SUPPLEMENTARY FILE

Biofilm-Associated Agr and Sar Quorum Sensing Systems of *Staphylococcus aureus* are Inhibited by 3-Hydroxybenzoic Acid Derived from *Illicium verum*

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Supplementary data

Table S1: Binding affinity calculated between AgrA from *Staphylococcus aureus* and phyto-constituents reported in GC-MS report

C. ID	Compound name	Binding Affinity (kcal/mol)	Amino Acid	Hydrogen Bonding	H-Bond Length (Å)	Hydrophobic Contacts
985	Palmitic acid	-3.9	ILE143,GLU144,LYS146,PHE182,TYR183,GLY184,ASN185,GLU188,LEU189&192	ASN185	3.58	ILE143,GLU144,LYS146,PHE182,TYR183,GLY184,,GLU188,LEU189&192
5281	Octadecanoic acid	-3.7	SER164&165,SER168,HIS169,ARG170,LEU171,ASN185,LEU186,LYS187,ARG198,ASN201	SER164&165	3.08, 3.40	SER168,HIS169,ARG170,LEU171,ASN185,LEU186,LYS187,ARG198,ASN201
7136	Eugenol acetate	-4.6	SER164&165,SER168,HIS169,ARG170,LEU171,ASN185,LEU186,LYS187,ARG198,ASN201	LEU186, LYS187	4.22, 4.17	SER164&165,SER168,HIS169,ARG170,LEU171,ASN185,LEU186,LYS187,ARG198,ASN201
7420	3-hydroxybenzoic acid	-4.4	ILE143,GLU144,LEU145,LYS146PHE182,TYR183,GLY184,ASN185,GLU188,LEU189,LEU192	GLU144,ASN185	3.01, 3.12	ILE143,LEU145,LYS146,PHE182,TYR183,GLY184,GLU188,LEU189,LEU192
11569	Benzaldehyde, 3-methoxy-	-4	ILE143,GLU144,LEU145,LYS146PHE182,TYR183,GLU188,LEU189,LEU192	GLU144	4.09	ILE143,LEU145,LYS146 PHE182,TYR183,GLU188,LEU189,LEU192
31244	Benzaldehyde,4-methoxy-	-3.8	SER164,LEU171,ASN185,LEU186,LYS187,ARG198	SER164,LYS187,ARG198	4.28, 3.69, 5.59	LEU171,ASN185,LEU186,
61007	Anisylacetone	-4.6	ILE143,GLU144,LYS146,TYR 156,PHE182,ASN185,GLU188,LEU189,LEU192	GLU144	3.91	ILE143,LYS146,TYR 156,PHE182,ASN185,GLU188,LEU189,LEU192
86608	alpha.-Cubebene	-4.7	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,GLU188,LEU189,LEU192			ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,GLU188,LEU189,LEU192
86609	alpha.-Cubebene	-5.2	THR142,ILE143,GLU144,LYS146,PHE182,TYR183,GLY184,GLU188,LEU189,LEU192			THR142,ILE143,GLU144,LYS146,PHE182,TYR183,GLY184,GLU188,LEU189,LEU192
112056	4-Methoxymandelic acid	-4.6	THR142,ILE143,GLU144,LYS146,PHE182,TYR183,GLY184,ASN185,GLU188,LEU189,LEU192	GLU144, TYR183,ASN145	4.28, 6.98, 4.01	THR142,ILE143,LYS146,PHE182,GLY184,GLU188,LEU189,LEU192
519764	Beta-Sesquiphellandrene	-4.8	ILE143,GLU144,LYS146,PH			ILE143,GLU144,LYS146,

			E182,TYR183,ASN185,GLU188,LEU189,LEU192			PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
556368	Tricyclo[4.3.1.0 2,5]decane	-4.7	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192			ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
637563	Anethole	-4.2	THR142,ILE143,GLU144,TYR183,GLY184ASN185,GLU188,LEU189,LEU192			THR142,ILE143,GLU144,TYR183,GLY184ASN185,GLU188,LEU189,LEU192
5317319	(Z)-.beta.-Farnesene	-4.2	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,GLY184ASN185,GLU188,LEU189,LEU192			ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,GLY184ASN185,GLU188,LEU189,LEU192
5356544	d-Nerolidol or Peruviol	-4.7	THR142,ILE143,GLU144,LYS146,TYR154,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192			THR142,ILE143,GLU144,LYS146,TYR154,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
5365626	(Z)6-Pentadecen-1-ol	-3.7	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192	ASN185	3.29	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,GLU188,LEU189,LEU192
6429302	trans-alpha-bergamotene	-4.7	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192			ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
10104370	1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-1-cyclohexene	-5	ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192			ILE143,GLU144,LEU145,LYS146,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
91746961	Feniculin	-4.7	THR142,ILE143,GLU144,LYS146,TYR156,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192	LYS146	4.21	THR142,ILE143,GLU144,TYR156,PHE182,TYR183,ASN185,GLU188,LEU189,LEU192
	p-Methoxy-N-methyl-mandelic acid amide	-4.6	THR142,ILE143,GLU144,LEU145,LYS146,TYR156,PHE182,TYR183,GLY184,GLU188,LEU189,LEU192	GLU144, LYS146, TYR183	4.47, 4.23, 6.43	THR142,ILE143,LEU145,TYR156,PHE182,GLY184,GLU188,LEU189,LEU192

Table S2: Binding affinity calculated between SarA from *Staphylococcus aureus* and phyto-constituents reported in GC-MS report

