1.995	2.500 1.250 0.625 0.313 0.156 0.039 0.020 0.020 114 3 200centration	Wells V E4 C E5 C E6 C E7 C E7 C E3 F F3 F E10 E11 F11 F	group# group# values	n Values 5 0.025 0.031 0.856 0.738 1.348 1.348 1.464 [m.+] [foo+] [4 (mg/m]) Mean Values	0.009 3 0.047 0.184 2 0.468 6 0.055 0.010 0.028 0.026	2.7 5.7 11.6 3.4 4.1 0.7 2.0 1.8	98,82 97,82 39,96 49,66 49,66 31,66 31,66 32,74 71,66 3,024 3,024 4,45 4,45 4,45 4,45 4,45 4,45 4,45 4,
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL8 C50 = 1.41 MIC = 2.233 F Grou	2.60 1.25 0.82 0.31 0.15 0.07 0.03 0.02	1 Wellis V 0 A4 B4 0 A5 B4 0 A6 B4 0 A5 B4 0 A4 B4 0 A4 B4	atLes Me 0.055 0.078 0.051 0.978 0.931 0.932 0.932 0.934 1.235 1.293 1.234 1.293 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475	an Values 3 0.067 1.094 0.915 0.945 1.244 1.296 1.475 1.281 * fe3* fe3*	0.018 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9	2 96 94 7 43 3 3 4 6 37 1 1 4 3 3 0 2 0 2 0 2 0 2 0 4 0 4 0 2 0 4 0 4 0 4 0 4 0 4 0 4 0 4 0 4	1866 6666 2006 2006 3001 301 8621 3041 3042 241 3042 241 3042 241 3042 3045 3045 3045 3045 3045 3045 3045 3045	BL1 BL2 BL3 BL4 BL4 BL4 BL4 BL4 BL4 BL4 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7	2.500 1.250 0.625 0.313 0.156 0.078 0.020 114 5 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.025 0.000 0.0250	Walls V. E4 C E5 C E6 C E7 C E8 C E9 C E11 C E11 C E11 C Walls V. H4 H4	STUES Me 0.021 0.024 0.034 0.797 0.565 0.407 1.310 1.357 1.504 1.357 1.426 STUES 0.009 0.005 0.0015	an Values S 0.028 0.028 0.821 0.866 0.738 1.248 1.811 1.386 1.464 (Ⅲ) (f0)+	0.009 3 0.047 0.184 2 0.468 6 0.065 0.028 0.028 0.028 0.028	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.8	98.82 97.83 39.99 44.00 44.00 44.00 9.25.74 71.09 9.02 3.04 4.5.42 4.43 4.43 4.43 4.43 4.43 4.43 5.23 0.20 0.20 9.33 9.93 9.93 9.93
	BL1 BL2 BL2 BL4 BL6 BL6 BL6 BL7 BL9 C50 = 1.41 MIC = 2.233 F III Grou	2 50 1.25 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2 concentration 2 50	1 Wells V 0 A4 B4 0 A6 B5 0 A5 B6 2 A7 B8 8 A9 B5 9 A10 0 0 A11 B11	atLess Me 0.055 0.078 0.051 0.315 1.371 0.934 1.253 1.253 1.253 1.253 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.294 Imm	<u>on Values S</u> <u>0.067</u> <u>1.094</u> <u>0.915</u> <u>0.945</u> <u>1.244</u> <u>1.295</u> <u>1.475</u> <u>1.281</u> <u>* [fos *] foc</u> <u>mg/ml)</u> <u>sen Values</u>	0.018 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9	2 96 3 37 4 3 3 37 3 37 3 37 3 37 3 1 4 3 3 37 3 1 4 3 3 7 3 1 1 1 4 3 3 7 3 7 1 1 4 3 3 7 1 1 4 1 1 1 4 1 1 1 1 1 1 1 1 1	1156 5000 12000 1791 1001 1011 10241 1042 1044 1044 1044 1050 1100	BL1 BL2 BL3 BL4 BL4 BL4 BL4 BL5 BL7 BL5 IC50 = 0.9 MIC = 1.995 MIC = 1.995 Sample