APPENDICES

Appendices

Appendix A

Ethanolic extract yield

No	Ethanolic Extract	Yield (%)
1	*Andrographis paniculata*	11.28
2	*Curcuma mangga* (Turmeric)	12.75
3	*Carica papaya* leaf (Papaya)	3.85
4	*Allium sativum* (Garlic)	11.05
5	*Cymbopogon citratus* (Lemon grass)	5.1
6	Fermented Bean	14.62
7	Functional Food Paste	29.81
8	Fermented Extract	44.65
9	Fermented vinegar with lactic acid bacteria	19.81

Appendix B

Protein/peptide extract yield

No	Plant samples (Protein/Peptide extracts)	Variant 1	Variant 2	Variant 3	Mean (mg/g)	SD
1	*Andrographis paniculata*	1.7622	1.7622	1.7799	1.7681	0.0102
2	*Curcuma mangga* (Turmeric)	1.3641	1.3626	1.3626	1.3631	0.0009
3	*Carica papaya* leaf (Papaya)	1.3514	0.9176	1.3596	1.2095	0.2528
4	*Allium sativum* (Garlic)	1.7644	1.9499	1.7579	1.8241	0.1090
5	*Cymbopogon citratus* (Lemon grass)	1.3536	1.3725	1.3649	1.3637	0.0095
6	*Zingiber officinale* (Ginger)	1.5651	1.5513	1.5488	1.5551	0.0088
7	*Beta vulgaris* (Beet root)	0.8301	0.8118	0.8005	0.8141	0.0149
8	*Allium cepa* (Big onion)	1.2019	1.2208	1.2110	1.2112	0.0094
9	*Allium cepa* (Small onion)	1.0634	1.0551	1.0569	1.0585	0.0044
10	Momordica charantia (Bitter gourd)	0.6459	0.6589	0.6893	0.6647	0.0223
11	*Momordica charantia* seeds	0.4890	0.4890	0.4975	0.4918	0.0049
12	*Agaricus bisporus* stem (Button mushroom)	0.8558	0.8408	0.8425	0.8463	0.0082
13	*Agaricus bisporus* fruiting body	0.5150	0.5139	0.5133	0.5141	0.0009

mg/g : milligram per gram of plant tissues

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.140	12	.595	100.432	.000
Within Groups	.154	26	.006		
Total	7.294	38			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
11.00	3	.4918							
13.00	3	.5141							
10.00	3		.6647						
7.00	3			.8141					
12.00	3			.8464					
9.00	3				1.0585				
3.00	3					1.2095			
8.00	3					1.2112			
2.00	3						1.3631		
5.00	3						1.3637		
6.00	3							1.5551	
1.00	3								1.7681

4.00	3								1.8241
Sig.		.726	1.000	.612	1.000	.979	.993	1.000	.381

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix C

16S rRNA sequences obtained from gene sequencing

Bacillus cereus

GGGAGAGCGCGGCTAACTGCAGTCGAGCGATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGC
CCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCAC
TTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCA
CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGT
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cgtcaaatcatcatgccccttatgacctgggctacacacgtgctacaatggacggtacaaagagctgcaagaccgcgaggtggagctaatctcataaaaccgttctcagttcggattgtaggctgcaactcgcctacatgaagctggaatcgct
agtaatcgcggatcagcatgccgcggtgaatacgttcccgggccttgtcacaccgcccgtcacaccacgagagtttgtaacacccgaagtcggtggggtaacctttgagccagccgctaagtgacgaagctt

Staphylococcus aureus

GGAGCGCGGCTATCTGCAGTCGAGCGACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTA
TAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTA
TAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTG
GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGA
TGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACG
GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTC
TGATGTGAAAGCCCACGGCTCAACCGTGGAGggtcattggaaactggaaaacttgagtgcagaagaggaaagtggaattccatgtgtagcggtgaaatgcgcagagatatggaggaacaccagtggcgaag
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Escherichia coli

GGGGGAGCGCAGCTACCTGCAGTCGAAGGTACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTG
CCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGG
ATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGA
AGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCT
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cgtcaagtcatcatggcccttacgaccagggctacacacgtgctacaatggcgcatacaaagagaagcgacctcgcgagagcaagcggacctcataaagtgcgtcgtagtccggattggagtctgcaactcgactccatgaagtcggaatc
gctagtaatcgtggatcagaatgccacggtgaatacgttcccgggccttgtacacaccgcccgtcacaccatgggagtgggtgcaaaagaagtaggtagctaaccttcggagggcgctaccacttgatcgcggg

Pseudomonas aeruginosa

TTTTCAGGGCGCTACCTGCAGTCGAGCGGATGAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTA
GTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCT
AGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCG
GATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGC
CAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCC
CCGGGCTCAACCTGggaactgcatccaaaactactgagctagagtacggtagagggtggtggaatttcctgtgtagcggtgaaatgcgtagatataggaaggaacaccagtggcgaaggcgaccacctggactgatactgac
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ccttacggccagggctacacacgtgctacaatggtcggtacaaagggttgccaagccgcgaggtggagctaatcccataaaaccgatcgtagtccggatcgcagtctgcaactcgactgcgtgaagtcggaatcgctagtaatcgtgaatc
agaatgtcacggtgaatacgttcccgggccttgtacacaccgcccgtcacaccatgggagtgggtgctccagaagtagctagtcaaccgcaaggggacggtaccacgaggtcgcggg

Appendix D

SOD activity of ethanolic extracts

No	Ethanolic Extract	Variant 1	Variant 2	Variant 3	Mean (%)	SD
1	Andrographis paniculata	81	80.1	80.13	80.4	0.51
2	Curcuma mangga (Turmeric)	89.22	89.99	88.78	89.3	0.61
3	Carica papaya leaf (Papaya)	80.62	81.12	80.08	80.6	0.52
4	Allium sativum (Garlic)	92.99	93.55	92.41	93.0	0.57
5	Cymbopogon citratus (Lemon grass)	91.34	92.45	90.85	91.5	0.82
6	Fermented Bean	88.22	86.89	86.55	87.2	0.88
7	Functional Food Paste	35.4	36	35.61	35.7	0.30
8	Fermented Extract	73	72.46	74.14	73.2	0.86
9	Fermented vinegar with lactic acid bacteria	62.47	64.1	61.63	62.7	1.26
	(+Control) Vitamin C (1mg/ml)	99.8	98.14	97.11	98.4	1.36
	(+Control) Vitamin C (10mg/ml)	98.89	99.41	99.3	99.2	0.27

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10334.968	10	1033.497	1622.784	.000
Within Groups	14.011	22	.637		
Total	10348.979	32			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
7.00	3	35.6700								
9.00	3		62.7333							
8.00	3			73.2000						
1.00	3				80.4100					
3.00	3				80.6067					
6.00	3					87.2200				
2.00	3						89.3300			
5.00	3							91.5467		
4.00	3								92.9833	
10.00	3									98.3500
11.00	3									99.2000
Sig.		1.000	1.000	1.000	.766	1.000	1.000	1.000	1.000	.206

