

APPENDICES

Appendix A – Material and Method

A-1) Phosphate Buffered Saline (PBS)

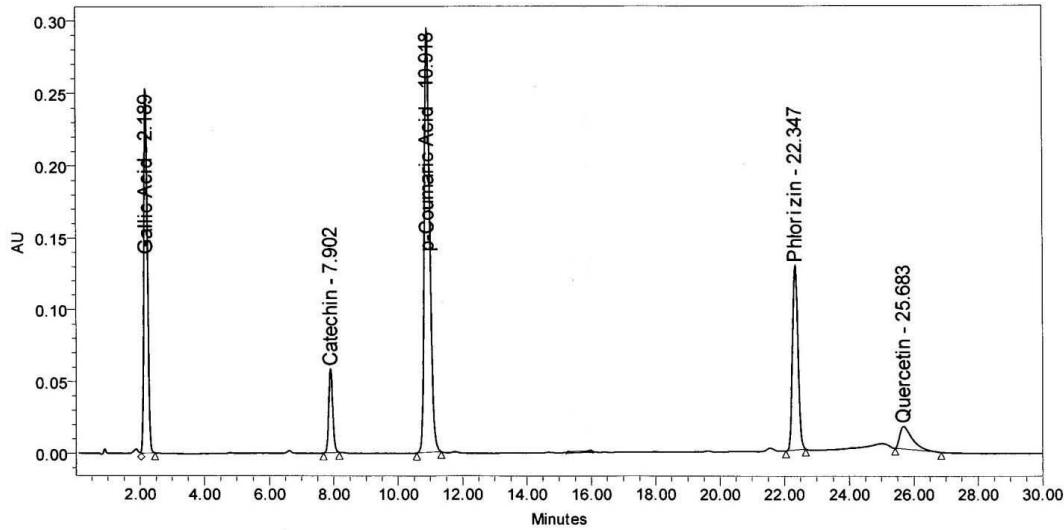
The below chemicals were added to 800 ml distilled water and dissolved. Finally, the volume adjusted to 1000 ml using distilled water and the buffer was autoclaved.

No.	Chemical	Amount (g)
1	Sodium chloride (NaCl)	8.00
2	Potassium chloride (KCl)	0.20
3	Disodium hydrogen phosphate (Na ₂ HPO ₄)	1.44
4	Potassium dihydrogen phosphate (KH ₂ PO ₄)	0.24

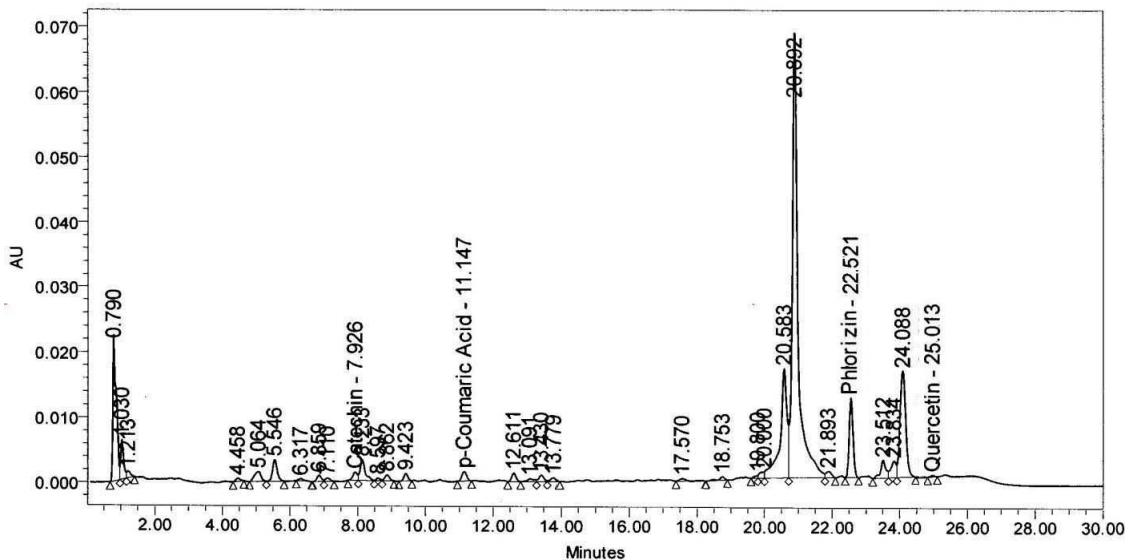
A-2) Bacterial Glycerol Stock Preparation

A single colony of bacteria from agar plate was cultured in nutrient broth to reach OD₆₀₀ ≈ 1.0. Then, 400 µl of cultured bacteria was added into 600 µl of 60% sterile glycerol in a 1.5 ml tube. The dilution was mixed and immediately freezed with liquid nitrogen and stored at -80° C until use.