C BL1 BL1 BL2	2.500 1.250 0.525 0.313 0.156 0.039 0.039 0.039 0.039 0.039 0.039 1.250 1.250	Wells V. E4 C F4 C E5 C F6 C F7 C E9 F7 F9 F1 Wells T G4 H4 H4 H4	stues Me 0.021 0.024 0.024 0.024 0.024 0.024 0.027 0.026 0.026 0.026 0.026 1.007 1.007 1.004 1.005 1.004 1.005 1.004 1.005 1.005 1.004 1.005	an Values 5 0.025 0.031 0.855 1.245 1.611 1.355 1.454 (m)+ (for+) 4 (mg/m) Mean Values 0.742	0.009 3 0.047 0.184 2 0.468 6 0.056 0.010 0.028 0.028 0.026 10.028	2.7 5.7 11.6 3.4 4.1 0.7 2.0 1.8	98,82 97,63 34,900 48,608 31,639 28,74 47,79 02 9,04 4,448 4,77 0,02 9,04 4,448 4,79 0,28 0,28 0,28 0,28 0,28 0,28 0,28 0,28
	BL1 BL2 BL2 BL4 BL5 BL5 BL5 BL5 BL5 BL5 C50 = 1.41 MIC = 2.233 F III Grou Sample C BL1 BL2	2.50 1.25 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2 concentration 2.50 1.25	1 Wellis V 0 A4 B4 0 A5 B5 0 A5 B5 0 A5 B5 0 A5 B5 0 A5 B1 0 C4 V 0 C4 C4	atLes Me 0.0656 0.075 0.0551 0.975 0.9932 0.9932 0.9934 1.293 1.293 1.293 1.475 1.475 1.294 1.294 0.0264 0.285 0.2954 1.294	or Values 2 0.067 1.094 0.915 0.945 1.244 1.295 1.475 1.391 (for)*) [for mg/ml) san Values 0.262 1.125	0.018 24 0.391 35 0.021 3 0.065 6 0.013 1 0.004 0 0.000 0 0.124 9 0.001 0 0.001 0 0.263 2	2 96 3 94 3 94 3 94 3 94 3 94 3 95 3 95 1 14 1 16 9 95 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0	1156 2006 2006 2007 101 2002 3001 101 2022 3001 3022 3021 471 471 4720 3021 4720	BL1 BL2 BL3 BL4 BL4 BL4 BL4 BL4 BL4 BL4 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7	2.500 1.250 0.625 0.313 0.156 0.078 0.020 114 5 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.020 0.025 0.000 0.0250	Wells X E4 C F4 C E5 C F6 C F7 C E6 C E7 C E7 C E8 C E9 C E9 C E9 C E9 C E9 C E10 F11 VWells X G44 H4 H5 Q6	stues Me 0.021 0.024 0.407 0.407 0.407 0.407 0.407 0.407 0.407 0.407 0.407 0.009 0.008 0.009 0.008	an Values S 0.028 0.028 0.821 0.866 0.738 1.248 1.811 1.386 1.464 (Ⅲ) (f0)+	0.009 3 0.047 0.184 2 0.468 6 0.056 0.010 0.028 0.028 0.026 10.028	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.8 7. 2.0 1.8	98,62 97,63 34,960 44,869 31,69 221,49 7,90 2,21,49 7,90 7,90 7,90 7,90 7,90 7,90 7,90 7,9
	BL1 BL2 BL3 BL4 BL6 BL6 BL6 BL7 BL8 C50 = 1.41 MIC = 2.233 MIC = 2.233 C50 = 1.41 MIC = 2.233	2.50 1.25 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2 concentration 2.50 1.25	1 Wells V 0 A4 B4 0 A6 B4 0 A5 B6 5 A6 B7 6 A5 B6 9 A7 B7 6 A5 B6 9 A9 B1 8 A9 B1 9 A10 B11 1 Wells V 0 C4 C4 0 C5 C6	atLess Me 0.055 0.078 0.051 0.315 1.371 0.934 1.253 1.253 1.253 1.253 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.475 1.294 Imm	on Values 3 0.067 1.084 0.915 0.945 1.244 1.295 1.475 1.381 ★ [fω★] [fw mg/ml) ean Values 0.262	0.018 24 0.391 35 0.021 