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix E

SOD activity of protein/peptide extracts

No	Protein/Peptide Extract	Variant 1	Variant 2	Variant 3	Mean (%)	SD
1	*Andrographis paniculata*	84.5	83.94	84.55	84.33	0.34
2	*Curcuma mangga* (Turmeric)	84.12	84.92	84.16	84.4	0.45
3	*Carica papaya* leaf (Papaya)	80.47	84	84.2	82.89	2.10
4	*Allium sativum* (Garlic)	91.8	94.2	92.19	92.73	1.29
5	*Cymbopogon citratus* (Lemon grass)	78.87	79.97	81.79	80.21	1.47
6	*Zingiber officinale* (Ginger)	81.27	86.42	82.12	83.27	2.76
7	*Beta vulgaris* (Beet root)	78.98	77.14	78.93	78.35	1.05
8	*Allium cepa* (Big onion)	75.9	73.1	73.42	74.14	1.53
9	*Allium cepa* (Small onion)	64.1	68.88	70.54	67.84	3.34
10	Momordica charantia (Bitter gourd)	85.9	87	86.27	86.39	0.56
11	*Momordica charantia* seeds	93.21	95.18	92.44	93.61	1.41
12	*Agaricus bisporus* stem (Button mushroom)	86.24	85.95	85.75	85.98	0.25
13	*Agaricus bisporus* fruiting body	63.56	62.22	61.63	62.47	0.99
	(+Control) Vitamin C (1mg/ml)	99.8	98.14	97.11	98.35	1.36
	(+Control) Vitamin C (10mg/ml)	98.89	99.41	99.3	99.2	0.27

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4449.339	14	317.810	132.166	.000
Within Groups	72.139	30	2.405		
Total	4521.478	44			

Duncan[a]

		Subset for alpha = 0.05								
VAR00001	N	1	2	3	4	5	6	7	8	9
13.00	3	62.4700								
9.00	3		67.8400							
8.00	3			74.1400						
7.00	3				78.3500					
5.00	3				80.2100					
3.00	3					82.8900				
6.00	3					83.2700	83.2700			
1.00	3					84.3300	84.3300	84.3300		
2.00	3					84.4000	84.4000	84.4000		
12.00	3						85.9800	85.9800		
10.00	3							86.3900		
4.00	3								92.7300	

11.00	3								93.6100	
14.00	3									98.3500
15.00	3									99.2000
Sig.		1.000	1.000	1.000	.152	.286	.058	.147	.492	.507

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix F

Hemolysis activity of ethanolic extracts

No	Ethanolic Extract	Variant 1 (%)	Variant 2 (%)	Variant 3 (%)	Mean (%)	SD
1	*Andrographis paniculata*	19.43	19.9	19.02	19.45	0.4403
2	*Curcuma mangga* (Turmeric)	22.22	20.42	21.71	21.45	0.9277
3	*Carica papaya* leaf (Papaya)	33.21	29.12	31.48	31.27	2.0531
4	*Allium sativum* (Garlic)	28.69	28.28	28.28	28.42	0.2333
5	*Cymbopogon citratus* (Lemon grass)	18.97	17.53	14.77	17.09	2.1343
6	Fermented Bean	28.34	29.7	28.66	28.90	0.7111
7	Functional Food Paste	38.88	35.21	35.53	36.54	2.0328
8	Fermented Extract	30.43	27.64	31.36	29.81	1.9359
9	Fermented vinegar with lactic acid bacteria	97.98	96.97	97.98	97.64	0.5832
	Non-pretreated	59.2	57.45	60.05	58.90	1.3257

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15982.299	9	1775.811	872.023	.000
Within Groups	40.729	20	2.036		
Total	16023.028	29			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
5.00	3	17.0900						
1.00	3	19.4500	19.4500					
2.00	3		21.4500					
4.00	3			28.4167				
6.00	3			28.9000	28.9000			
8.00	3			29.8100	29.8100			
3.00	3				31.2700			
7.00	3					36.5400		
10.00	3						58.9000	
9.00	3							97.6433
Sig.		.056	.102	.271	.067	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix G

Hemolysis activity of protein/peptide extracts

No	Plant samples (Protein/Peptide extracts)	Variant 1 (%)	Variant 2 (%)	Variant 3 (%)	Mean (%)	SD
1	Andrographis paniculata	30.707	30.707	31.717	31.044	0.583
2	Curcuma mangga (Turmeric)	30.707	31.717	30.707	31.044	0.583
3	Carica papaya leaf (Papaya)	44.848	44.848	44.848	44.848	0.000
4	Allium sativum (Garlic)	38.788	36.768	38.788	38.114	1.166
5	Cymbopogon citratus (Lemon grass)	30.707	31.717	30.707	31.044	0.583
6	Zingiber officinale (Ginger)	18.586	18.990	18.990	18.855	0.233
7	Beta vulgaris (Beet root)	23.636	24.040	24.040	23.906	0.233
8	Allium cepa (Big onion)	20.606	20.606	20.606	20.606	0.000
9	Allium cepa (Small onion)	20.606	20.606	18.586	19.933	1.166
10	Momordica charantia (Bitter gourd)	14.545	14.545	12.525	13.872	1.166
11	Momordica charantia seeds	20.606	20.606	20.606	20.606	0.000
12	Agaricus bisporus stem (Button mushroom)	13.535	13.737	13.535	13.603	0.117
13	Agaricus bisporus fruiting body	26.667	26.667	26.667	26.667	0.000
	Non-pretreated	59.20	57.45	60.05	58.90	1.326
	100% hemolysis	100	100	100	100.000	
	Vitamin C (1mg/ml)	0	0	0	0.000	

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6156.543	13	473.580	949.818	.000
Within Groups	13.961	28	.499		
Total	6170.504	41			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
12.00	3	13.6023								
10.00	3	13.8717								
6.00	3		18.8553							
9.00	3		19.9327	19.9327						
8.00	3			20.6060						
11.00	3			20.6060						
7.00	3				23.9053					
13.00	3					26.6670				
1.00	3						31.0437			
2.00	3						31.0437			
5.00	3						31.0437			

4.00	3							38.1147		
3.00	3								44.8480	
14.00	3									58.9000
Sig.		.644	.072	.280	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix H

DPPH scavenging activity of ethanolic extracts

No	Ethanolic Extract	Variant 1 (%)	Variant 2 (%)	Variant 3 (%)	Mean	SD
1	*Andrographis paniculata*	48.4375	48.4375	48.4375	48.4375	0
2	*Curcuma mangga* (Turmeric)	87.5000	87.5000	84.3750	86.4583	1.8042
3	*Carica papaya* leaf (Papaya)	48.4375	53.1250	51.5625	51.0417	2.3868
4	*Allium sativum* (Garlic)	51.5625	54.6875	52.3438	52.8646	1.6263
5	*Cymbopogon citratus* (Lemon grass)	28.1250	29.6875	28.1250	28.6458	0.9021
6	Fermented Bean	81.2500	79.6875	81.2500	80.7292	0.9021
7	Functional Food Paste	7.8125	7.8125	18.7500	11.4583	6.3148
8	Fermented Extract	21.8750	21.8750	23.4375	22.3958	0.9021
9	Fermented vinegar with lactic acid bacteria	53.1250	53.1250	54.6875	53.6458	0.9021
	BHT 10mg/ml	98.4375	98.4375	98.4375	98.4375	0.0000