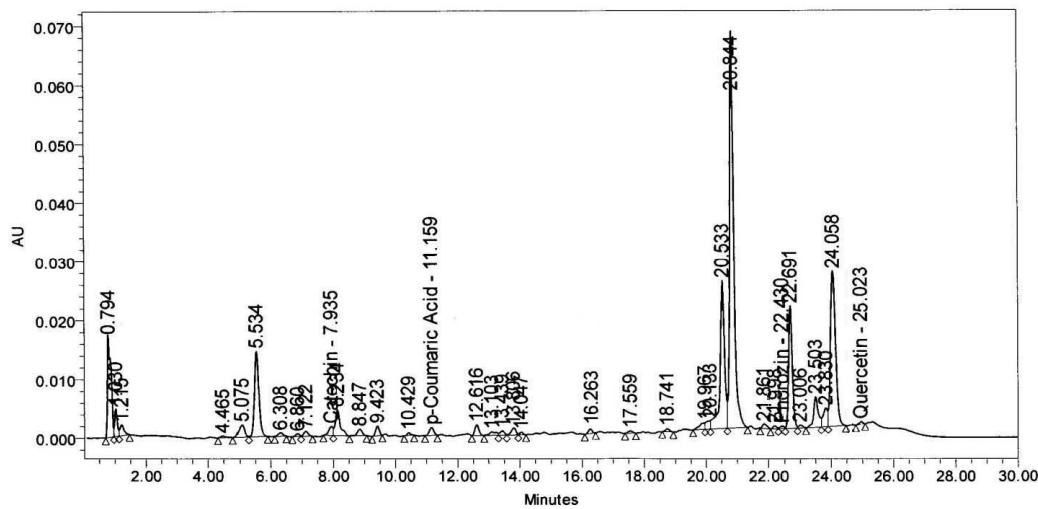
Appendix B – HPLC Chromatograms



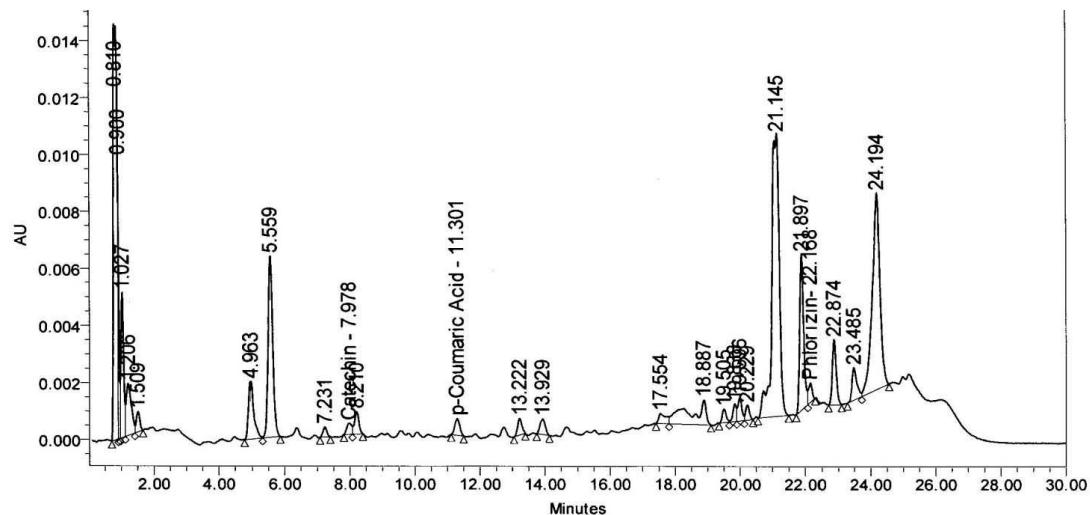
B-1) HPLC Chromatogram of gallic acid, catechin, p-coumaric acid, phlorizin, and quercetin as the phenolic compound standards.



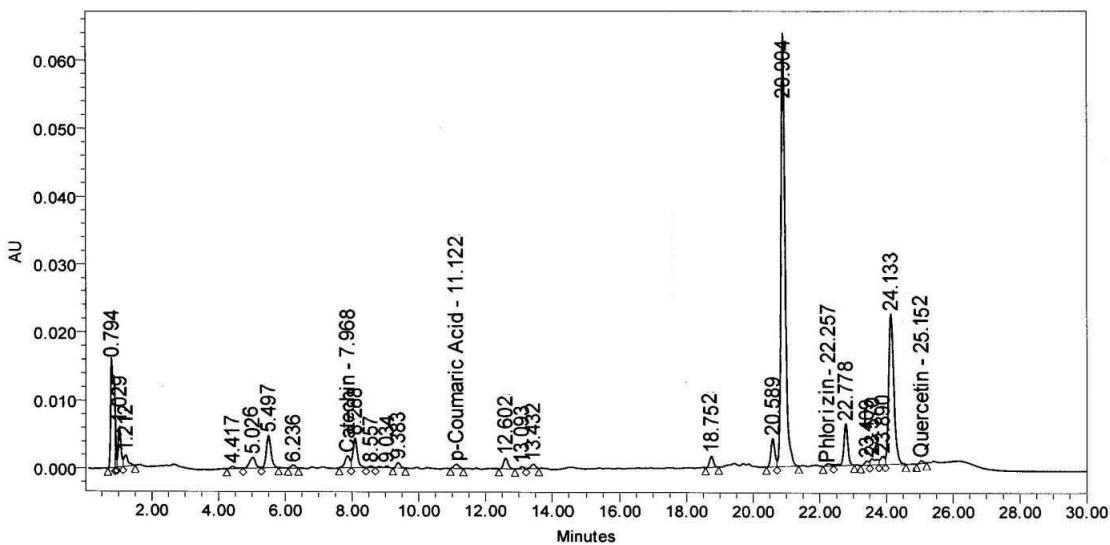
B-2) HPLC chromatogram of the phenolic compounds of leaf extract of the control *Centella asiatica* plant (Pathogenic bacteria inoculation study).



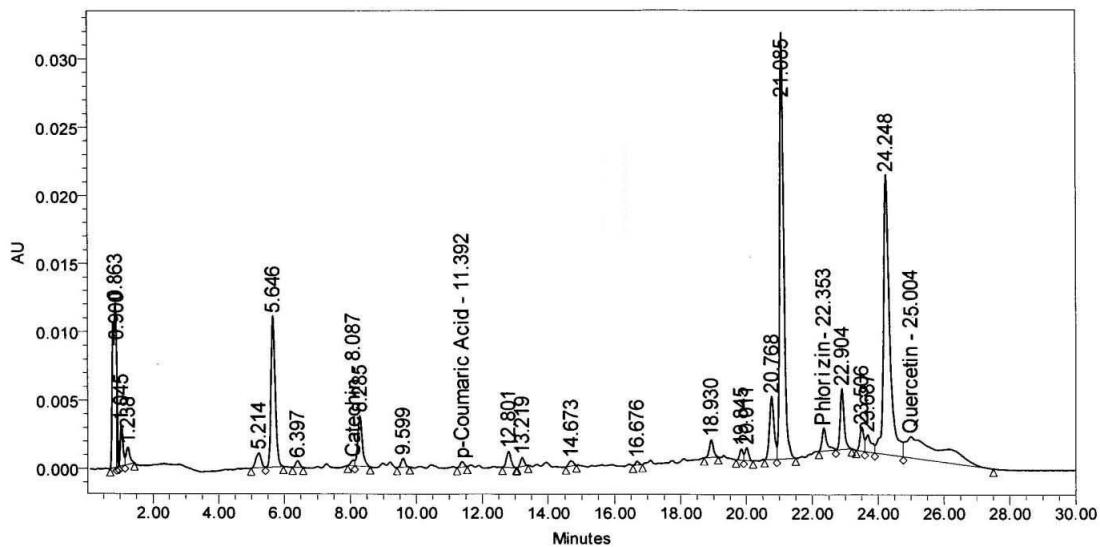
B-3) HPLC chromatogram of the phenolic compounds of leaf extract of the *Centella asiatica* plant 3 days after inoculation with pathogenic bacteria.