C. ID	Name of the compounds	Binding Affinity (kcal/mol)	Amino Acid	Hydrogen Bonding	H-Bond Length (Å)	Hydrophobic Contacts
10104370	Palmitic acid	-4.8	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
112056	Octadecanoic acid	-4.3	LYS121,SER124,LEU125,LYS128,ALA218,GLU221,ILE222	GLU221	2.79,4.41	LYS121,SER124,LEU125,LYS128,ALA218,ILE222
11569	Eugenol acetate	-3.9	ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
31244	3-hydroxybenzoic acid	-4.1	LYS121,SER124,LEU125,LYS128,GLU221,ILE222	GLU129,ARG210	3.22, 3.10	LYS121,SER124,LEU125,LYS128,GLU221,ILE222
519764	Benzaldehyde, 3-methoxy-	-4.7	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			THR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
5281	Benzaldehyde,4-methoxy-	-4.2	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,GLU145,LEU160,ILE215	GLU145	5.46	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,LEU160,ILE215
5317319	Anisylacetone	-4.4	MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,GLU145,LEU160,ILE215			MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,GLU145,LEU160,ILE215
5356544	alpha.-Cubebene	-4.8	MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,GLU145,LEU160,ILE215			MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,GLU145,LEU160,ILE215
5365626	alpha.-Cubebene	-4.1	MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,TYR142,LEU160,ILE215			MET115,TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,TYR142,LEU160,ILE215
556368	4-Methoxymandelic acid	-4.9	ALA119,LEU122,LYS123,ILE126,PHE134,PHE13			ALA119,LEU122,LYS12

			7,ILE215			3,ILE126,PHE134,PHE137,ILE215
61007	Beta-Sesquiphellandrene	-4.3	LYS121,SER124,LEU125,LYS128,GLU221,ILE222,GLU223	LYS128	4.51	LYS121,SER124,LEU125,LYS128,GLU221,ILE222,GLU223
637563	Tricyclo[4.3.1.0 2,5]decane	-4.5	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
6429302	Anethole	-5.7	ALA119,ASP120,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			ALA119,ASP120,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
7136	(Z)-.beta.-Farnesene	-4.9	LYS121,SER124,LEU125,LYS128,GLU221,ILE222,GLU223	LYS128	4.39	LYS121,SER124,LEU125,GLU221,ILE222,GLU223
7420	d-Nerolidol or Peruviol	-4.1	GLU129,PHE130,ARG210,LYS213,ARG214,GLU217	ARG210	4.90	GLU129,PHE130,ARG210,LYS213,ARG214,GLU217
86608	(Z)6-Pentadecen-1-ol	-5.7	ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
86609	trans-alpha-bergamotene	-5.4	ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215			ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ILE215
91746961	1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-1-cyclohexene	-5.2	LYS121,SER124,LEU125,LYS128,GLU129,GLU221,ILE222,GLU223			LYS121,SER124,LEU125,LYS128,GLU129,GLU221,ILE222,GLU223
985	Feniculin	-4	TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,TYR162,ILE215			TYR118,ALA119,LEU122,LYS123,ILE126,PHE134,PHE137,ALA138,THR141,TYR162,ILE215
	p-Methoxy-N-methyl-mandelic acid amide	-4.3	LYS121,SER124,LEU125,LYS128,ALA218,GLU221,ILE222,GLU223	GLU221	4.07	LYS121,SER124,LEU125,LYS128,ALA218,ILE222,GLU223

Table S3: Antibiogram profiles of *S. aureus* (SA-01) and *S. aureus* (SA-02) strains employed in the investigation.

S. No	Antibiotics	SA-01		SA-02	
		SD value	R/S	SD value	R/S
1	Penicillin G	-	R	31.3 ± 1.1	S
2	Methicillin	-	R	24 ± 1.15	S
3	Amoxycillin	-	R	36.3 ± 0.5	S
4	Ampicillin	0.8 ± 0.1	R	34.1 ± 0.15	S
5	Colistin	-	R	-	R
6	Tigecycline	15.6±0.5	IM	20.6±0.5	S
7	Imipenem	10±0	R	11±1.73	R
8	Kanamycin	-	R	19±1	S
9	Ciprofloxacin	-	R	8.6±0.5	R
10	Streptomycin	-	R	14±0	IM
11	Ceftazidime	-	R	12.6±0.5	R
12	Cephalothin	-	R	38.8±1.04	S
13	Chloramphenicol	16±1.7	R	19±0	S
14	Gentamicin		R	20.3±0.5	S