Vit-C 10mg/ml	87.5000	87.5000	90.6250	88.5417	1.8042

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25061.146	10	2506.115	475.434	.000
Within Groups	115.967	22	5.271		
Total	25177.113	32			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05							
		1	2	3	4	5	6	7	8
7.00	3	11.4583							
8.00	3		22.3958						
5.00	3			28.6458					
1.00	3				48.4375				
3.00	3				51.0417	51.0417			
4.00	3					52.8646			
9.00	3					53.6458			
6.00	3						80.7292		
2.00	3							86.4583	
11.00	3							88.5417	
10.00	3								98.4375

Sig.		1.000	1.000	1.000	.179	.202	1.000	.278	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix I

DPPH scavenging activity of protein/peptide extracts

No	Plant samples (Protein/Peptide extracts)	Variant 1	Variant 2	Variant 3	Mean	sd
1	*Andrographis paniculata*	15.238	16.190	15.238	15.556	0.549857
2	*Curcuma mangga* (Turmeric)	17.143	17.143	18.095	17.460	0.549857
3	*Carica papaya* leaf (Papaya)	25.714	27.619	28.571	27.302	1.454786
4	*Allium sativum* (Garlic)	-166.667	-171.429	-171.429	-169.841	2.749287
5	*Cymbopogon citratus* (Lemon grass)	18.095	19.048	18.095	18.413	0.549857
6	*Zingiber officinale* (Ginger)	18.095	18.095	19.048	18.413	0.549857
7	*Beta vulgaris* (Beet root)	1.905	0.952	1.905	1.587	0.549857
8	*Allium cepa* (Big onion)	25.714	24.762	25.714	25.397	0.549857
9	*Allium cepa* (Small onion)	10.476	12.381	11.429	11.429	0.952381
10	Momordica charantia (Bitter gourd)	6.667	7.619	7.619	7.302	0.549857
11	*Momordica charantia* seeds	3.810	4.762	2.857	3.810	0.952381
12	*Agaricus bisporus* stem (Button mushroom)	4.762	3.810	4.762	4.444	0.549857
13	*Agaricus bisporus* fruiting body	3.810	4.762	3.810	4.127	0.549857
	BHT 1mg/ml	23.4375	29.6875	32.8125	28.6458	4.773516
	Vit-C 1mg/ml	76.5625	70.3125	73.4375	73.4375	3.125

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	110536.488	13	8502.807	4966.931	.000
Within Groups	47.933	28	1.712		
Total	110584.421	41			

Duncan[a]

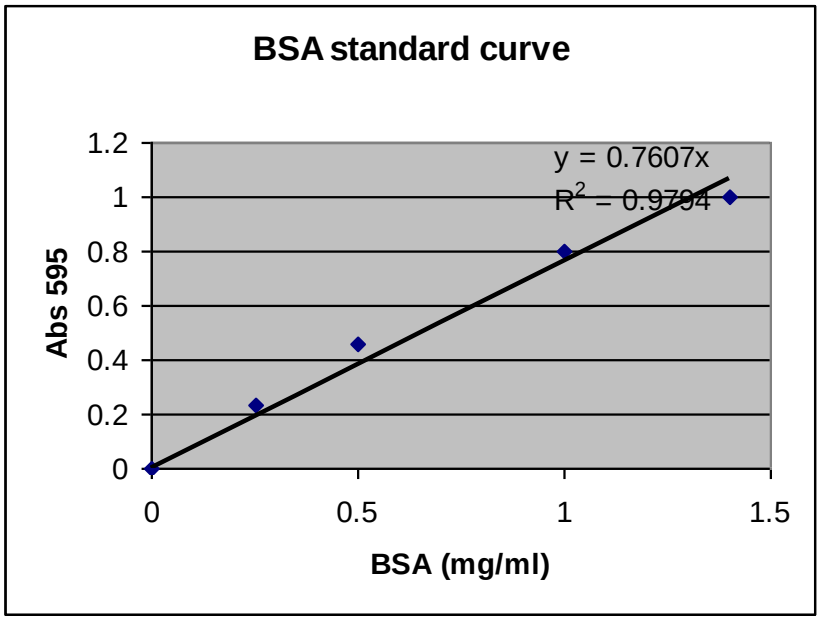
VAR00001	N	Subset for alpha = 0.05								
		1	2	3	4	5	6	7	8	9
4.00	3	-169.8433								
7.00	3		1.5900							
11.00	3			3.8100						
13.00	3			4.1267						
12.00	3			4.4433						
10.00	3				7.3033					
9.00	3					11.4300				
1.00	3						15.5553			
2.00	3						17.4600	17.4600		
5.00	3							18.4167		
6.00	3							18.4167		

8.00	3								25.3933	
3.00	3								27.3000	
14.00	3									73.4367
Sig.		1.000	1.000	.582	1.000	1.000	.085	.406	.085	1.000

Means for groups in homogeneous subsets are displayed.

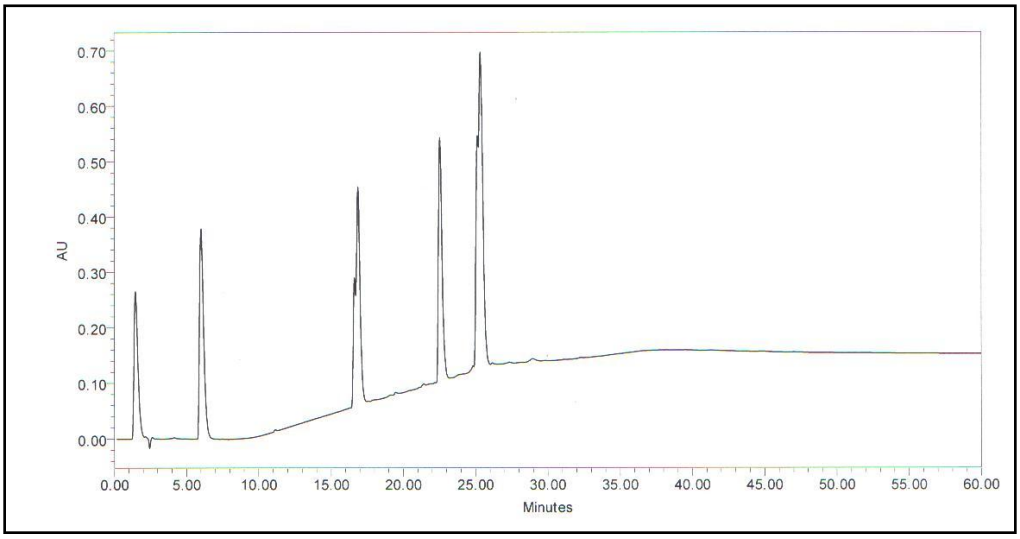
a. Uses Harmonic Mean Sample Size = 3.000.