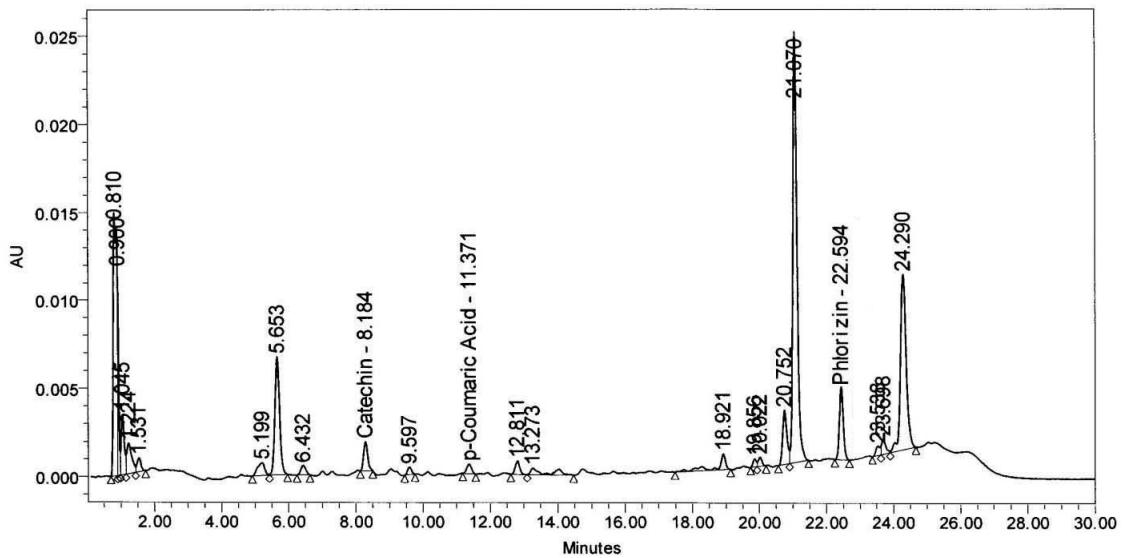
B-4) HPLC chromatogram of the phenolic compounds of leaf extract of the *Centella asiatica* plant 7 days after inoculation with pathogenic bacteria (the infected plant).



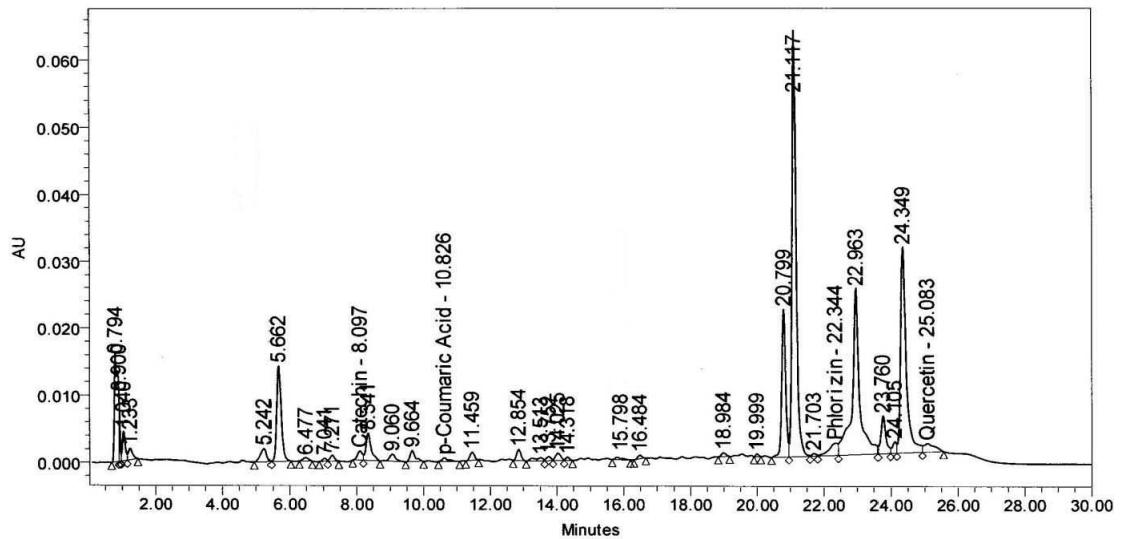
B-5) HPLC chromatogram of the phenolic compounds of petiole-stem extract of the control *Centella asiatica* plant (pathogenic bacteria inoculation study).



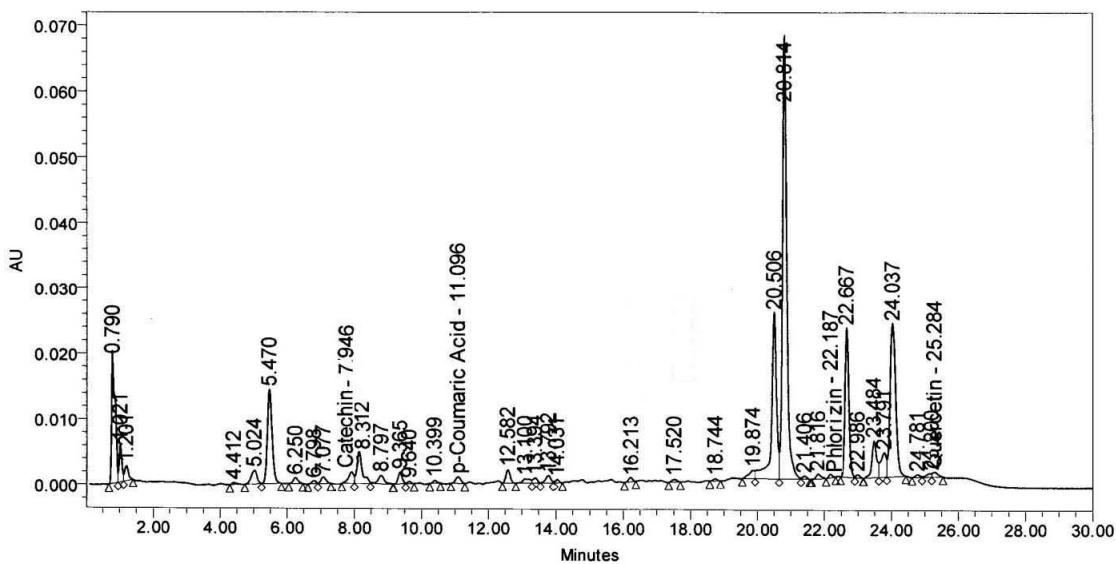
B-6) HPLC chromatogram of the phenolic compounds of petiole-stem extract of the *Centella asiatica* plant 3 days after inoculation with pathogenic bacteria.