3 0.065 6 0.013 1 0.004 0 0.000 0 0.124 9 0.001 0 0.001 0 0.263 2	2 96 3 37 4 4 5 94 5 94 5 95 5 95 1 1 1 5 95 1 0 5 95 1 0 5 95 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0	1156 5000 12000 1791 1001 1011 10241 1042 1044 1044 1044 1050 1100	BL1 BL2 BL3 BL4 BL4 BL4 BL4 BL5 BL7 BL5 IC50 = 0.9 MIC = 1.995 MIC = 1.995 Sample C BL1 BL1 BL2	2.500 1.250 0.525 0.313 0.156 0.039 0.039 0.039 0.039 0.039 0.039 1.250 1.250	Wells X E4 C F4 C E5 C F6 C F7 C E9 F7 F7 C E9 F10 F10 F11 X F11 X F11 X F11 X F11	alues Me 0.021 0.024 0.025	an Values 5 0.025 0.031 0.855 1.245 1.611 1.355 1.454 (m)+ (for+) 4 (mg/m) Mean Values 0.742	0.0049 3 0.047 0.184 2 0.468 6 0.0468 6 0.010 0.028 0.026 0.026 500 2 0.000 2 0.000 2 0.264	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.8 4.1 0.7 2.0 1.8 5 5 6.6	98 62 97 633 4 4 60 4 8 68 3 3 5 6 4 7 1 6 9 9 004 4 8 68 3 3 5 6 4 7 1 6 9 9 004 4 4 6 4 7 1 6 9 9 004 4 4 6 4 7 1 6 9 9 004 4 7 1 6 9 9 004 7 1 6 9 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	BL1 BL2 BL2 BL4 BL5 BL5 BL5 BL5 BL5 BL5 C50 = 1.41 MIC = 2.233 F III Grou Sample C BL1 BL2	2.50 1.25 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2 concentration 2.50 1.25	1 Weills V 0 A4 BA 0 A4 BA 0 A6 A 2 B7 C 3 B7 B 6 A5 B 9 A10 D 0 A4 O 0 B11 O 1 O C6 5 C6 C6	alus Me 0.056 0.078 0.058	or Values 2 0.067 1.094 0.915 0.945 1.244 1.295 1.475 1.391 (for)*) [for mg/ml) san Values 0.262 1.125	0.018 24 0.391 25 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9 Std Dev. C 0.001 0 0.253 22 0.025	2 80 3 3 4 4 3 3 4 4 5 3 3 4 6 3 3 4 7 3 4 4 7 3 7 4 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1166 2209 2209 961 961 961 101 683 244 206 881 4471 9851 4471 9429 4421 9429 9420 9420 9420 9420 9420 9420 9420	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL1 BL1 BL2 BL3 BL4	2.600 1.260 0.625 0.313 0.155 0.075 0.035 0.020 1.4 0.020 1.4 0.020 1.260 1.260 0.255 0.020 0.313 0.020 0.020 0.020 0.020 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.025 0.035 0.025 0.025 0.035 0.025	Wells V/ E4 C F4 C F4 C F4 C F6 C F6 C F8 C F8 C F8 C F8 C F8 C F9 C F10 F11 Wells N G44 G4 H65 G66 H66 H7	Group# Values Values Values Values Values 0.021 0.021 0.024 0.024 0.024 0.026 0.0	an Values 5 0.025 0.021 0.855 1.248 1.811 1.385 1.464 (IIII) (IIIII) (IIII) (0.047 0.047 0.047 0.047 0.046 0.056 0.046 0.056 0.010 0.256 0.026 0.026 \$60,260 0.000 \$0.026 0.000 \$0.000 0.000 \$0.000 0.000 \$0.000 0.000 \$0.000 0.000 \$0.000 0.000 \$0.000 0.000	2.7 5.7 5.4 4.1 0.7 2.0 1.8 7 4.2 2.0 1.8 7 5 5 6.6 1 11.1	98 62 97,63 34,800 44,80 34,800 34,800 44,00 5,000 44,000 44,0000 44,0000 44,0000 44,0000 44,0000 44,00000000