Appendix J



Standard curve for the net absorbance at 595nm versus the protein

Appendix K



HPLC chromatograms of protein/peptide compound separated from peptide standard

1mg/ml of peptide standard compound is injected with the volume of 10µl every injection

Appendix L

Animal experimental ethical number (Toxicity Studies Using ICR mice)

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PEJABAT KETUA

6 Ogos 2009

Saravana Kumar A/L Sinniah
Institut Sains Biologi
Fakulti Sains
Universiti Malaya

Tuan,

STUDIES ON THE BIOACTIVE PROPERTIES OF SELECTED PLANT AND FERMENTED EXTRACTS

Dengan sukacitanya Jawatankuasa Etika Penjagaan dan Penggunaan Haiwan, Fakulti Perubatan, Universiti Malaya telah meluluskan permohonan untuk penyelidikan tersebut di atas.

No rujukan etika: **ISB/05/08/2009/SKS (R)**

Sila ambil perhatian bahawa nombor rujukan etika yang diberi adalah sah bermula dari **5 Ogos 2009 sehingga 4 Ogos 2011.**

Sila lengkapkan borang yang dilampirkan bersama dengan surat ini (Animal Traffic Record) dan hendaklah dikembalikan kepada pihak kami setelah penyelidikan tamat.

Sekian, terima kasih.

Yang benar,

b/p

Dr. Haji Azizuddin Bin Haji Kamaruddin
Ketua
Pusat Haiwan Makmal
Fakulti Perubatan
Merangkap Setiausaha Jawatankuasa Etika Penjagaan dan Penggunaan Haiwan

SK : Puan Zura Syazleena Hamizan
Setiausaha MCRC
Pejabat Dekan
Fakulti Perubatan

MS ISO 9001:2000 REG. NO. AR 2760

Ketua, Pusat Haiwan Makmal
Fakulti Perubatan, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
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AppendixM

Animal experimental ethical number (Hemolysis Assay, erythrocyte from rabbit)

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PEJABAT KETUA

6 Ogos 2009

Teoh Wuen Yew
Institut Sains Biologi
Fakulti Sains
Universiti Malaya

Tuan,

STUDY OF BACTERIAL DIVERSITY IN THE ORAL CAVITY OF MALAYSIAN SUBJECTS

Dengan sukacitanya Jawatankuasa Etika Penjagaan dan Penggunaan Haiwan, Fakulti Perubatan, Universiti Malaya telah meluluskan permohonan untuk penyelidikan tersebut di atas.

No rujukan etika: **ISB/05/08/2009/TWY (R)**

Sila ambil perhatian bahawa nombor rujukan etika yang diberi adalah sah bermula dari **5 Ogos 2009 sehingga 4 Ogos 2011**.

Sila lengkapkan borang yang dilampirkan bersama dengan surat ini (Animal Traffic Record) dan hendaklah dikembalikan kepada pihak kami setelah penyelidikan tamat.

Sekian, terima kasih.

Yang benar,

b/p

Dr. Haji Azizuddin Bin Haji Kamaruddin
Ketua
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Appendix N

Biotechnology 8 (2): 248-253, 2009
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Antimicrobial Peptides in Aqueous and Ethanolic Extracts from Microbial, Plant and Fermented Sources

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Abstract: The objective of this research was to isolate novel peptides from extracts prepared from native microbial, plant and fermented sources. The antimicrobial properties of these extracts were initially tested using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*. The exact species and strains of these test microorganisms were confirmed by identifying its 16S RNA sequence. The most pronounced inhibition zone for ethanolic extracts was obtained with *Andrographis paniculata*. For peptide/protein extracts only *Allium sativum* showed promising results. The particular compound responsible for the inhibition in each case is undergoing characterization by using High Performance Liquid Chromatography (HPLC) and mass spectrometry.

Key words: Antimicrobial peptides, antibiotics, ethanolic extracts

INTRODUCTION

Biologically active peptides and polypeptides occur in a vast range of sizes and no generalization can be made about the molecular weights in relation to their functional properties. Naturally occurring peptides range in length from two amino acids to many thousands of residues. Even the smallest peptides can have biologically important effects.

A variety of peptides and proteins have been used to produce biopesticides, biopesticidal microbes and pest-resistant crops. These compounds derive from a number of sources including the venoms of predatory or parasitoid animals (Taniai, 2002), arthropod-pathogenic microbes including bacterial symbiotes of entomopathogenic nematodes (Beard, 2001), plant lectins, protease inhibitors (Brunelle *et al*., 2005) or ribosome inactivating proteins (Sharma, 2004), arthropod hormones and neuropeptides (Altstein, 2004; Borovsky, 2003), plant defensins (Lay and Anderson, 2005) and plant hormones (Dinan, 2001).

The gene-encoded cationic antimicrobial peptides (AMPs) are important mediators in the primary host defense system against pathogenic microorganisms, which are widely distributed in nature. In the last few years, the burgeoning reports of the occurrence and characterization of low-molecular-mass AMPs from a wide variety of organisms have been accumulating at a rapid rate because of their biochemical diversity, broad specificity against bacteria or fungi (Sitaram and Nagaraj, 2002) and also because some of them have anti-viral (Sitaram and Nagaraj, 2002), anti-tumoral (Rozek *et al*., 2000) or wound-healing effects (Fernandes *et al*., 2002).

On the other hand, the resistance to antibiotics of bacteria has also risen dramatically and the resistance to most or all available agents has appeared in the clinic over the past decade. There is a growing need to discover and introduce new drugs and AMPs provide new promising candidates for screening of new antibiotics.

This study was undertaken to isolate novel peptides and secondary metabolites from selected Malaysian indigenous microbial, plant and fermented sources. Subsequently, these peptides and secondary metabolites were tested *in vitro* using test microorganisms.

MATERIALS AND METHODS

This study was conducted at the Fermentation Technology Laboratory, Division of Microbiology, Institute of Biological Sciences, Faculty of Science and Department of Molecular Medicine, Faculty of Medicine, University of Malaya. The research project was conducted from September 2007 to August 2008.

Sample preparation: Plant samples were cleaned and dried at a temperature not exceeding 40°C and pulverised to powder form. Fermented samples were prepared by exposing them to solid state lactic acid fermentation at

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20% moisture. These test samples were prepared in triplicates and also used for the subsequent part of this study.

Ethanol extraction: The powder was soaked in 95% ethanol for a week. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C in a rotary evaporator and then weighed to determine the total extractable compounds. The crude extracts were then transferred to vials and kept at -4°C. These crude extracts were dissolved in water or solvents and used for the assessment of antimicrobial activity (Severo *et al*., 2008).