Note: “R”- Resistant, “S”- Sensitive, “IM”- Intermediate, SD

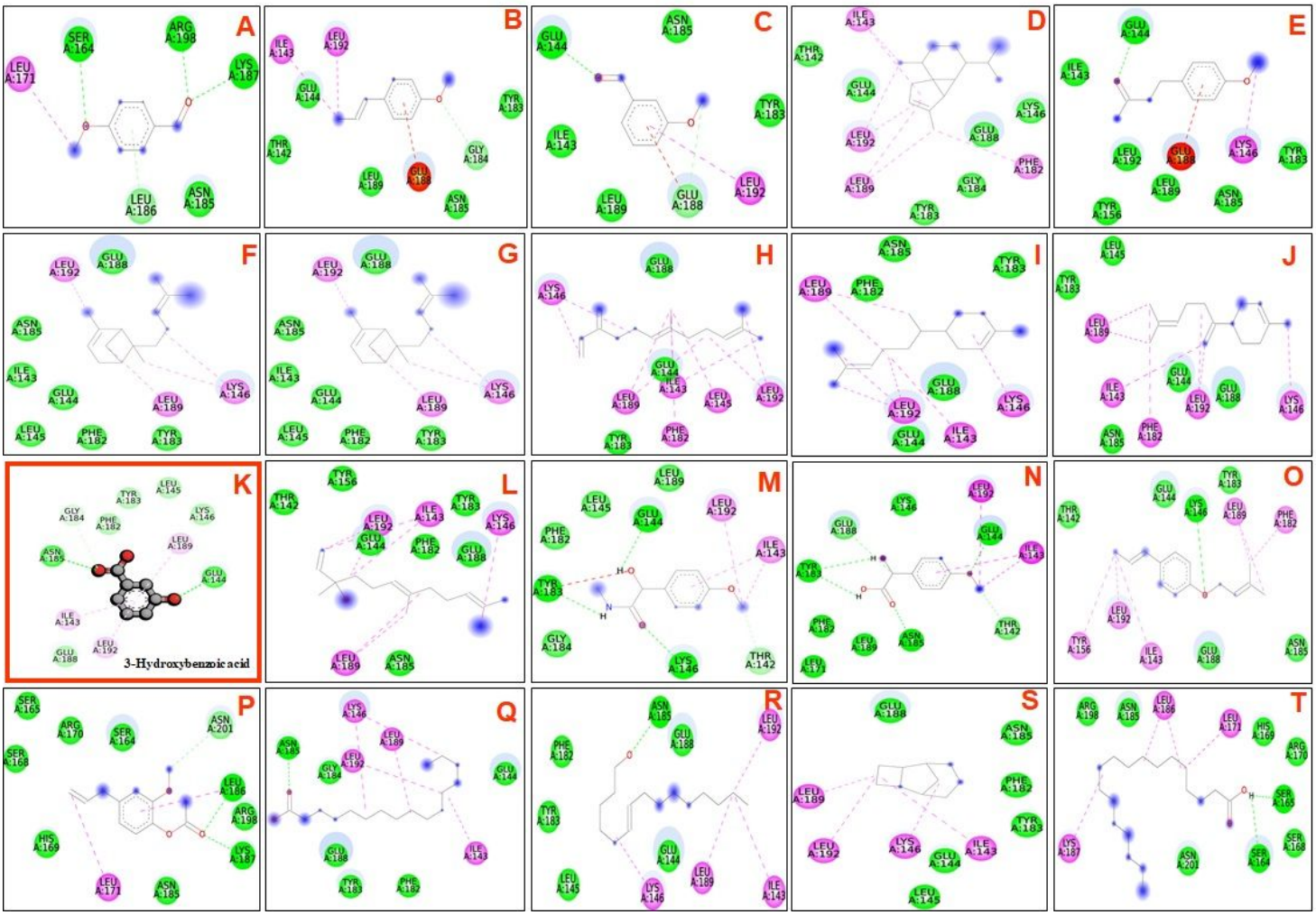

Figure S1.3-Hydroxybenzoic acid bound binding site amino acids (AA) in AgrA protein were shown in 2D interaction. Amino acids were colored and represented as its role in biochemical reaction with small molecules. Hydrogen bonding and hydrophobic interaction in protein complex was modeled and revealed by dashed lines. **(A)** Benzaldehyde,4-methoxy-, **(B)** Anethole, **(C)** Benzaldehyde, **(D)** 3-methoxy-, alpha.-Cubebene, **(E)** Anisylacetone, **(F)** alpha-bergamotene, **(G)** trans-alpha-bergamotene, **(H)** (Z)-.beta.-Farnesene, **(I)** Beta-Sesquiphellandrene, **(J)** 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-1-cyclohexene, **(K)** 3-hydroxybenzoic acid (Ball and stick), **(L)** d-Nerolidol or Peruviol, **(M)** p-Methoxy-N-methyl-mandelic acid amide, **(N)** 4-Methoxymandelic acid, **(O)** Feniculin, **(P)** Eugenol acetate, **(Q)** Palmitic acid, **(R)** (Z)6-Pentadecen-1-ol, **(S)** Tricyclo[4.3.1.0 2,5]decane, **(T)** Octadecanoic acid.