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7	2 800 1 28 0 82 0 31 0 18 0 07 0 03 0 02 4 up#2 0 ncentration 2 80 0 82 0 82 0 0 0 0 0 0 0 0	Weilis V 0 A4 0 A4 0 A4 0 A6 0 A6 6 A6 8 A6 9 A10 0 A11 811 B11 0 A4 0 A4 0 C4 0 C4 0 C4 0 C5 5 C6 0 C5 5 C6 0 C5 5 C67	alus Me 0.058 0.058 0.076 0.058	D Values S O 007 1.094 0.915 0.945 1.295 1.475 1.291 1.291 1.281 1.281 0.282 1.475 1.281 0.282 1.128 1.284 1.285 1.475 1.281 1.842	0.018 24 0.391 25 0.021 2 0.065 5 0.013 1 0.004 0 0.000 0 0.124 9 <u>516 Dev. C</u> 0.263 2 0.025 2 0.025 2 0.048 1	2 80 3 3 4 4 3 3 4 4 5 3 3 4 6 3 3 4 7 3 4 4 7 3 7 4 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1166 7.661 7.61 7.61 9.62 9.62 9.62 2.41 2.001 2.41 2.001 2.41 2.001 2.41 2.001 3.41 1.150	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL1 BL1 BL1 BL2 BL1 BL2 BL4 BL4 BL4 BL4	2 800 1 280 0 818 0 318 0 318 0 075 0 038 0 020 114 2 800 1 280 0 818 0 918 0 91	Wells X F4 C F4 C F4 C F4 C F6 C F6 C F6 C F7 F7 F8 C F8 C F8 C F8 C F8 C F8 C F9 C F11 C G4 G G4 G G4 G G4 G G7 G H6 G H6 G	group# group# 0.01 0.021 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.024 0.025 0.724 0.886 1.280 1.280 1.280 1.280 0.018<	an Values S 0.023 0.031 0.886 1.346 1.464 (me) [so+] (4 (mg/ml) Mean Value 0.0742 1.285 1.285 1.464 (me) [so+] 0.012 0.0742 1.285 1.285 1.484	0 0.047 0 0.184 0 0.184 0 0.468 0 0.184 0 0.468 0 0.665 0 0.10 0 0.28 0 0.26 \$ 0.002 \$ 0.026 \$ 0.026 \$ 0.026	2.7 5.7 5.4 4.1 0.7 2.0 1.8 4.2 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5	98 62 97 635 44 60 45 65 3 25 74 9 004 4 8 65 9 004 4 8 65 9 004 4 7 1 69 9 004 4 4 65 9 004 9 0004 9 004 9 0000000000
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL7 BL1 BL2 BL3 BL4 BL6 BL4 BL6	2.60 1.26 0.62 0.31 0.16 0.07 0.03 0.02 4 up#2 2.60 1.26 0.62 0.62 0.31 0.15	Wells V 0 A4 0 A4 0 A4 0 A4 0 A4 0 B5 0 B6 0 B6 0 B7 0 A4 0 A4 0 A4 0 C4 0 C5 2 C7 2 C7 2 C7 2 C7	atus Ms 0.056 0.076 0.076 0.076 0.056 0.076 0.056 0.00	N values (0 067 1.094 0.915 0.945 1.244 1.265 1.475 1.281 (1.475 1.281 (1.475 1.281 (1.475 1.281 (1.426 1.126 1.126 1.126 1.126 1.126 1.126 1.424 1.126 1.424 1.126 1.424 1.126 1.444 1.444 1.444	0.016 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9 Std Dev. C1 0.001 1 0.263 2 0.026 2 0.026 2 0.026 2 0.026 2	2 90 3 17 4 4 3 27 4 4 4 3 4 3 4 3 4 3 4 3 4 3 4 3	11000 11000 111000	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL1 BL1 BL2 BL3 BL4	2.600 1.260 0.625 0.313 0.155 0.075 0.035 0.020 1.4 0.020 1.4 0.020 1.260 1.260 0.255 0.020 0.313 0.020 0.020 0.020 0.020 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.035 0.025 0.025 0.035 0.025 0.025 0.035 0.025	Wells V/ E4 C F4 C F4 C F4 C F4 C F6 C F7 C F7 C Walls V Walls V Walls C G0 G G0 G G0 G G0 G G0 G G0 G	state in the second secon	an Values 5 0.025 0.021 0.855 1.248 1.811 1.385 1.464 (IIII) (IIIII) (IIII) (0 0.047 0 0.184 0 0.184 0 0.468 0 0.184 0 0.468 0 0.665 0 0.10 0 0.28 0 0.26 \$ 0.002 \$ 0.026 \$ 0.026 \$ 0.026	2.7 5.7 5.4 4.1 0.7 2.0 1.8 4.2 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5	98 62 97 638 44 609 24 7 1 62 24 7 1