Peptide/protein extraction: Tissue was placed in a cold mortar and pestle. Approximately 2 mL of extraction buffer was added for every 1 g of tissue. The extraction buffer consists of 5 mL of KPO_4, 0.5 M of EDTA, 1 mL of triton X-100, 12.5 mL of 80% (v/v) glycerol and 15.4 mg of DTT for every 100 mL. The tissue was grounded till no more tissue was visible. All steps were carried out at 4°C. The ground tissues were transferred into a centrifuge tube and centrifuged at 12,000 rpm for 15 min. The pellet was discarded and the supernatant was collected and stored at -20°C. The samples were freeze dried prior and reconstituted into 5 mL of buffer before protein determination.

Protein quantification: Protein standards of appropriate concentration in the same buffer as the sample was prepared using bovine serum albumin (Sigma-Aldrich Inc., Saint Louis). The protein standards ranged from 0.1 till 1.4 mg mL^{-1}. After adding 3 mL of Bradford Reagent to each tube, these were vortexed gently for thorough mixing (Bradford, 1976). The samples were incubated at room temperature for 15 min and absorbance was measured at 595 nm. The protein concentration was determined by comparison of the measured absorbance to a standard curve prepared using the protein standards (Bradford Reagent product manual, Sigma-Aldrich, Inc., Saint Louis).

Microbial strain identification of test microorganisms: The microbial DNA was extracted using either the i-genomic BYF DNA Extraction Kit for gram positive bacteria or the i-genomic CTB DNA Extraction Kit for gram negative bacteria, (iNtRON Biotechnology, Seongnam). The extracted genomic DNA was examined by electrophoresis in a 1% agarose gel. Universal primers used to amplify 16S rRNA gene were (27f : 5'-AGA GTT TGA TCA TGG CTC AG and 1492r : 5'-TAC GGC TAC CTT GTT ACG ACTT) (Bioneer Corporation, Daejeon). PCR conditions used for amplification were: initial denaturation at 94°C for 5 min followed by denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1.5 min and a final extension at 72°C for 10 min. Reaction mixtures of 20 μL in total contained 2 μL of 10x PCR buffer, 2 μL of dNTP mix (2.5 mM each), 1 μL of each primer (10 pmoles), 50 ng of DNA template, 0.5 μL of i-Taq™ DNA polymerase (5U μL^{-1}) (iNtRON Biotechnology, Seongnam). The PCR products of approximately 1.4 Kbp were examined by electrophoresis in a 1.5% agarose gel. The PCR product was purified using PCRquick-spin™ (iNtRON Biotechnology, Seongnam). The 16S rRNA gene sequencing was done by Macrogen Inc. (Seoul) which uses ABI 3730xl DNA analyzer. The 16S rRNA sequences obtained were compared with the NCBI database using Blastn. Identity of ≥98% was the criterion used to identify the microbial species and strain.

Antimicrobial tests: Antimicrobial tests were performed based on the recommendation of the British Society for Antimicrobial Chemotherapy and National Committee for Clinical Laboratory Standards (2005) guidelines. Bacterial test cultures were grown overnight on Mueller Hinton broth (Becton, Dickinson and Company, Franklin Lakes). The inocula suspension concentration was diluted with 0.85% sterile saline solution to achieve an optical density between 0.08 to 0.1 units at 625 nm. Before inoculation, the inocula was diluted 10 times to make it approximately 10^7 colony forming unit per mL. Mueller Hinton agar plates were prepared in advance. Sterile cotton swabs were used to streak entire plates with the inoculum suspension. Sterile 6 mm filter paper discs (Whatman International Ltd, Maidstone) were used to place the samples on agar plates. The samples concentration was adjusted to 1 mg mL^{-1} for protein/peptide extract and 50 mg mL^{-1} for ethanolic extract. All the plates were incubated at 37°C for 16 h. The positive control used was Tetracycline (Oxoid, Basingstoke).

RESULTS AND DISCUSSION

Identification of test microorganisms: The microbes were identified using 16S RNA sequences as *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* RF122, *Escherichia coli* UTI89 and *Pseudomonas aeruginosa*.

The results (Table 1, 2; Fig. 3) show the inhibition of various plant and fermented samples that were tested against gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*). The four bacteria obtained from the Microbiology Department were further analysed for their exact species/strain by 16S rRNA sequence determination and comparison to existing

Table 1: Inhibition zones of various plant and fermented ethanolic extracts

	Inhibition zone (mm) against			
Plant species/Fermented samples	*E. coli*	*S. aureus*	*B. cereus*	*P. aeruginosa*
Andrographis paniculata	No inhibition	11±1.0	14±1.0	11.5±1.5
Curcuma mangga (Tumeric)	No inhibition	8.5±1.5	10.5±0.5	9.5±0
Carica papaya (Papaya leaf)	No inhibition	No inhibition	No inhibition	No inhibition
Allium sativum (Garlic)	No inhibition	No inhibition	7.0±1.0	7.0±1.0
Cymbopogon citratus (Lemon grass)	No inhibition	No inhibition	No inhibition	No inhibition
Fermented bean	No inhibition	No inhibition	No inhibition	7.0±1.0
Functional food paste	No inhibition	No inhibition	No inhibition	No inhibition
Fermented extract	No inhibition	No inhibition	No inhibition	8.0±1.0
Fermented vinegar with lactic acid bacteria	No inhibition	No inhibition	No inhibition	No inhibition
+ control	15±1.0	16±0	19±1.0	16.5±0.5
- control	No inhibition	No inhibition	No inhibition	No inhibition

All tests were done in triplicates

Table 2: Inhibition zone of various protein/peptide extracts

	Inhibition zone (mm) against			
Plant species/Fermented samples	*E. coli*	*S. aureus*	*B. cereus*	*P. aeruginosa*
Andrographis paniculata	No inhibition	No inhibition	No inhibition	No inhibition
Curcuma mangga (Tumeric)	No inhibition	No inhibition	No inhibition	No inhibition
Carica papaya (Papaya leaf)	No inhibition	No inhibition	No inhibition	No inhibition
Allium sativum (Garlic)	15±1.5	28±2.1	16.3±1.5	9±0.9
Cymbopogon citratus (Lemon grass)	No inhibition	No inhibition	No inhibition	No inhibition
Zingiber officinale (Ginger)	No inhibition	No inhibition	No inhibition	No inhibition
Beta vulgaris (Beetroot)	No inhibition	No inhibition	No inhibition	No inhibition
Allium cepa (Big onion)	No inhibition	No inhibition	No inhibition	No inhibition
Allium cepa (Small onion)	No inhibition	No inhibition	No inhibition	No inhibition
Momordica charantia (Bitter gourd)	No inhibition	No inhibition	9.0±0.3	10.0±0.9
Momordica charantia seeds	No inhibition	No inhibition	No inhibition	No inhibition
Agaricus bisporus stem (Button mushroom)	No inhibition	No inhibition	No inhibition	No inhibition
Agaricus bisporus fruiting body	No inhibition	No inhibition	No inhibition	No inhibition
+ ve Control	18±0.6	30±0	34±0	26±0
- ve Control	No inhibition	No inhibition	No inhibition	No inhibition

All tests were done in triplicates

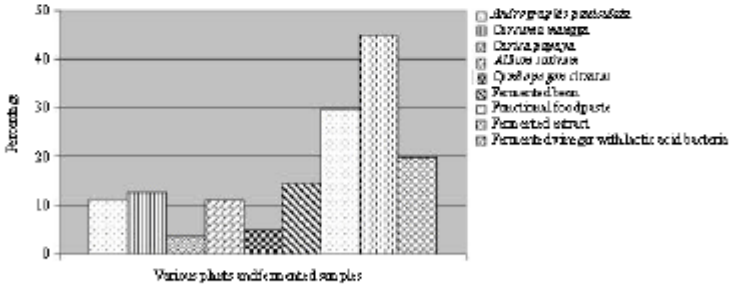

Fig. 1: Ethanolic extract yield. % (w/w) yield refers to gravimetric determination of total extractable compounds expressed as a percentage of the sample weight

databases. The amount of secondary metabolite and peptide extracted from various plant and fermented extracts are shown in the bar charts (Fig. 1, 2).