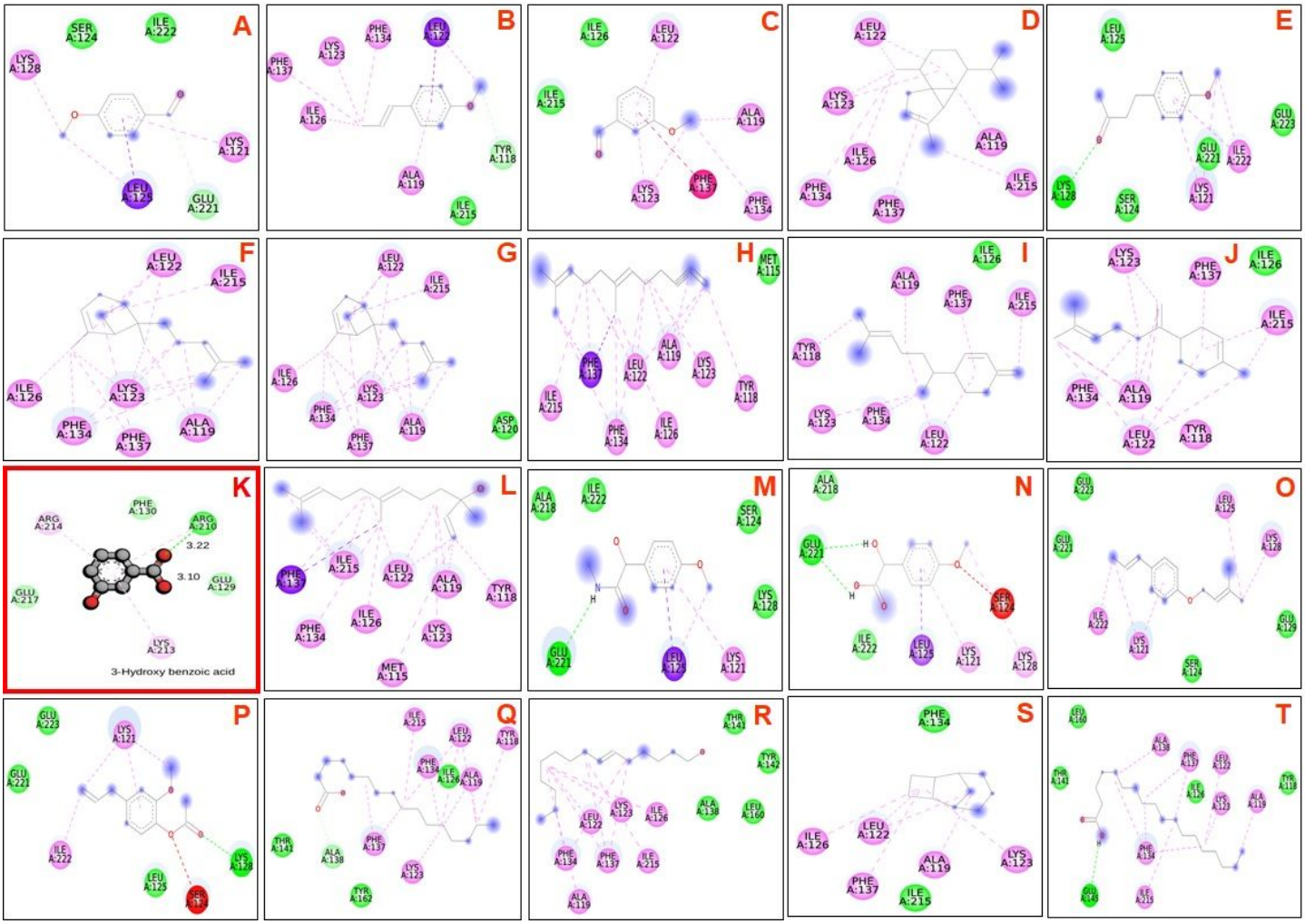

Figure S2. 3-Hydroxybenzoic acid interacting amino acids (AA) in SarA protein were shown in 2D interaction. Amino acids were colored and represented as its role in biochemical reaction with small molecules. Hydrogen bonding and hydrophobic interaction in protein complex was modeled and revealed by dashed lines. **(A)** Benzaldehyde,4-methoxy-, **(B)** Anethole, **(C)** Benzaldehyde, **(D)** 3-methoxy-, alpha.-Cubebene, **(E)** Anisylacetone, **(F)** alpha-bergamotene, **(G)** trans-alpha-bergamotene, **(H)** (Z)-.beta.-Farnesene, **(I)** Beta-Sesquiphellandrene, **(J)** 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-1-cyclohexene, **(K)** 3-hydroxybenzoic acid (Ball and stick), **(L)** d-Nerolidol or Peruviol, **(M)** p-Methoxy-N-methyl-mandelic acid amide, **(N)** 4-Methoxymandelic acid, **(O)** Feniculin, **(P)** Eugenol acetate, **(Q)** Palmitic acid, **(R)** (Z)6-Pentadecen-1-ol, **(S)** Tricyclo[4.3.1.0 2,5]decane, **(T)** Octadecanoic acid.