	BL1 BL2 BL3 BL4 BL6 BL6 BL6 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7 BL7	2 800 1 28 0 82 0 31 0 18 0 07 0 03 0 02 4 up#2 0 ncentration 2 80 0 82 0 82 0 0 0 0 0 0 0 0 0 0 0 0 0	Wells V 0 A4 0 A4 0 A4 0 A4 0 A4 0 B5 0 B6 0 B6 0 B7 0 A4 0 A4 0 A4 0 C4 0 C5 3 C7 3 C7 6 C6 0 C5	alus Me 0.058 0.058 0.076 0.058	values 5 vore vo	0.016 24 0.391 35 0.021 3 0.055 5 0.013 1 0.004 0 0.000 0 0.124 9 Std Dev. C1 0.001 1 0.263 2 0.026 2 0.026 2 0.026 2 0.026 2	2 6 6 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	11000 11000 11000 1	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL1 BL1 BL1 BL2 BL1 BL2 BL4 BL4 BL4 BL4	2 800 1 280 0 818 0 318 0 318 0 075 0 038 0 020 114 2 800 1 280 0 818 0 918 0 91	Wells V. Wells V. FF C FF C FF C E C FF C E C F7 C E F7 C E F1 C F11 C F11 C F11 C G0 F11 G0 G H6 G G1 G G1 G G1 G1 G1 G1 G1 G1	group# group# values	an Values S 0.023 0.031 0.886 1.346 1.464 (me) [so+] (4 (mg/ml) Mean Value 0.0742 1.285 1.285 1.464 (me) [so+] 0.012 0.0742 1.285 1.285 1.484	0.047 0.047 0.047 0.184 2 0.468 6 0.056 0.026 0.026 0.026 0.026 0.026 2 0.004 2 0.068 2 0.068 2 0.068 0.0580000000000	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.0 4.27 2.0 1.0 5.5 6.6 5.6 6.6 1.1,1 1.1,1 2.4,1 7.2,1	98 62 97 633 44 669 44 669 33 87 7 1 69 9 0 0 4 3 8 7 4 4 4 6 9 0 0 4 9 0 0 1 0 0 1 0 0 1 0 0 1 0 1 0 0 1 0 0 1 0 0 10 0 0 10 0 0 0
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL7 BL1 BL2 BL3 BL4 BL6 BL4 BL6	2.60 1.26 0.62 0.31 0.16 0.07 0.03 0.02 4 up#2 2.60 1.26 0.62 0.62 0.31 0.15	N Wells V 0 A4 0 0 A4 0 0 A4 0 0 A5 0 0 B5 B5 5 A6 0 0 B7 0 0 A11 0 0 A11 0 0 C4 0 0 C4 0 0 C4 0 0 C5 0 0	autor Ms 0.058 0.076 0.058 0.076 0.058 0.076 0.058 0.076 0.890 0.890 0.890 0.890 0.890 1.294 1.294 1.294 1.294 1.294 0.201 0.201 0.204 1.294 1.294 1.294 1.294 1.294 1.294 1.294 1.294 1.294 1.294 1.294	N values (0 067 1.094 0.915 0.945 1.244 1.265 1.475 1.281 (1.475 1.281 (1.475 1.281 (1.475 1.281 (1.426 1.126 1.126 1.126 1.126 1.126 1.126 1.424 1.126 1.424 1.126 1.424 1.126 1.444 1.444 1.444	0.016 24 0.391 26 0.031 3 0.066 6 0.013 1 0.004 0 0.000 0 0.124 9 Std Dev. C 0.001 1 0.001 1 0.026 2 0.026 2 0.026 3 0.026 3 0.027 3 0.026 3 0.027 3 0	2 66 2 66 3 6 37 3 7 4 4 3 3 4 4 4 3 3 4 4 4 3 3 3 7 4 4 4 3 7 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	