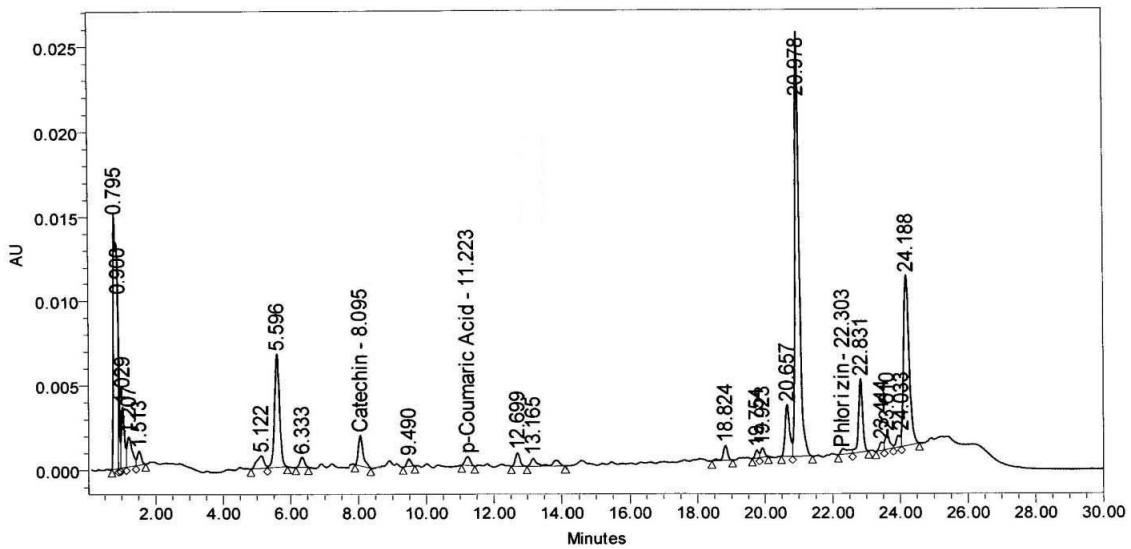
B-7) HPLC chromatogram of the phenolic compounds of petiole-stem extract of the *Centella asiatica* plant 7 days after inoculation with pathogenic bacteria (the infected plant).



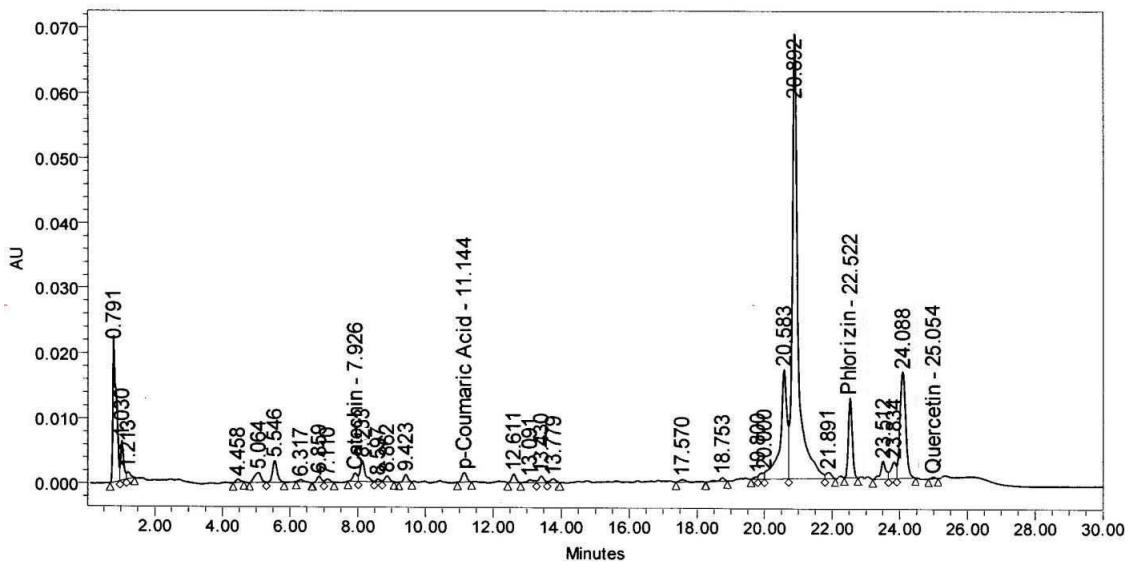
B-8) HPLC chromatogram of the phenolic compounds of root extract of the control *Centella asiatica* plant (pathogenic bacteria inoculation study).



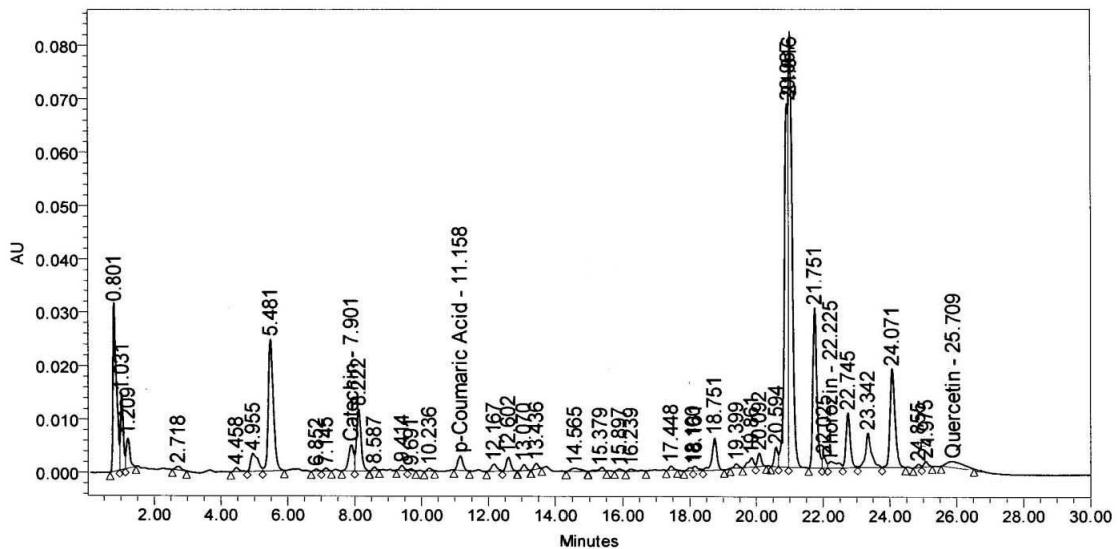
B-9) HPLC chromatogram of the phenolic compounds of root extract of the *Centella asiatica* plant 3 days after inoculation with pathogenic bacteria.