Fermented extract followed by functional food paste showed highest total extractable compounds from the ethanolic extract with 45 and 30%, respectively. Total extractable compound from fermented vinegar was 20% and the rest of the sample was below 20%. On the other hand, *Andrographis paniculata* and *Allium sativum* showed the highest protein content with 1.77 and 1.80 mg g^{-1}, respectively. These were followed by *Zingiber officinale* with 1.56 mg g^{-1}

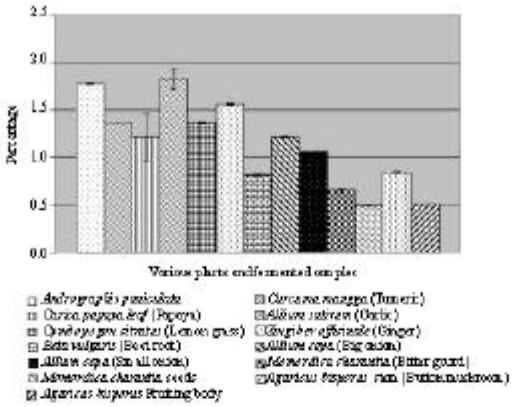

Fig 2: Protein/peptide extract yield. Concentration refers to protein or peptide concentration in mg g^{-1} tissue

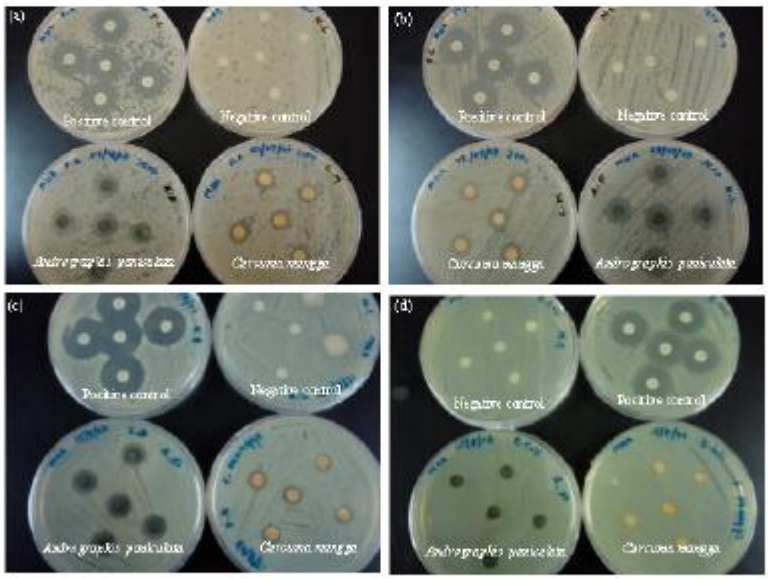

Fig 3: Effect of ethanolic extracts on bacterial growth. (a) *Pseudomonas aeruginosa*, (b) *Bacillus cereus*, (c) *Staphylococcus aureus* and (d) *E. coli*

and both *Curcuma mangga* and *Cymbopogon citratus* with 1.36 mg g^{-1}. The rest of the samples have protein content below 1.30 mg g^{-1}.

The most pronounced inhibition zone for ethanolic extract was obtained with *Andrographis paniculata* producing inhibition zones of 11 mm against *S. aureus*, 14 mm against *B. cereus* and 11.5 mm against *P. aeruginosa*. In studies done by Singha *et al.* (2003), the aqueous extract and the arabinogalactan protein fractions showed inhibition against *E. coli* and *P. aeruginosa* but

not towards *S. aureus*. Their 80% methanol and chloroform extraction of *Andrographis paniculata* did not show inhibition against *E. coli*, *P. aeruginosa* or *S. aureus*. Hence, these findings are contradictory to present findings. These differences could be attributed to the solvents used in the current study for extraction. Moreover, the used strain of the bacteria could also affect the results significantly.

Ethanolic extracts from *Curcuma mangga* produced inhibition zones of 8.5 mm against *S. aureus*, 10.5 mm against *B. cereus* and 9.5 mm against *P. aeruginosa*. Extracts from *Allium sativum* inhibits both *B. cereus* and *P. aeruginosa*, respectively with 7 mm inhibition zones. Ethanolic extracts prepared from fermented bean and fermented extract only inhibited *P. aeruginosa* with 7 and 8 mm inhibition zones, respectively. Ethanolic extracts from many plant sources have been shown to have biological activity against bacteria. For instance, the ethanolic extracts from *Rhus* (Nassar-Abbas and Halkman, 2004) are inhibitory towards gram positive and gram negative bacteria. A comprehensive review of the biological activities of *Rhus* extracts details the promising potential of the extracts of parts of this plant (Rayne and Mazza, 2007).

Peptide/protein extracts from *Allium sativum* showed promising results with 15 mm inhibition zones against *E. coli*, 28 mm against *S. aureus*, 16.3 mm against *B. cereus* and 9 mm against *P. aeruginosa*. Extracts from *Momordica charantia* were more selective and showed inhibition only against *B. cereus* and *P. aeruginosa* with 9 and 10 mm inhibition zones, respectively. Present results are in agreement with those of Gosh *et al*. (2008) who showed that aqeous extracts are generally less potent in their bioactivity than methanolic extracts.

CONCLUSION

Ethanolic extracts of *Andrographis paniculata* exhibited some degree of antibacterial activity towards *P. aeruginosa*, *S. aureus* and *B. cereus*. However, its peptide/protein extract did not produce any inhibition towards the bacteria species tested. Peptide/protein extract of *Allium sativum* exhibited a strong inhibition zone against both gram negative and gram positive bacteria but its ethanolic extract only produced a small degree of inhibition against *B. cereus* and *P. aeruginosa*. The particular compound responsible for the inhibition in each case is undergoing characterization by using High Performance Liquid Chromatography (HPLC) and mass spectrometry.