1100 1100 1100 111000 111000 111000 111000 111000 111000 111000 11100	BL1 BL2 BL2 BL4 BL4 BL6 BL6 BL6 BL6 BL6 BL6 BL6 BL6 BL6 BL1 BL2 BL2 BL2 BL4 BL6 BL6 BL7	2.600 1.260 0.625 0.313 0.152 0.075 0.035 0.020 1.4 0.020 1.260 0.55 0.020 1.260 0.655 0.313 0.166 0.075 0.020 0.015 0.020 0.015 0.020 0.015 0.020	Wells X E4 C FE C FE C FE C FE C E8 C E9 F F7 C E9 F F1 C E10 F C4 C H0 C H2 C H3 C H3 C H4 C In	Use Ms 0.021 0.024 0.024 0.024 0.027 0.024 0.027 0.024 0.027 0.024 0.027 0.024 0.027 0.024 0.027 0.724 0.020 0.027 1.310 1.310 1.311 1.416 1.416 1.416 1.420 0.016 0.930 0.932 0.932 1.422 1.223 1.423 1.223 1.423 1.223 1.423	an Values S 0.025 0.031 0.035 1.248 1.248 1.4511 1.365 1.464 4 (mg/m) Mean Value 0.012 0.742 1.268	0.0047 0.047 0.047 0.047 0.048 0 0.048 0 0.048 0 0.048 0 0.048 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.047 0 0.048 0 0.049 0	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.8 	99 63 97 63 94 4 66 4 4 66 2 2 6 74 2 2 6 74 3 3 64 4 4 55 3 3 64 4 55 3 56 4 57 3 56 4 57 3 56 4 57 3 56 4 57 3 56 4 57 56 56 56 56 57 56 56 57 56 56 57 56 56 56 56 56 56 56 56 56 56 56 56 56
	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL8 C50 = 1.41 MiC = 2.233 BL1 BL1 BL2 BL1 BL2 BL4 BL4 BL4 BL6 BL6 BL6	2.60 0.62 0.31 0.15 0.07 0.03 0.02 4 up#2 0.000 1.26 0.62 0.31 0.31 0.62 0.31 0.62 0.31 0.65 0.62 0.31 0.65 0.	N No.119 V 0 A4 0 A4 0 0.4 0 C4 0 0.4 0 C4 0 0.4 0 C4 0 0.4 0.4 0 0 0.4 0.4 0 0 0.4 0.4 0 0 0.4 0.4 0 0 0.4 0.4 0 0 0.4 0.4 0.4 0 0.4 0.4 0.4 0 0.4 0.4 0.4	atus Ms 0 058 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Noices Noice	0.016 24 0.391 36 0.021 3 0.025 3 0.025 5 0.013 1 0.004 0 0.000 0 0.124 9 Std Dev. C 0.004 1 0.026 2 0.026 2 0.026 3 0.046	2 900 3 100 5 1000 5 1000 5 1000 5 1000 5 1000 5 1000 5 1000 5 1000 5	11000 111000 111000 111000 111000 11100 11100 11100 11100 11100	BL1 BL2 BL3 BL4 BL6 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL6 BL7 BL7 BL2 BL1 BL2 BL2 BL2 BL2 BL2 BL2 BL2 BL2 BL2 BL2	2 600 1 200 0 625 0 313 0 166 0 076 0 025 0 025 1200 1 200 1 200 0 625 0 315 0 120 0 0 120 0 0 0 0 0 0 0 0 0	Wells X E4 C FE C FE C FE C FE C E8 C E9 F F7 C E9 F F1 C E10 F C4 C H0 C H2 C H3 C H3 C H4 C In	group# group# values	an Values S 0.021 0.021 0.865 1.248 1.248 1.348 1.454 1.355 1.454 4 (mg/ml) Mean Values 0.742 1.263	0.0047 0.047 0.047 0.047 0.048 0 0.048 0 0.048 0 0.048 0 0.048 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.028 0 0.047 0 0.048 0 0.049 0	2.7 5.7 1.6 3.4 4.1 0.7 2.0 1.8 	%innibition 97 98 34 35 36 4 350 360 360 350 360 360 4 4 4 4 4 4 4

Fig9. Different values of different drugs against V. cholerae obtained from SpectramaxPlus 384 molecular Device USA.

