

APPENDIX A

Materials and Equipments

1. Equipments

Micro pipette, Microscope, Incubator, Refrigerator, Digital Balance, Laminar Airflow, Water Bath Shaker, Autoclave, Hot air oven.

2. Materials

Measuring cylinder, Glass Slides, Cover slips, Petri plates, Inoculating loop, Beakers, Spatula, Wire gauge, Test tubes, Screw cap tubes, Pipettes, Glass rods, Filter paper, Funnel, Conical flasks etc.

3. Chemicals

Gram's iodine, Crystal violet, DMSO, Barium chloride, Conc. H_2SO_4 , Conc. HCl, Lead acetate, Potassium iodide, HNO_3 , Ferric chloride, Chloroform, Mercuric iodide, Saffranin, LPCB etc.

4. Media

Nutrient Broth,(NB), Nutrient Agar (NA), Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), MacConkey Agar etc.

5. Antibiotic discs

Antibiotics disc of Ampicillin, Amoxicillin, Chloramphenicol, Gentamycin, Kanamycin and Tetracycline as well as Chloramphenicol in powder form.

Appendix B

Test Organisms

S.N.	Plant pathogen	Sample	Disease	Confirmation	
1	Bacteria	<i>X. oryzae</i> pv <i>oryzae</i>	Rice	BLB	Koch postulate
		<i>X. axonopodis</i> pv <i>citri</i>	Lemon	Citrus cancker	Koch Postulate
2	Fungi	<i>F. oxysporum</i> f.sp. <i>cubense</i>	Banana leaf	Fusarium wilt	Koch Postulate
		<i>B. oryzae</i>	Rice	Brown spot	Koch Postulate

Biochemical test of Bacterial isolates

Plant Pathogenic Bacteria	Microscopic		Biochemical Tests											
	Gram stain	Cat	Oxi	H ₂ S	M	I	MR	VP	C	TSI				
										L	D	S	C0 ₂	
<i>X. oryzae</i> pv <i>oryzae</i>	-	Rod	+	-	-	+	-	+	-	+	+	+	+	+
<i>X.</i> <i>axonopodis</i> pv <i>citri</i>	-	Rod	+	-	-	+	-	+	-	+	+	+	+	+

Cat = Catalase, Oxi= Oxidase, M= Motility, I=Indole, C= Citrate,
L=Lactose, D =Dextrose, S=Sucrose.

APPENDIX C

The collection site and Parts of selected plants for the study

S.N.	Botanical Name	Common Name	Part used	Place of collection	Date of collection
1	<i>Azadirachta indica</i>	Neem	Leaf	Dharan	Dec,2018
2	<i>Allium sativum</i>	Garlic	Bulb	Dharan	Nov,2018
3	<i>Capsicum annum</i>	Chili	Fruit	Dharan	Nov,2018

APPENDIX D

Zone Size Interpretative Chart for Antibiotic Sensitivity Test of Selected Antibiotics Discs

S. N.	Antibiotic disc	Code	Disc Potency (μg)	Sensitivity		Resistant \geq mm
				\leq mm	mm	
1	Ampicillin	AMP	10	17	14-16	13
2	Amoxicillin	AMX	30	18	14-17	13
3	Chloramphenicol	C	30	18	13-17	12
4	Gentamycin	GEN	10	15	13-14	12
5	Kanamycin	K	30	18	14-17	13
6	Tetracyclin	TE	30	19	15-18	14

Source: Product Information Guide, HiMedia Laboratories Pvt. Ltd, Mumbai, India

APPENDIX E

Statistical Analysis

Neem extract vs ZOI Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.000 ^a	1	.014		
Continuity Correction ^b	2.667	1	.102		
Likelihood Ratio	8.318	1	.004		
Fisher's Exact Test				.100	.050
N of Valid Cases	6				

a. 4 cells (100.0%) have expected count less than 5. The minimum expected count is 1.50.

b. Computed only for a 2x2 table

Test is statistically significant, $p=0.14$ ($p<0.05$)

H_0 : There is no significant difference between Treatment and Antimicrobial Activity.

H_1 : There is significant difference between Treatment and Antimicrobial Activity.

Conclusion: $p<0.05$. So we fail to accept Null Hypothesis. There is significant difference in treatment with Case and control

Garlic Extract (case and control)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.000 ^a	1	.005		
Continuity Correction ^b	4.500	1	.034		
Likelihood Ratio	11.090	1	.001		
Fisher's Exact Test				.029	.014
N of Valid Cases	8				

Test is statistically significant

Chillis extract (Case and Control)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.000 ^a	1	.014		
Continuity Correction ^b	2.667	1	.102		
Likelihood Ratio	8.318	1	.004		
Fisher's Exact Test				.100	.050
N of Valid Cases	6				

a. 4 cells (100.0%) have expected count less than 5. The minimum expected count is 1.50.

b. Computed only for a 2x2 table

Antibiotics AST vs ZOI

ANOVA

ZOI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	509.667	5	101.933	35.976	.000
Within Groups	17.000	6	2.833		
Total	526.667	11			

Analysis of Variance among Test fungal species with MIC

ANOVA

MIC fungi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	114843750.000	1	114843750.000	3.706	.127
Within Groups	123958333.333	4	30989583.333		
Total	238802083.333	5			

Analysis of Variance among different Test samples with MIC

ANOVA

MIC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14648437.500	1	14648437.500	1.510	.286
Within Groups	38802083.333	4	9700520.833		
Total	53450520.833	5			

ZOI of plant extract (aqueous) against bacterial plant pathogens.

ANOVA

Score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.213	1	28.213	20.619	.001
Within Groups	13.683	10	1.368		
Total	41.897	11			

Test is statistically significant.

MIC against Test fungi

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	500.667	6	83.445	35.976	0.047
Within Groups	26.000	5	13		
Total	526.667	11			

Test is statistically significant.

ANOVA

MIC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	400.667	1	400.667	35.976	0.047
Within Groups	24.000	4	6.00		
Total	424.667	5			

Test is statistically significant.