ACKNOWLEDGMENT

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Appendix O

American Journal of Applied Sciences 6 (8): 1613-1617, 2009
ISSN 1546-9239

Antimicrobial Activity of Some Medicinal Plants from Malaysia

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Abstract: **Problem statement:** About 32 extracts from eight selected medicinal plants, namely *Pereskia bleo, Pereskia grandifolia, Curcuma aeruginosa Roxb., Curcuma zedoria, Curcuma mangga, Curcuma inodora aff. Blatter, Zingiber officinale var. officinale* (jahe gajah) and *Zingiber officinale var. rubrum* (jahe emprit) used by Malaysia traditional health care systems were screened for their antimicrobial activity against both Gram-positive bacteria and Gram-negative bacteria using agar disc diffusion assay. **Approach:** The efficacy of the extracts was compared to the commercially prepared antibiotic diffusion discs. **Results:** No inhibition was observed with the water fractions. **Conclusion/Recommendations:** None of the plants tested showed inhibition against *Escherichia coli*. *Curcuma mangga* showed some remarked inhibition against the bacteria used in this study.

Key words: Antimicrobial activity, agar disc diffusion assay, Malaysia medicinal plants

INTRODUCTION

Natural products perform various functions, and many of them have interesting and useful biological activities[3]. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. In Peninsular Malaysia, 1,200 species of higher plants and 2,000 species in Sabah and Sarawak are reported to have medicinal value and have been used for generations in various traditional health care systems[5]. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections[3,4,15]

This study reports a screening programme of 32 methanolic extracts and from eight medicinal plants in Malaysia for their antimicrobial properties against two Gram-positive bacteria and two Gram-negative bacteria. These plants included two families namely Cactaceae (*Pereskia bleo* and *Pereskia grandifolia*) and Zingiberaceae (*Curcuma aeruginosa Roxb., Curcuma zedoria, Curcuma mangga, Curcuma inodora aff. Blatter, Zingiber officinale* var. *officinale* and *Zingiber officinale* var. *rubrum*). There were no previous reports of antimicrobial study on *Pereskia bleo, Pereskia grandifolia, Curcuma mangga* and *Curcuma inodora aff. Blatter*.

There were numerous antimicrobial studies conducted on both essential oils and extracts of common ginger (*Zingiber officinale* var. *officinale*). However, there is no report on the antimicrobial activity of the variants of *Zingiber officinale* such as jahe emprit (*Zingiber officinale* var. *rubrum*) and jahe gajah (*Zingiber officinale* var. *officinale*). Sofia *et al.*[14] reported that the ginger extract showed insignificant antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*. In 2005, Lopez *et al.*[7]. reported that essential oil of ginger showed weakest inhibition against selected bacteria and fungi whilst Rath *et al.*[13]. reported that essential oil of ginger did not show any inhibition on the tested pathogens in their study.

Extracts of *Curcuma aeruginosa* obtained from supercritical fluid extraction have shown negligible inhibition activity against Gram negative bacteria *Escherichia coli* and yeast *Malassezia furfur*[8,9]. from Vietnam, however, isolated sesquiterpene constituents from the petroleum ether extract of *Curcuma aeruginosa* and found that these compounds have a broad spectrum of antimicrobial activity. There are a number of papers reported the antimicrobial activity of the essential oil of *Curcuma zedoaria* against Gram positive and negative pathogenic microorganism[2,6,12,16] reported that petroleum ether, hexane, chloroform, acetone and ethanolic extracts of *Curcuma zedoaria*

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exhibited antibacterial and antifungal activity whilst Phan *et al.*[8] isolated sesquiterpene constituents from the petroleum ether extract of *Curcuma zedoaria* which showed active inhibition against *Candida albicans*.

MATERIALS AND METHODS

Plant material: Eight traditional medicinal plants used in this study were *Pereskia bleo*, *Pereskia grandifolia*, *Curcuma aeruginosa Roxb.*, *Curcuma zedoria*, *Curcuma mangga*, *Curcuma inodora aff. Blatter*, *Zingiber officinale var. officinale* and *Zingiber officinale var. rubrum*. These medicinal plants were chosen based on their traditional medicinal use and reported biological activities. The fresh leaves of *Pereskia bleo* and *Pereskia grandifolia* were collected from Petaling Jaya, Selangor, Malaysia in May 2007. The rhizomes of *Curcuma aeruginosa*, *Curcuma mangga*, *Zingiber officinale var. officinale* and *Zingiber officinale var. rubrum* were obtained from Jogjakarta, Indonesia in 2006. Whereas both rhizomes of *Curcuma zedoaria* and *Curcuma inodora* were obtained from MARDI Kluang, Johor, Malaysia in January 2007. They were identified by Professor Dr. Halijah Ibrahim of Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia and voucher specimens were deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Extraction of plant material: The fresh samples were washed, dried and ground to fine powders using a blender. The dried, ground samples were then soaked in methanol (1.5 L) for 3 days at room temperature. The solvent-containing extracts were then decanted and filtered. The extractions of the ground samples were further repeated (2x) with methanol (1.5 L each time). The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure using a rotary evaporator to give crude methanol extracts. The methanol extracts were further extracted with hexane to give hexane-soluble fractions and hexane insoluble residues. The hexane-insoluble residues were further partitioned between ethyl acetate-water (ratio 1:1) to give ethyl acetate-soluble fractions. The water layers were freeze-dried to give water fractions. All the extracts and fractions were stored at 4°C for determination of antibacterial activity.

Test microorganisms and microbial culture: Four bacterial strains were used in this study: Gram negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, Gram positive bacteria; *Staphylococcus aureus*, *Bacillus subtilis*. The test microorganisms were obtained from the Microbiology Laboratory, Microbiology Division, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia. Bacterial strains were cultivated at 37°C and maintained on nutrient agar (Difco, USA) slant at 4°C.

Antimicrobial activity assay: Antimicrobial activity was determined against four bacterial pathogens by the agar disc diffusion assay (NCCLS (National Committee for Clinical Laboratory Standards), 2005). The crude methanol and fractionated extracts were dissolved in Dimethyl Sulfoxide (DMSO) with the exception of the water fraction and then antimicrobial effect of crude methanol and fractionated extracts were tested using two different concentrations. Petri dishes (measuring 90 mm each side) containing 20 mL of mueller hinton agar (OXOID). At the same time, 6 mm diameter sterile Whatman Antibiotic disc were placed on the surface of the inoculated agar plates, and then appropriate concentration of the extracts in DMSO and water were applied onto the discs, 50 and 500 mg final concentrations were obtained for each discs. The plates were incubated at 37°C for 16-18 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. Standard discs of the antibiotic gentamycin (10 µg) and ampicilin (10 µg) served as the positive antibacterial controls. Negative controls were done using paper discs loaded with 20 µL of DMSO and water. After that, the diameter of inhibition zone was measured in millimeters by Vernier Calipers. All tests were repeated three times to minimize test error. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity[11].

RESULTS AND DISCUSSION

This study reports the antimicrobial activity of 32 extracts from eight selected medicinal plants in Malaysia against two Gram positive bacteria and two Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The results of the antimicrobial activity of the investigated extracts are shown in Table 1. None of the extracts showed activity against *Escherichia coli*. All the water fractions of the eight selected plants showed no inhibition against all the bacteria tested in this study. Generally, among the investigated extracts the ethyl acetate fractions exhibited the highest antibacterial effect followed by the methanol extracts.