V. CONCLUSION

It can be concluded from the present study that all the three Gymnospermous essential oil have some synergistic activity against V. cholerae. It was first time in our best knowledge that essential oil of P. roxburghii shows remarkable effect against V. cholerae. Chir oil shows remarkable efficiency over bald cypress oil and white cedar oil against bacteria. Pinus oil shows great efficiency against V. cholerae and other microbes (25). The components (terpenes) of essential oil of P. roxburghii needles are highly active against microbes. As this oil significantly inhibited the growth of certain bacteria tested. The main oil component of P. roxburghii essential oil are monoterpene and sequiterpene hydrocarbons and their derivatives. These derivatives act as antibacterial and antifungal



International Journal for Research in Applied Science & Engineering Technology (IJRASET) ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887 Volume 6 Issue III, March 2018- Available at www.ijraset.com

substance, the most well-known of which being terpenes and phenolics in general (26). The essential oil from the leaves and cones of bald cypress trees grown exhibited potent antimicrobial activities against bacteria (27). Essential oils from needles and foliages of these gymnosperms plants viz., P. roxburghii, T. distichum and T. occidentalis, exhibit great potential eco-friendly, non-toxic, cost-efficient and antibacterial herbal formulations.

VI. ACKNOWLEDGEMENTS

I would like to give special thanks to HOD Botany and Director of Biological Product Laboratory (BPL), Department of Botany, University Of Allahabad, for providing me laboratory facilities; to Dr. Anand Pandey, Dr. Rajesh kumar and Mr. Sharad Kumar Tripathi for their valuable suggestions.

REFERENCES

- [1] Press JR, Shrestha KK, Sutton DA. Annotated Checklist of the Flowering Plants of Nepal. The Natural History Museum. 2000
- [2] Wu Z, Raven PH. Flora of China. Vol. 4. Beijing Science Press; 1999.
- [3] Gewali MB. Institute of Natural Medicine. Japan: University of Toyama; 2008. Aspects of Traditional Medicine in Nepal; pp. 19–20.
- [4] Vallejo MCN, Evandro A, Sergio ALM. Volatile wood oils of the Brazilian Pinus caribaea var. hondurensis and Spanish Pinus pinaster var. mediterranea. J Braz Chem Soc. 1994;5:107–112.
- [5] Asta J, Jurgita S, Aida S, Eugenija K. Characteristics of essential oil composition in the needles of young scots pine (Pinus sylvestris L.) stands growing along and ariel ammonia gradient. Chemija. 2006;17:67–73.
- [6] Hassan A, Amjid I. Gas chromatography-mass spectrometric studies of essential oil of Pinus roxburghaii stems and their antibacterial and antifungal activities. J Med Plant Res. 2009;3:670–3.
- [7] Ravikant Singh, Ashutosh Pathak, Anupam Dikshit, Rohit Kumar Mishra, "COMPARISION OF BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL OF THREE GYMNOSPERMS AGAINST SALMONELLA TYPHIMURIUM", INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT), ISSN:2320-2882, Volume.6, Issue 1, Page No pp.1420-1426, February 2018, Available at : http://www.ijcrt.org/IJCRT1802181.pdf
- [8] Sidiqui KM, Iqbal M and Mohammad A. Forest ecosystem climate change impact assessment and adaptation strategies for Pakistan. Clim. Res., 1999; 12: 195-203.