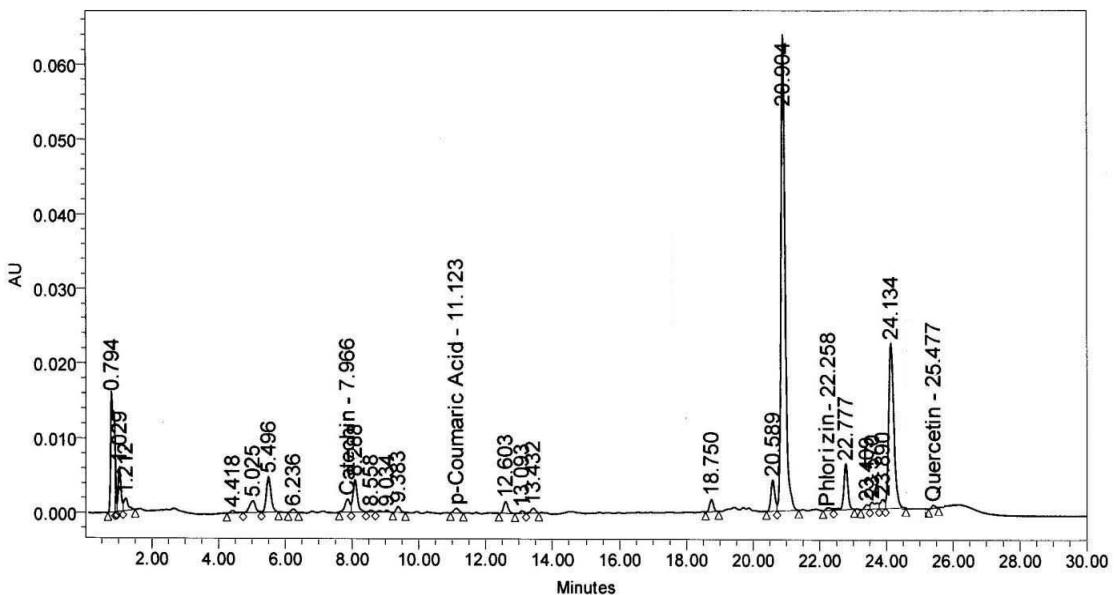
B-10) HPLC chromatogram of the phenolic compounds of root extract of the *Centella asiatica* plant 7 days after inoculation with pathogenic bacteria (the infected plant).



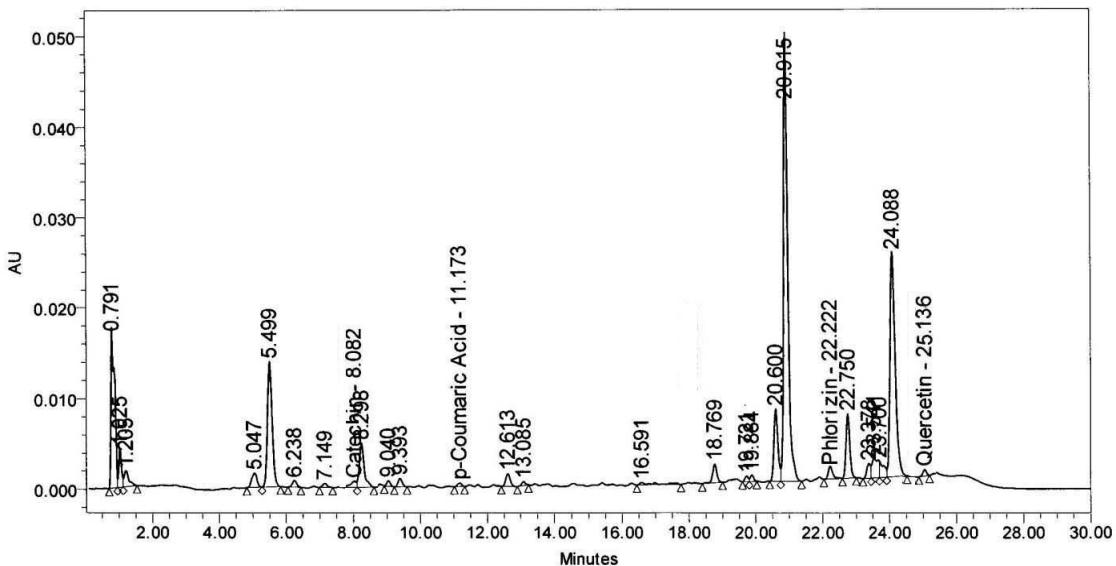
B-11) HPLC chromatogram of the phenolic compounds of leaf extract of the control *Centella asiatica* plant (beneficial bacteria treatment study).



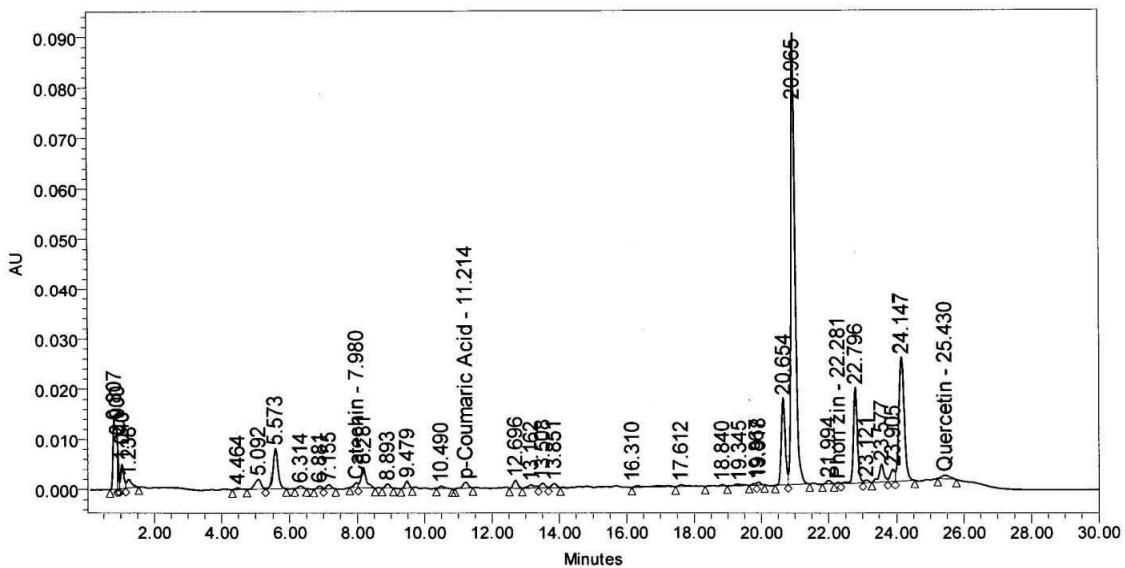
B-12) HPLC chromatogram of the phenolic compounds of leaf extract of the treated *Centella asiatica* plant with beneficial bacteria.