Table 1: Results of the antimicrobial tests of the investigated plants in agar diffusion assay

Plant species	Extracts /fractions	Concentration (mg mL^{-1})	Inhibition zone (mm)[a] against			
			E. c	*P. a*	*S. a*	*B. s*
Pereskia bleo	Methanol	50	-	8.3	-	-
		500	-	9.8	-	-
	Hexane	50	-	-	-	-
		500	-	9.5	-	8.2
	Ethyl acetate	50	-	7.3	-	-
		500	-	8.5	-	7.8
	Water	50	-	-	-	-
		500	-	-	-	-
Pereskia grandifolia	Methanol	50	-	-	-	-
		500	-	-	-	-
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	8.0	9.2	8.5
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma aeruginosa Roxb	Methanol	50	-	-	-	-
		500	-	7.0	-	-
	Hexane	50	-	7.2	-	-
		500	-	7.5	7.5	-
	Ethyl acetate	50	-	-	-	7.0
		500	-	7.8	6.7	9.0
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma zedoaria	Methanol	50	-	-	-	-
		500	-	7.0	-	-
	Hexane	50	-	-	7.5	-
		500	-	7.7	8.5	8.5
	Ethyl acetate	50	-	-	-	-
		500	-	-	-	8.2
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma mangga	Methanol	50	-	7.2	7.5	9.3
		500	-	13.0	10.5	19.3
	Hexane	50	-	8.5	7.7	11.3
		500	-	15.0	9.5	13.5
	Ethyl acetate	50	-	-	7.0	8.7
		500	-	11.5	9.0	13.7
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma inodora aff. Blatter	Methanol	50	-	-	-	7.3
		500	-	7.8	8.3	8.0
	Hexane	50	-	-	-	-
		500	-	7.7	6.7	-
	Ethyl acetate	50	-	-	-	7.5
		500	-	7.8	10.0	9.0
	Water	50	-	-	-	-
		500	-	-	-	-
Zingiber officinale var. rubrum	Methanol	50	-	-	-	-
		500	-	-	-	-
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	-	-	7.5
	Water	50	-	-	-	-
		500	-	-	-	-
Zingiber officinale var. officinale	Methanol	50	-	-	-	-
		500	-	7.2	-	7.3
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	-	7.3	7.8
	Water	50	-	-	-	-
		500	-	-	-	-
Gentamycin, 10 µg/disc			20.7	18.0	22.0	19.0
Ampicilin, 10 µg/disc			NT	32.5	37.0	38.5

E.c: Escherichia coli; P.a: Pseudomonas aeruginosa; S.a: Staphylococcus aureus; B.s: Bacillus subtilis. -: no activity; NT: Not Tested, Negative controls did not show any activity, [a] Inhibition zones including the diameter of the paper disc (6 mm)

The most pronounced activity with inhibition zones of more than 14.0 mm was shown by methanol extract (inhibition zone 19.3 mm against *Bacillus subtilis* at concentration 500mg mL^{-1}) and hexane fraction (inhibition zone 15.0 mm against *Pseudomonas aeruginosa* at concentration 500mg mL^{-1}) of *Curcuma mangga*. In addition, the methanol extract of *Curcuma mangga* had a remarked sensitivity towards *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones 13.0 and 10.5 mm at concentration 500 mg mL^{-1} respectively. The hexane fraction of *Curcuma mangga* also showed significant antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 9.5 and 13.5 mm at concentration 500 mg mL^{-1}respectively whilst the ethyl acetate fraction showed inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 11.5, 9.0, 13.7 mm respectively at concentration 500 mg mL^{-1}. When the concentration of the extracts were decreased from 500-50 mg mL^{-1}, slight decrease in inhibition zones were observed. A recent phytochemical study of *Curcuma mangga* revealed the presence of labdane-type diterpene compounds and these compounds are similar to those that have been reported to possess strong antimicrobial activity against Gram positive, Gram negative bacteria and pathogenic fungi[1,10]. It is likely that the presence of this type of compounds may have contributed to the antimicrobial activity of *Curcuma mangga*.

At concentration 500 mg mL^{-1}, the methanol, hexane and ethyl acetate extracts of *Curcuma inodora* showed inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The methanol and ethyl acetate extracts of *Curcuma inodora* also showed modest inhibition against *Bacillus subtilis* at both concentrations of 500 and 50 mg mL^{-1}.

The methanol, hexane and ethyl acetate extracts of *Pereskia bleo*, at the concentration of 500 mg mL^{-1}, exhibited modest inhibition against *Pseudomonas aeruginosa* at 9.8, 9.5 and 8.5 mm, respectively. When the concentrations of these three extracts are lowered to 50 mg mL^{-1}, a slight decline in the inhibition zone were shown by the methanol and ethyl acetate extracts whilst the hexane extract showed no inhibition at all (Table 1). The hexane and ethyl acetate extracts of *Pereskia bleo*, at the concentration of 500 mg mL^{-1}, also showed modest inhibition against *Bacillus subtilis* at 8.2 and 7.8 mm, respectively. However, only the ethyl acetate extract of *Pereskia grandifolia* showed some antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1}.

Antimicrobial activity of jahe gajah (*Zingiber officinale* var. *officinale*) showed no inhibition against all the bacteria used in this study except a small inhibition zone of 7.5 mm against *Bacillus subtilis* at concentration 500 mg mL^{-1}. The methanol extract of jahe emprit (*Zingiber officinale* var. *rubrum*) showed inhibition against *Pseudomonas aeruginosa* and *Bacillus subtilis* whilst its ethyl acetate extract inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1} (Table 1).

At concentration of 500 mg mL^{-1}, the methanolic extract of *Curcuma zedoaria* exhibited antimicrobial activity against *Pseudomonas aeruginosa* whilst the ethyl acetate extract of *Curcuma zedoaria* showed antimicrobial activity against *Bacillus subtilis*. The hexane extract of *Curcuma zedoaria*, however, is observed to inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1}. There is a slight decrease in inhibition of *Staphylococcus aureus* by the hexane extract of *Curcuma zedoaria* concentration of 50 mg mL^{-1}.

The ethyl acetate extract of *Curcuma aeruginosa* showed inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1} while at 50 mg mL^{-1}, its ethyl acetate extract showed inhibition against *Bacillus subtilis*. Both hexane and methanolic extract of *Curcuma aeruginosa* showed antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with inhibition zones of 7.5 mm each, at concentration of 500 mg mL^{-1}. The hexane extract of *Curcuma aeruginosa* also showed antimicrobial activity against *Pseudomonas aeruginosa* with inhibition zone of 7.2 mm at concentration of 50 mg mL^{-1}.

CONCLUSION

Curcuma mangga exhibit some degree of antibacterial activity towards *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Thus, it shows that some of the medicinal plants used in traditional medicine are potentially effective antimicrobial agents. None of the plants tested in this study inhibited the growth of *Escherichia coli*. Investigation of the antimicrobial compounds in *Curcuma mangga* is now underway. The resulting information will contribute to a better understanding of the antimicrobial activity of the plant.

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