- [9] Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allurred Publishing Corporation, pp. 1–456.
- [10] El Tantawy ME, El Sakhawy FS, El Sohly MA, Ross SA (1999): Chemical composition and biological activity of the essential oil of the fruit of Taxodium distichum. L. growing in Egypt. J Essent Oil Res 11: 386–392 (and references cited therein).
- [11] Flamini G, Luigi C, Morelli I (2000): Investigation of the essential oil of feminine cones, leaves and branches of Taxodium distichum. from Italy. J Essent Oil Res 12: 310–312.
- [12] Geiger H, de Groot-Pfleiderer W (1979): Die flavon- und flavonolglykoside von Taxodium distichum.. Phytochemistry 18: 1709–1710
- [13] Adams RP (2001): Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Carol Stream, IL, Allurred Publishing Corporation, pp. 1–456.
- [14] Denny, G.C. Evaluation of selected provenances of Taxodium distichum for drought, alkalinity and salinity tolerance. PhD Thesis, A&M University: Texas, 2007.
- [15] Kusumoto, N.; Ashitani, T.; Murayama, T.; Ogiyama, K.; Takahashi, K. Antifungal abietane-type diterpenes from the cones of Taxodium distichum Rich. J. Chem. Ecol., 2010, 36 (12), 1381-1386.
- [16] Peng, D.; Wang, X.-Q. Reticulate evolution in Thuja inferred from multiple gene sequences: Implications for the study of biogeographical disjunction between easren Asia and North America. Mol. Phylogenet. Evol. 2008, 47, 1190–1202.
- [17] Kamden, P.D.; Hanover, J.W. "Inter-Tree variation of essential oil composition of Thuja occidentalis L." J. Essent. Oil Res. 1993, 5, 279–282.
- [18] Duke, J.A. Handbook of Medicinal Herbs; CRC Press, Inc.: Boca Raton, FL, USA, 1985.
- [19] FAO (Food and Agriculture Organization of the United Nations). Non-Wood Forest Products from Conifers. Chapter 7-Essential Oils; FAO: Rome, Italy, 1995; Vol. 12, p. 86.
- [20] Von Rudloff, E. Gas-liquid chromatography of terpenes VI. The volatile oil of Thuja plicata Donn. Phytochemistry 1962, 1, 195–202.
- [21] Keita, M.S.; Vincent, Ch.; Schmidt, J.-P.; Arnasson, J.T. Insecticidal effects of Thuja occidentalis (Cupressaceae) essential oil on Callosobruchus maculates (Coleoptera: Bruchidae). Can. J. Plant Sci. 2001, 81, 173–177.
- [22] Isiaka A. Ogunwande, Nureni O. Olawore, Oluranti O. Ogunmola, Tameka M. Walker, Jennifer M. Schmidt & William N. Setzer (2007) Cytotoxic Effects of Taxodium distichum. Oils, Pharmaceutical Biology, 45:2, 106-110
- [23] McFarland J. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 1907; 14:1176-8.
- [24] Satyal P, Paudel P, Lamichhane B, Setzer WN. Volatile constituents and biological activities of the leaf essential of Jasminum mesnyi growing in Nepal. J Chem Pharm Res. 2012;4:437–9.
- [25] Von Rudloff, E. Volatile Leaf Oil Analysis in Chemosystematic Studies of North American Conifers. Biochem. Sys. Ecol. 1975, 2, 131–167.
- [26] Gulten, T. G., Branden, A.N., Sahika, A. G., and Mehmat, K. (2012). "Antimicrobial activity of oregano oil on Iceburg lettuce with different attachment conditions." J. Food Sci 77(7), 412-415.
- [27] EI Tantawy, M.E.; El Sakhawy, F.S.; EI Sow, M.; Ross, S.A. Chemical Composition and Biological Activity of the Essential Oil of the Fruit of Taxodium distichurn L. Rich Growing in Egypt. J. Essent. Oil Res., 1999, 11, 386-392.











45.98



IMPACT FACTOR: 7.129







INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 🕓 (24*7 Support on Whatsapp)