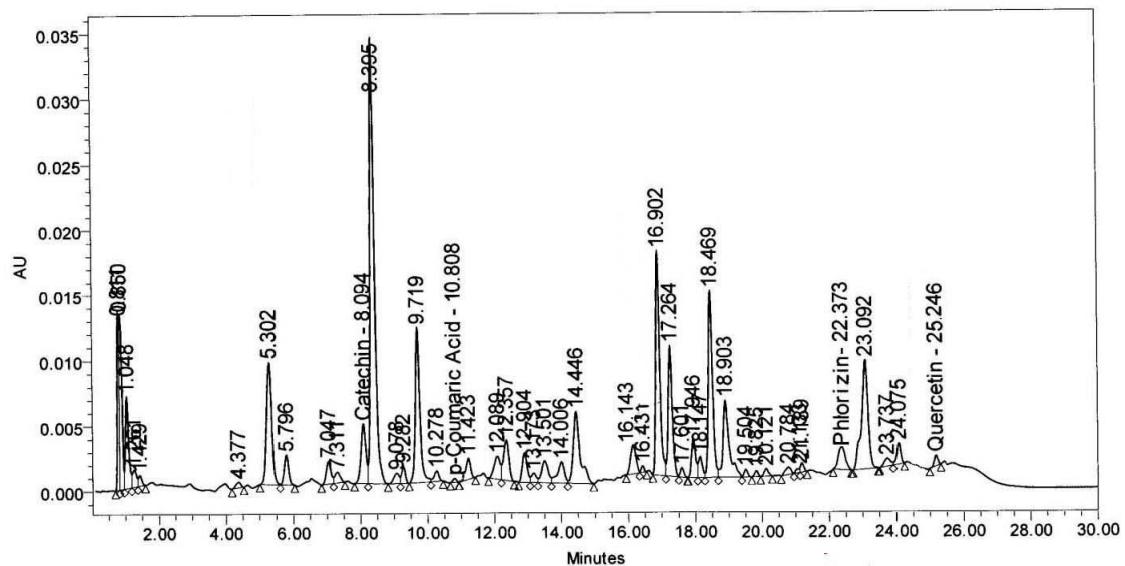
B-13) HPLC chromatogram of the phenolic compounds of petiole-stem extract of the control *Centella asiatica* plant (beneficial bacteria treatment study).



B-14) HPLC chromatogram of the phenolic compounds of petiole-stem extract of the treated *Centella asiatica* plant with beneficial bacteria.



B-15) HPLC chromatogram of the phenolic compounds of root extract of the control *Centella asiatica* plant (beneficial bacteria treatment study).



B-16) HPLC chromatogram of the phenolic compounds of root extract of the treated *Centella asiatica* plant with beneficial bacteria.

Appendix C – 16S rRNA Gene Sequences

C-1) Partial 16S rRNA gene sequence of *Bacillus gibsonii* strain AR_PBSTSB (NCBI LOCUS: HM582875, Size: 1445bp)

cggcgngcta tacatgcgaag tcgagcggac gttttgaag cttgcttcaa aaacgttagc
ggcggacggg ttagtaaacac gtgggcaacc taccttatcg actggataa ctccggaaa
ccggggctaa taccgataa catctagcac ctccgtgtgc cgattaaaa gagggcttct
tgctctcactg atgagatggg cccgcggcgc attagctgt tggagaggtt acggctcccc
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agcaacgccc cgtgagtgtt gaagggttc ggctcgtaaa gctctgttat gagggaaagaa
cacgtaccgt tcgaataggg cggtaccttgc acggtacctc atcagaaagc cacggctaac
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cgcgccgagg cgccttaag tctgatgtga aatcttgcgg ctcaaccgca agcggccatt
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agtttgtaac aaccgaagtc ggtgaggtt cttttggag ccagccggc aagtgacana
nnnnng

C-2) Partial 16S rRNA gene sequence of *Methylobacterium radiotolerans* strain AR_PSLBHI (NCBI LOCUS: HM582876, Size: 1374bp)

gcggcttaca catgcagtcg agcggccct tcgggttcg cggcggacgg gtgagtaac
cgtgggaacg tgcctctgg ttcggataa ccctggaaa ctaggctaa taccggata
gcctttgg ggaaagggtt actgccggaa gatcgcccg cgtctgatta gctagtttgt
ggggtaacgg cttaccaagg cgacgtacg tagctggctt gagaggatgtt tcagccacac

tgggactgag acacggccca gactcctacg ggaggcagca gtggggata ttggacaatg
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gccacggta atacgttccc gggccttgta cacaccgccc gtcacaccat gggagttgt
cttacccgac ggctgcgc caaccgcaag gaggcaggcg accacgnagt cnna

C-3) Partial 16S rRNA gene sequence of *Pantoea agglomerans* strain AR_PSBH2 (NCBI LOCUS: HM582877, Size: 1446bp)

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cgagtggcgg acgggtgagt aatgtctggg gatctgccc atggagggggataactactg
gaaacggtag ctaataccgc ataacgtcgc gagaccaaag tgggggaccc tcgggcctca
caccatcgga tgaaccaga tgggattagc tagtaggtgg gtaatggct caccctaggcg
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tgggttgcaa aagaagttagg taggttagac tcntcgntcg nngcatncc nctntngatt
nnncng

**C-4) Partial 16S rRNA gene sequence of *Pantoea agglomerans* strain AR_PINLBH4
(NCBI LOCUS: HM582878, Size: 1460bp)**

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ccatgggagt gggttgcaaa agaagttagga nngtttagat ctacgntcga gctgcntacg
ctgctttgn tncnnnnnng

C-5) Partial 16S rRNA gene sequence of *Erwinia tasmaniensis* strain AR_PINLTS5 (NCBI LOCUS: HM582879, Size: 1459bp)

gnnnannngc gggaggctac acatgcagtc gtacnaatga gtcannncna ngagcttgc
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gtaatcggtt atcagaatgc cacggtaat acgttccccgg gccttgtaca caccggccgt
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aacgctgctg ntcgnntnag

C-6) Partial 16S rRNA gene sequence of *Erwinia soli* strain AR_PINLTS1 (NCBI LOCUS: HM582880, Size: 1446bp)

tncngcgggc tacacatgca agtcggacgg tagcacaggt anagcttgc ctccgggtga
cgagtggcgg acgggtgagt aatgtctggg aaactgcccg atggaggggg ataactactg
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atggcgcata caaagagaag cgacctcgcg agagcaagcg gacctcataa agtgcgtcgt
agtccggatc ggagcttgca actcgactcc gtgaagtcgg aatcgctagt aatcgtagat
cagaatgcta cggtaatac gttccgggc cttgtacaca ccgcgggtca caccatggga
gtgggttgca aaagaagtag gtagctttaga ctcntcgggt cgggcgcatt accannntng
atcnag

C-7) Partial 16S rRNA gene sequence of *Providencia vermicola* strain AR_PSBH1 (NCBI LOCUS: HM582881, Size: 1441bp)

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gcggcggacg ggtgagtaat gtatggggat ctgcccata gagggggata actactggaa
acggtgttca ataccgcata atctctttagg agcaaaagcag gggaaacttcg gtccttgcgc
tatcggatga acccatatgg gattagctag taggtggggat aatggctcac ctaggcgacg
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ttgttgccag cgattcggc gggaaactcaa aggagactgc cggtgataaa ccggaggaag
gtggggatga cgtcaagtca tcatggccct tacgagtagg gctacacacg tgctacaatg

gcgtatacaa agagaagcga cctcgcgaga gcaagcggaa ctcataaagt acgtcgttagt
ccggatttggg gtctgcaact cgactccatg aagtccgaaat cgcttagtaat cgttagatcag
aatgctacgg tgaatacgtt cccgggcctt gtacacaccg cccgtcacac catgggagtg
ggttgcaaaa gaagtaggta gcttaacctt cgggaggcg ctaccactn gntncannn
g