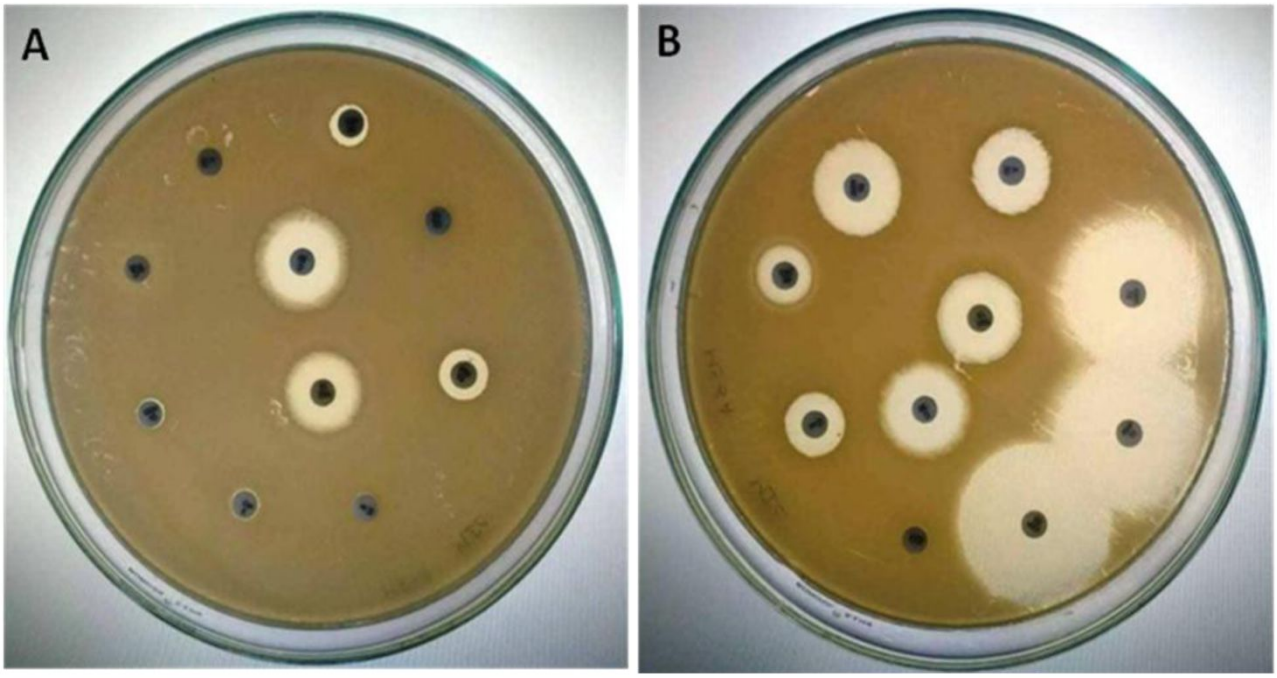

Figure S3: Antibiogram profile of test *S. aureus* strains by Kirby–Bauer disk diffusion method. A) *S. aureus* SA-01 B) *S. aureus* SA-02.

Figure S4: Electrospray ion chromatogram and mass spectrum of methanol extract of *I. verum* constituents (Peak # 1 to 20) as analyzed by GC-MS.

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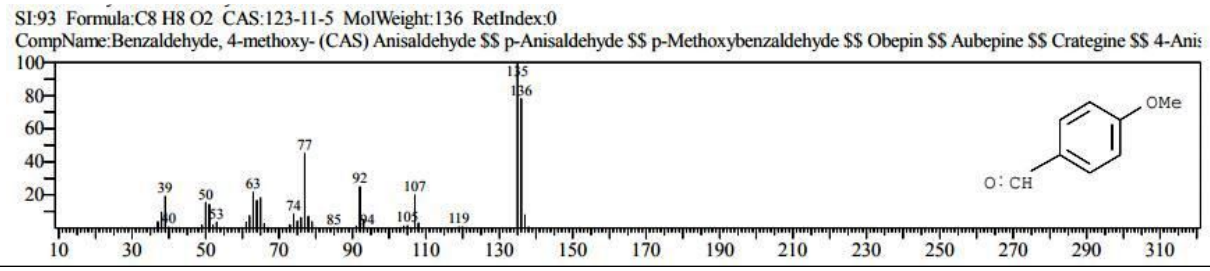

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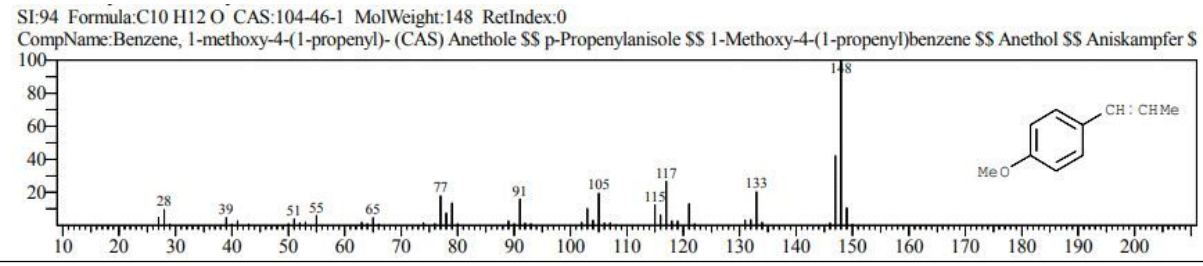

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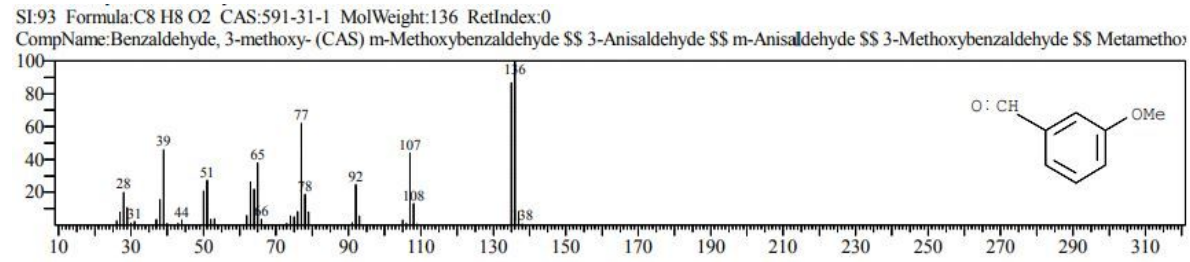

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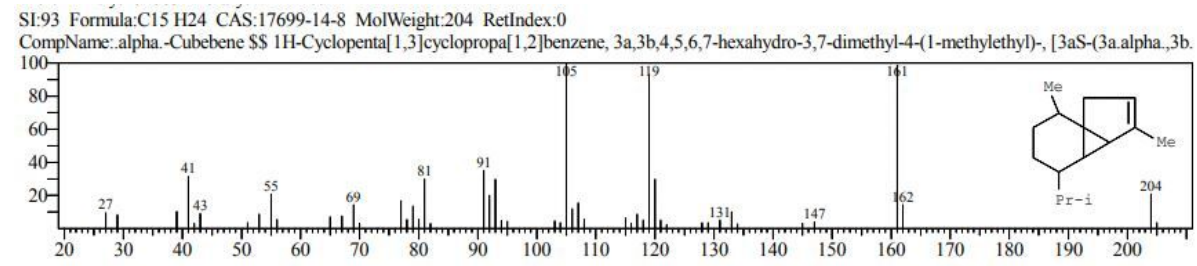

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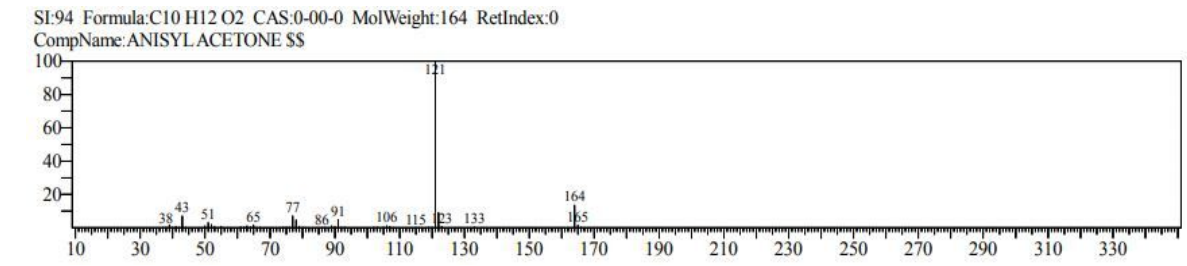

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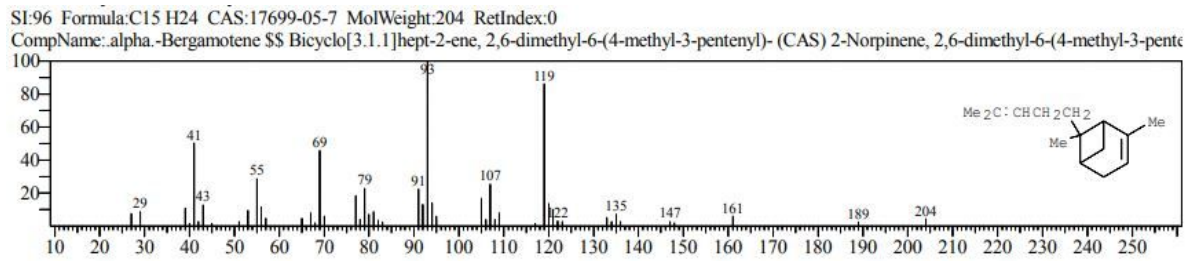

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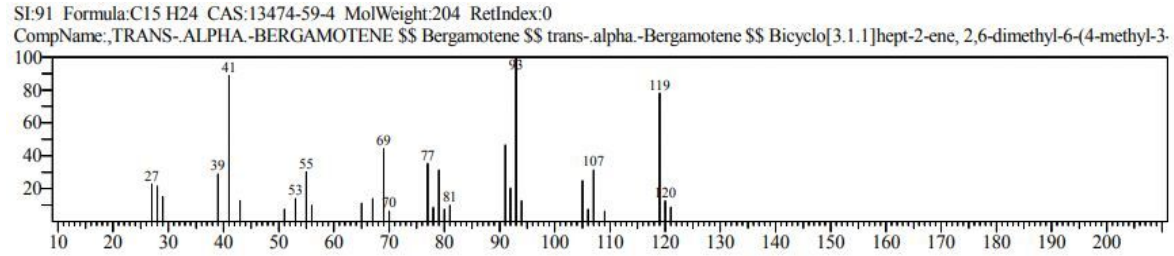

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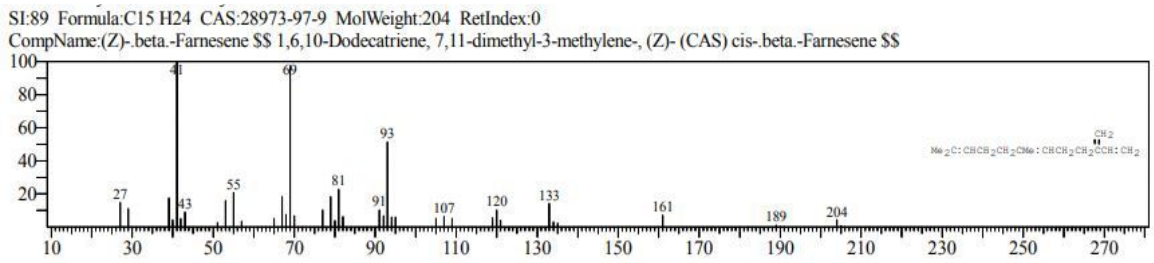

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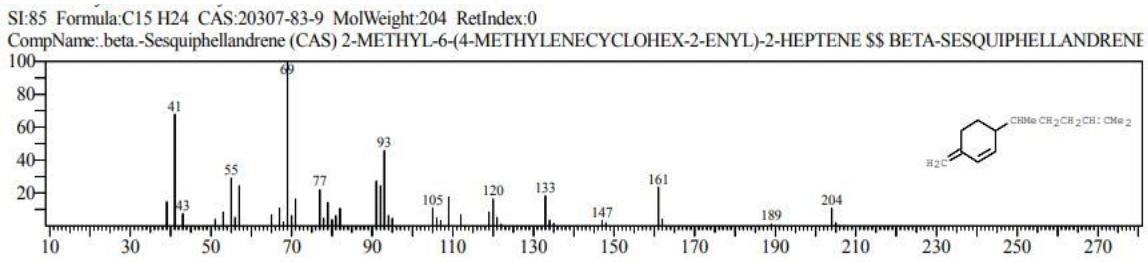