C-8) Partial 16S rRNA gene sequence of *Pseudomonas fulva* strain AR_PSTS1 (NCBI LOCUS: HM582882, Size: 1436bp)

ggnnnggcct acacatgcaa gtcgagcggt tgacgggagc ttgctccctg attcagcggc
ggacgggtga gtaatgccta ggaatctgcc tattagtggg ggacaacgtt tcgaaaggaa
cgctaataacc gcatacgtcc tacgggagaa agcaggggac cttcggcct tgcgctaata
gatgagccta ggtcggatta gcttgggtt gaggtaatgg ctcaccaagg cgacgatccg
taactggtct gagaggatga tcagtcacac tggaactgag acacggtcca gactcctacg
ggaggcagca gtgggaata ttggacaatg ggcgaaagcc tgatccagcc atgccgcgtg
tgtgaagaag gtcttcggat tgtaaagcac tttaagttgg gaggaagggt ttagattaa
tactctgcaa ttttgcgtt accgacagaa taagcaccgg ctaactctgt gccagcagcc
gcggtaataac agagggtgca agcgtaatcg gaattactgg gctgtaaagcg cgcttaggtg
gtttagttaag ttggatgtga aagccccggg ctcaacctgg gaactgcac caaaactggc
aagctagagt acggttagagg gtggtggaaat ttcctgtgtc gctgtgaaat gcgtagatat
aggaaggaac accagtggcg aaggcgacca cctggactga tactgacact gaggtgcgaa
agcgtgggaa gcaaacagga ttagataccc tgtagtcca cgccgtaaac gatgtcaact
agccgttggaa atccttgaga ttttagtggc gcagctaacg cattaagttt accgcctggg
gagtacggcc gcaaggtaa aactcaaatg aattgacggg ggcccgacca agcggtaggag
catgtggttt aattcgaagc aacgcgaaga accttacctg gccttgacat gctgagaact
ttccagagat ggattggcgc ctccggagc tcagacacag gtgctgcac gctgtcgtca
gctcgtgtcg tgagatgttgg gtttaagtcg cgtaaacgac gcaacccttgc tccttagtta
ccagcacctc gggtagggaaac tctaaggaga ctgcgggtc gaaaccggag gaaggtgggg
atgacgtcaa gtcatcatgg cccttacggc cagggctaca cacgtgtac aatgggtcggt
acaaagggtt gccaagccgc gaggtggagc taatcccata aaaccgatcg tagtccggat
cgctgtctgc aactcgactg cgtaaagtcg gaatcgctag taatgtgtc gaaatgtc
acggtaata cgttccccggg cttgtacac accgccccgtc acaccatggg agtgggttgc
accagaagta gcttagtcta accttcgggaa ggacggtacc ncggngannn ngnnnng

C-9) Partial 16S rRNA gene sequence of *Xanthomonas axonopodis* strain AR_PINLBH3 (NCBI LOCUS: HM582883, Size: 1444bp)

gngcagctac catgcagtcg aacggcagca cagtaagagc ttgctcttat gggtagggcag
tggcggacgg gtgaggaata catcgaaatc tactcttcg tggggataa cgtagggaaa
cttacgctaa taccgcatac gacctacggg taaaagcggaa ggaccttcgg gcttcgcgc

gttgaatgag ccgatgtcgg attagctagt tggcgggta aaggcccacc aaggcgacga
 tccttagctg gtctgagagg atgatcagcc acactggaac tgagacacgg tccagactcc
 tacgggaggc agcagtgggg aatattggac aatgggcgc a gcctgatcc agccatgccc
 cgtgggtgaa gaaggccttc gggttgtaaa gccctttgt tgggaaagaa aagcagtcgg
 ttaatacccg attgttctga cggtaacca a agaataagca ccggctaact tcgtgccagc
 agccgcggta atacgaaggg tgcaagcgtt actcgaatt actgggcgt a a g c g t g c g t
 aggtgggtgg ttaagtctgt tgtgaaagcc ctggcgtcaa cctgggatt gcagtggata
 ctggatcact agagtggtt agagggttagc ggaattcccg gtgttagcagt gaaatgcgt
 gagatcggga ggaacatccg tggcgaaggc ggctacctgg accaacactg acactgaggc
 acgaaagcgt ggggagcaaa caggattaga taccctggta gtccacgccc taaacatgc
 gaactggatg ttgggtgcaa ttggcacgc agtatcgaag ctaacgcgtt aagttcgccg
 cctggggagt acggtcgcaa gactgaaact caaaggaatt gacgggggcc cgacacaagcg
 gtggagttatg tggtaatt ccatgcaacg cgaagaacct tacctggtct tgacatccac
 ggaactttcc agagatggat tggcgttc gggaccgtg agacaggtgc tgcatggctg
 tcgtcagctc gtgtcgtgag atggtgggaa aagtccgc a acgagcgc a cccttgcct
 tagttgccag cacgtaatgg tgggactct aaggagaccg ccggtgacaa accggaggaa
 ggtggggatg acgtcaagtc atcatggccc ttacgaccag ggctacacac gtactacaat
 gtagggaca gaggcgtca aaccgcgag ggcaagccaa tcccagaaac cctatctcag
 tccggattgg agtctgcaac tcgactccat gaagtcggaa tcgctagtaa tcgcagatca
 gcattgctgc ggtgaatacg ttcccggcc ttgtactaca ccgcgggtca caccatggga
 gtttggca ccagaagcag gtagcttaac ctcgggaggc cgcttgcnc ggtggnn can
 ngng

Appendix D – ANOVA Tables

D-1) ANOVA on antibacterial activity of *Centella asiatica* subspecies A and B against the growth of *Bacillus cereus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1199.625	7	171.375	1096.800	0.000
Within Groups	2.500	16	0.156		
Total	1202.125	23			

D-2) ANOVA on antibacterial activity of *Centella asiatica* subspecies A and B against the growth of *Escherichia coli*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1640.625	7	234.375	625.000	0.000
Within Groups	6.000	16	0.375		
Total	1646.625	23			

D-3) ANOVA on antibacterial activity of *Centella asiatica* subspecies A and B against the growth of *Pseudomonas aeruginosa*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	941.625	7	134.518	153.735	0.000
Within Groups	14.000	16	0.875		
Total	955.625	23			

D-4) ANOVA on antibacterial activity of *Centella asiatica* subspecies A and B against the growth of *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2362.500	7	337.500	2700.000	0.000
Within Groups	2.000	16	0.125		
Total	2364.500	23			

D-5) ANOVA on erythrocytes hemolysis prevention potential of *Centella asiatica* subspecies A and B

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	3601.852	7	514.550	468.216	0.000
Within Groups	17.583	16	1.099		
Total	3619.435	23			

D-6) ANOVA on DPPH scavenging activity of *Centella asiatica* subspecies A and B

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	7889.528	7	1127.075	180.485	0.000
Within Groups	99.915	16	6.245		
Total	7989.443	23			

D-7) ANOVA on SOD activity of *Centella asiatica* subspecies A and B

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	189.348	7	27.050	70.967	0.000
Within Groups	6.099	16	0.381		
Total	195.446	23			

D-8) ANOVA on total phenolic contents of *Centella asiatica* subspecies A and B

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	7.670	5	1.534	69.727	0.000
Within Groups	0.264	12	0.022		
Total	7.934	17			

D-9) ANOVA on antibacterial activity of *Centella asiatica* inoculated with *Enterobacter* sp. against the growth of *Bacillus cereus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1412.727	10	141.273	188.364	0.000
Within Groups	16.500	22	0.750		
Total	1429.227	32			

D-10) ANOVA on antibacterial activity of *Centella asiatica* inoculated with *Enterobacter* sp. against the growth of *Escherichia coli*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2125.712	10	212.571	572.641	0.000
Within Groups	8.167	22	0.371		
Total	2133.879	32			

D-11) ANOVA on antibacterial activity of *Centella asiatica* inoculated with *Enterobacter* sp. against the growth of *Pseudomonas aeruginosa*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	859.636	10	85.964	102.227	0.000
Within Groups	18.500	22	0.841		
Total	878.136	32			

D-12) ANOVA on antibacterial activity of *Centella asiatica* inoculated with *Enterobacter sp.* against the growth of *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2620.909	10	262.091	3844.000	0.000
Within Groups	1.500	22	0.068		
Total	2622.409	32			

D-13) ANOVA on DPPH scavenging of *Centella asiatica* inoculated with *Enterobacter sp.*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	10967.418	10	1096.742	139.118	0.000
Within Groups	173.438	22	7.884		
Total	11140.855	32			

D-14) ANOVA on SOD activity of *Centella asiatica* inoculated with *Enterobacter sp.*

	Sum of Squares	Df	Mean Square	F. value	Pr > F
Between Groups	121.636	10	12.164	31.953	0.000
Within Groups	8.375	22	0.381		
Total	130.010	32			

D-15) ANOVA on total phenolic contents of *Centella asiatica* inoculated with *Enterobacter sp.*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	11.425	8	1.428	44.019	0.000
Within Groups	0.584	18	0.032		
Total	12.009	26			

D-16) ANOVA on antibacterial activity of *Centella asiatica* treated with *Pseudomonas sp.* against the growth of *Bacillus cereus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1344.000	7	192.000	558.545	0.000
Within Groups	5.500	16	0.344		
Total	1349.500	23			

D-17) ANOVA on antibacterial activity of *Centella asiatica* treated with *Pseudomonas sp.* against the growth of *Escherichia coli*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1774.500	7	253.500	624.000	0.000
Within Groups	6.500	16	0.406		
Total	1781.000	23			

D-18) ANOVA on antibacterial activity of *Centella asiatica* treated with *Pseudomonas sp.* against the growth of *Pseudomonas aeruginosa*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1070.625	7	152.946	271.905	0.000
Within Groups	9.000	16	0.563		
Total	1079.625	23			

D-19) ANOVA on antibacterial activity of *Centella asiatica* treated with *Pseudomonas sp.* against the growth of *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2522.625	7	360.375	549.143	0.000
Within Groups	10.500	16	0.656		
Total	2533.125	23			

D-20) ANOVA on DPPH scavenging of *Centella asiatica* treated with *Pseudomonas sp.*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	9668.363	7	1381.195	48.109	0.000
Within Groups	459.358	16	28.710		
Total	10127.722	23			

D-21) ANOVA on SOD activity of *Centella asiatica* treated with *Pseudomonas sp.*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	94.691	7	13.527	31.746	0.000
Within Groups	6.818	16	0.426		
Total	101.509	23			

D-22) ANOVA on total phenolic contents of *Centella asiatica* treated with *Pseudomonas sp.*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	43.338	5	8.668	123.618	0.000
Within Groups	0.841	12	0.070		
Total	44.180	17			

D-23) ANOVA on antibacterial activity of the isolated endophytic bacteria associated with *Centella asiatica* against the growth of *Bacillus cereus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1320.000	10	132.000	1452.000	0.000
Within Groups	2.000	22	0.091		
Total	1322.000	32			

D-24) ANOVA on antibacterial activity of the isolated endophytic bacteria associated with *Centella asiatica* against the growth of *Escherichia coli*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	1843.636	10	184.364	2028.000	0.000
Within Groups	2.000	22	0.091		
Total	1845.636	32			

D-25) ANOVA on antibacterial activity of the isolated endophytic bacteria associated with *Centella asiatica* against the growth of *Pseudomonas aeruginosa*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	724.909	10	72.491	88.600	0.000
Within Groups	18.000	22	0.818		
Total	742.909	32			

D-26) ANOVA on antibacterial activity of the isolated endophytic bacteria associated with *Centella asiatica* against the growth of *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2620.909	10	262.091	3844.000	0.000
Within Groups	1.500	22	0.068		
Total	2622.409	32			

D-27) ANOVA on erythrocytes hemolysis prevention potential of the isolated endophytic bacteria associated with *Centella asiatica*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	2231.659	10	223.166	14.934	0.000
Within Groups	328.759	22	14.944		
Total	2560.418	32			

D-28) ANOVA on DPPH scavenging of the isolated endophytic bacteria associated with *Centella asiatica*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	12887.306	10	1288.731	126.228	0.000
Within Groups	224.611	22	10.210		
Total	13111.917	32			

D-29) ANOVA on SOD activity of the isolated endophytic bacteria associated with *Centella asiatica*

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	21291.049	10	2129.105	190.237	0.000
Within Groups	246.220	22	11.192		
Total	21537.270	32			

D-30) ANOVA on percentage of callus induction from *Centella asiatica* leaf explant

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	25000.000	3	8333.333	20.000	0.000
Within Groups	5000.000	12	416.667		
Total	30000.000	15			

D-31) ANOVA on fresh weight of callus produced from *Centella asiatica* leaf explant

	Sum of Squares	df	Mean Square	F. value	Pr > F
Between Groups	16710.453	3	5570.151	516.710	0.000
Within Groups	129.360	12	10.780		
Total	16839.814	15			