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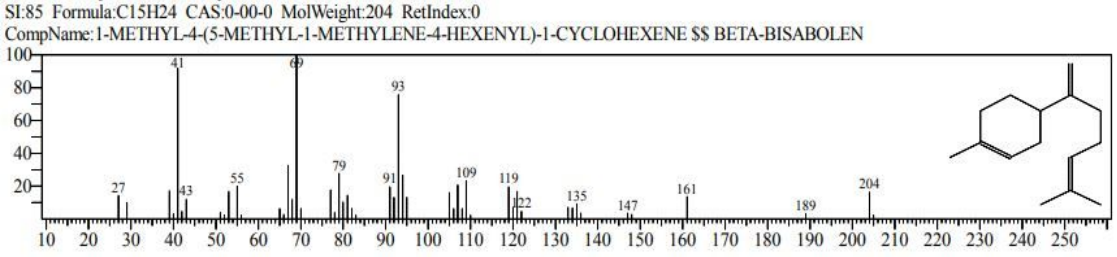

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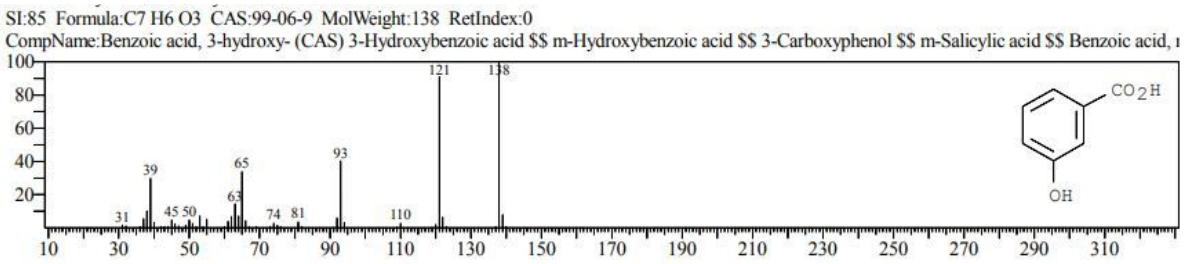

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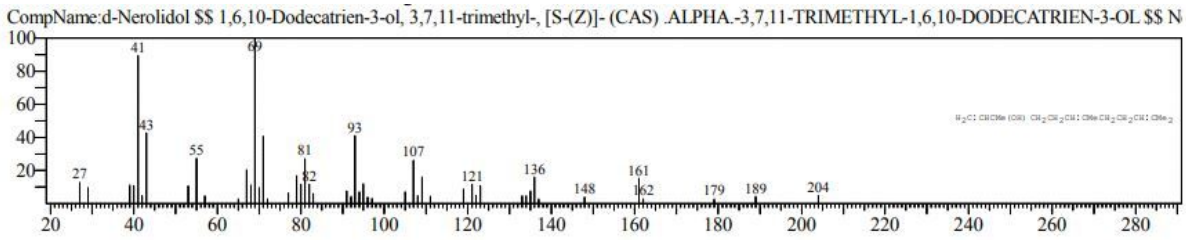

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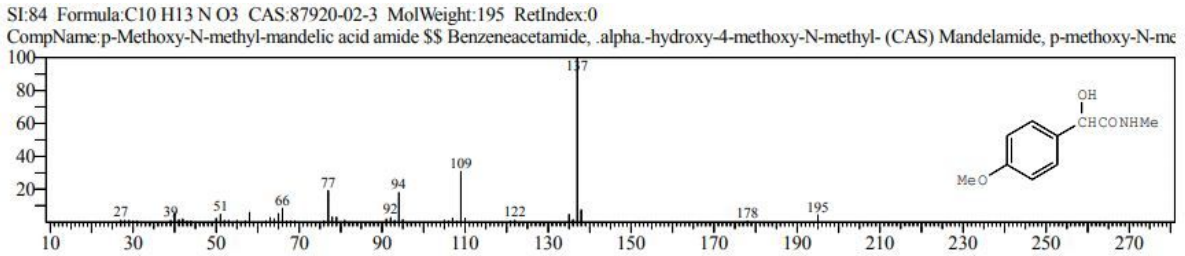

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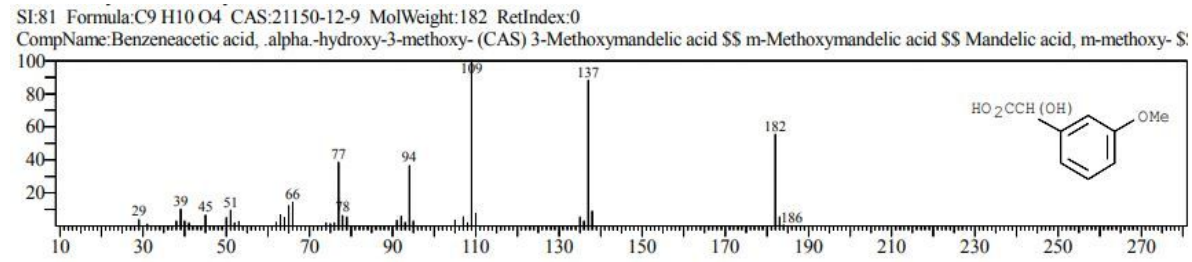

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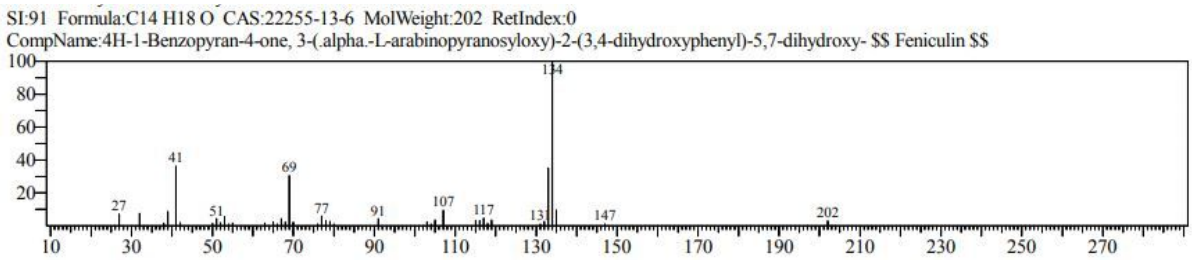

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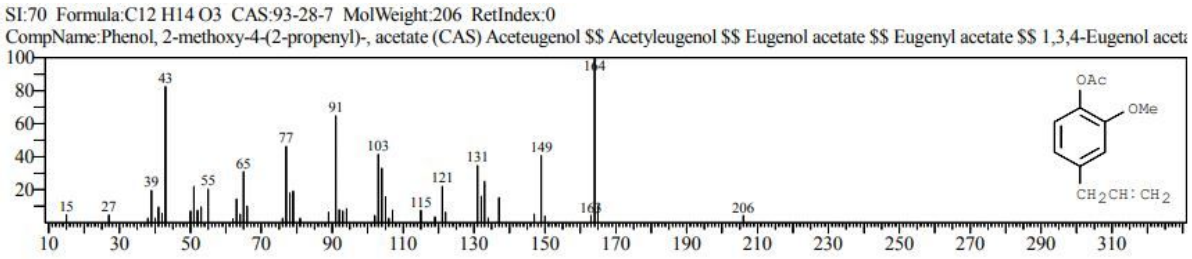
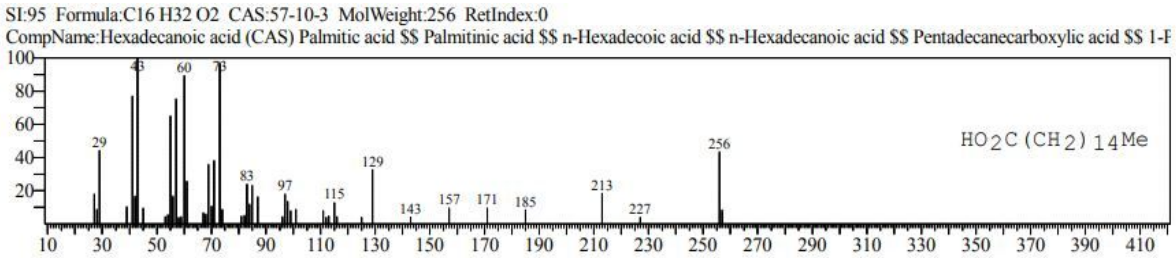
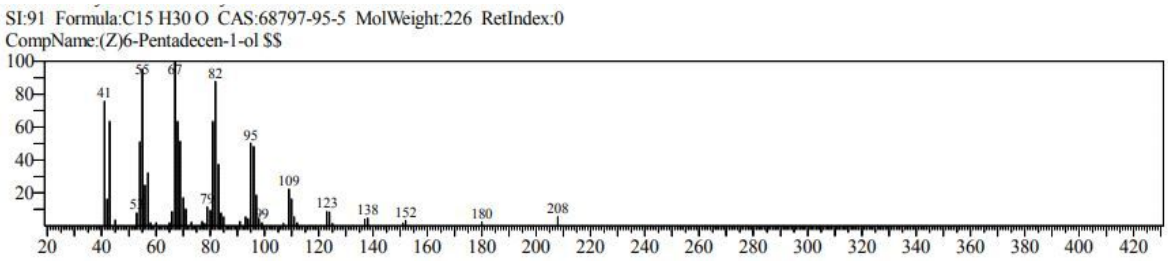


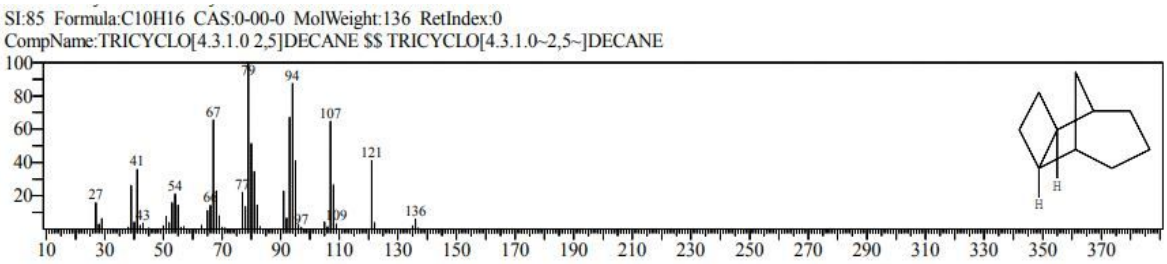
Peak # 17:



Peak # 18:



Peak # 19:



Peak # 20: