

Supplementary Information

Bioactive compound from *Micrococcus luteus* associated with *Datura stramonium* L. seeds

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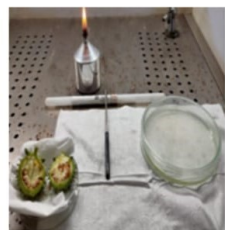
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Figure S1 Isolated endophytic bacterial colonies



Collection of Datura seeds



Petri-plates inoculated with seeds



Fermentation of isolated organism



Extraction of crude extract

Figure S2 Isolation and purification of bioactive compound from endophytic bacteria

>907R Seq153_DS5

GCATGGACTACCAGGGTATCTAATCCTGTTTCGCTCCCCATGCTTTCGCTCCTCA
GCGTCAGTTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCCTCCTGATATC
TGCGCATTCCACCGCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTC
TGCCCGTACCCACCGCAGATCCGGGGTTAAGCCCCGGACTTTCACGACAGACG
CGACAAACCGCCTACGAGCTCTTTACGCCAATAATTCCGGATAACGCTCGCA
CCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTCTTCTGCAG
GTACCGTCACTTTCGCTTCTTCCCTACTGAAAGAGGTTTACAACCCGAAGGCCG
TCATCCCTCACGCGGCGTTCGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCC
CCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGG
TCACCCTCTCAGGCCGGCTACCCGTCGTCGCTTGGTGAGCCATTACCTCACCA
ACAAGCTGATAGGCCGCGAGTCCATCCAAAACCGATAAATCTTTCCAACACCC
ACCATGCGGTGGACGCTCCTATCCGGTATTAGACCCAGTTTCCCAGGCTTATCC
CAGAGTTAAGGGCAGGTTACTCACGTGTTACTACCCGTTTCGCCACTAATCCAC
CC

Figure S3 Genotypic characterisation of the isolate DS5. The DS5 isolate was subjected to molecular identification and characterization based on their 16S rRNA sequence.

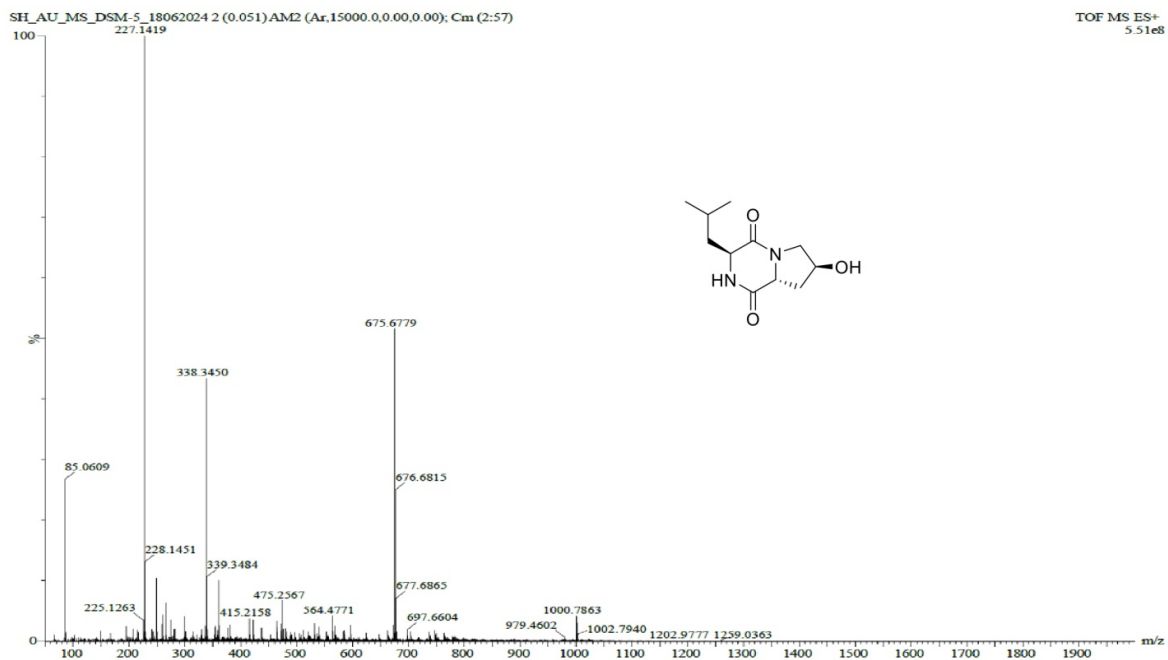
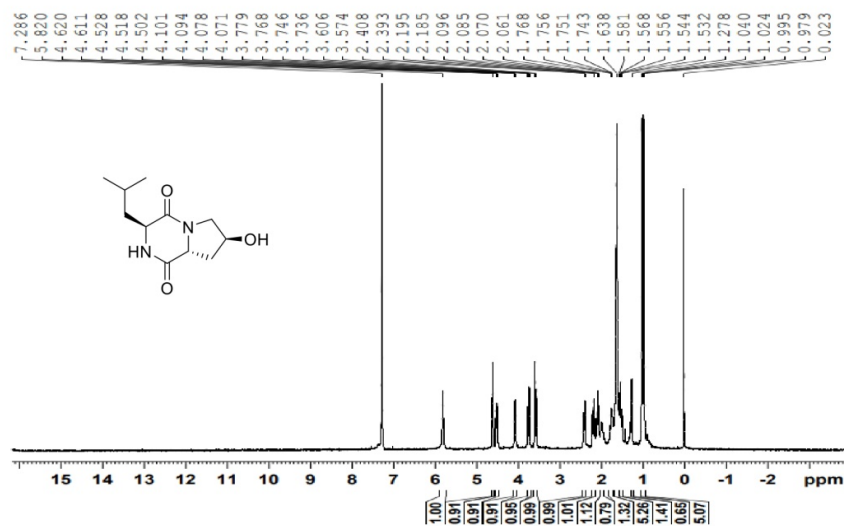


Figure S4 HRMS of compound DSM-05

F11-14-40%FRACTION-CDCL3-PROTON



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PROCNO 1

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PULPROG zg30
TD 65536
SOLVENT CDCl3
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DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 162.09
DW 62.400 usec
DE 6.50 usec
TE 298.2 K
D1 3.00000000 sec
TDO 1
SFO1 400.1324708 MHz
NUC1 1H
P1 15.00 usec
PLW1 10.21100044 W

F2 - Processing parameters
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LB 0.30 Hz
GB 0
PC 1.00

Figure S5 ¹H NMR of compound DSM-05

F11-14-40%FRACTION-CDCL3-CARBON

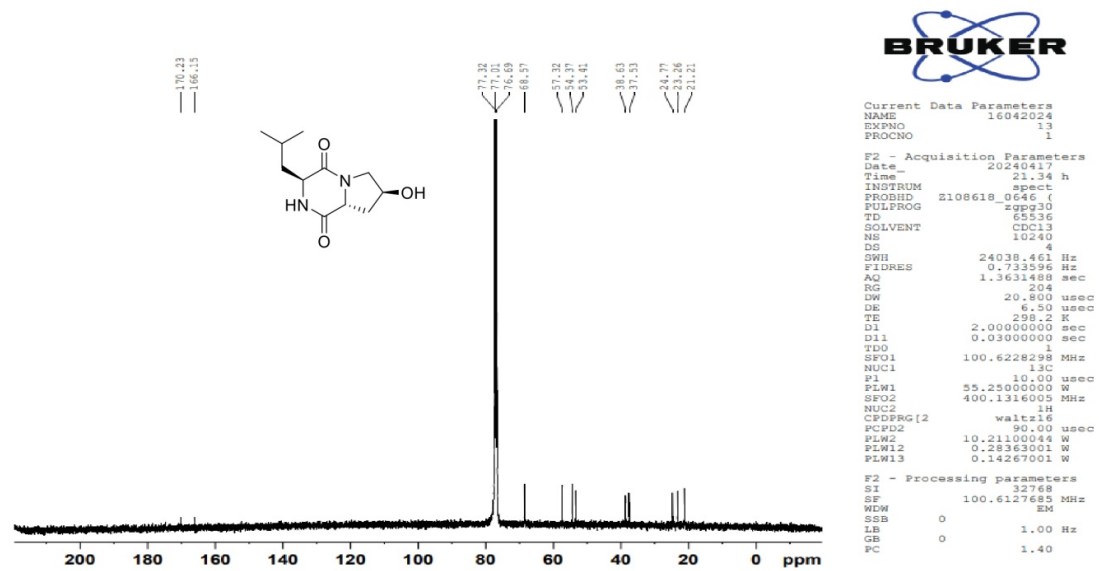


Figure S6 ¹³CNMR of compound DSM-05

F11-14-40%FRACTION-CDCL3-COSY

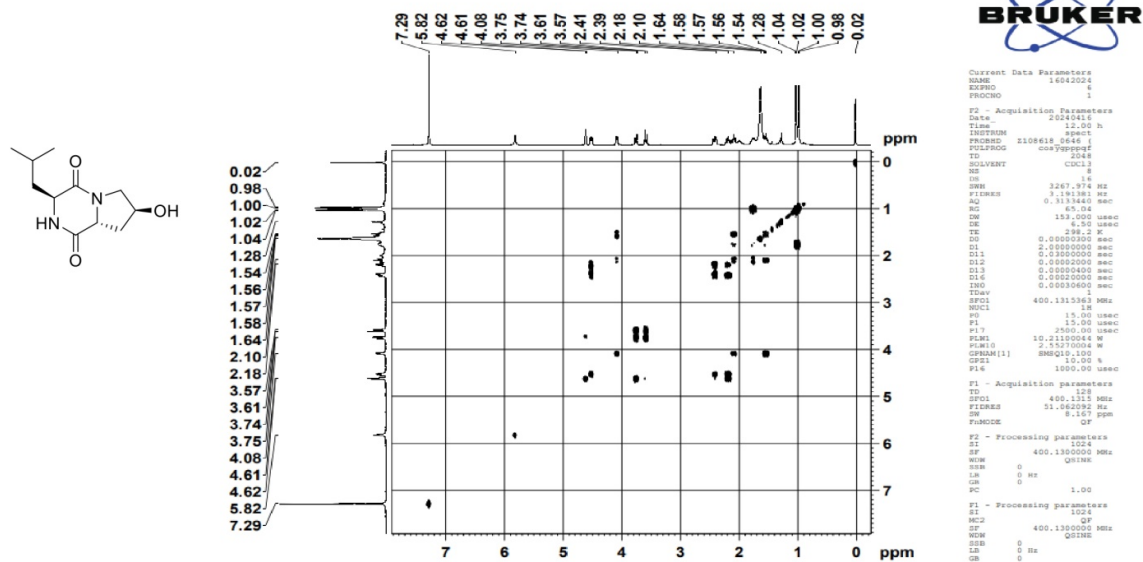
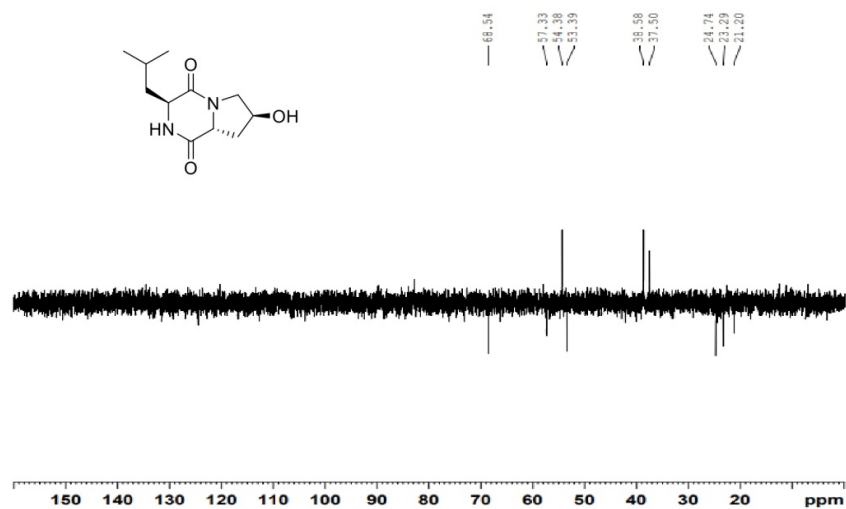


Figure S7 COSY of compound DSM-05

F11-14-40%FRACTION-CDCL3-DEPT



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PROCNO    1

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PULPROG   zgpg135
ZD        65536
SOLVENT   CDCl3
NS        256
DS        6
SWH        16129.032 Hz
FIDRES     0.492219 Hz
AQ         2.0316160 sec
RG         204
SQ         31.000 usec
DE         6.50 usec
TE         298.1 K
CNST2     145.0000000
D1         2.0000000 sec
D2         0.00344828 sec
D12        0.00002000 sec
TD         1
SFO1       100.6208175 MHz
NUC1       13C
P1         10.00 usec
P2         20.00 usec
PLM1       55.2500000 W
SFO2       400.1316005 MHz
NUC2       1H
CPDPRG2    waltz16
PS         15.00 usec
P4         30.00 usec
PCPD2     90.00 usec
PLM2      10.21100044 W
PLM12     0.28363001 W

F2 - Processing parameters
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PC         1.40
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Figure S8 DEPT of compound DSM-05

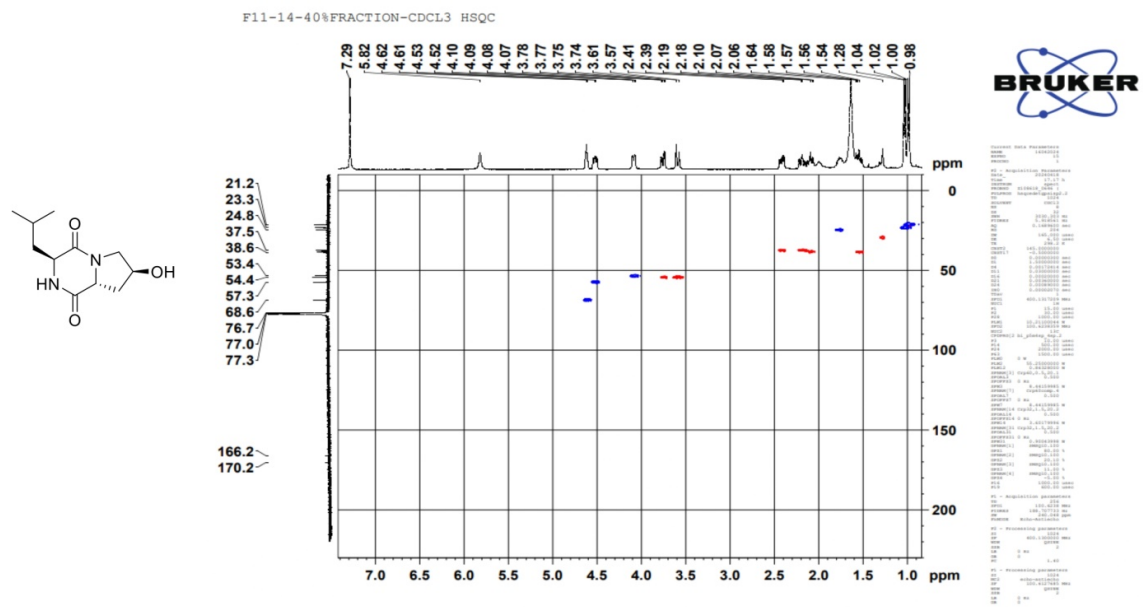


Figure S9 HSQC of compound DSM-05

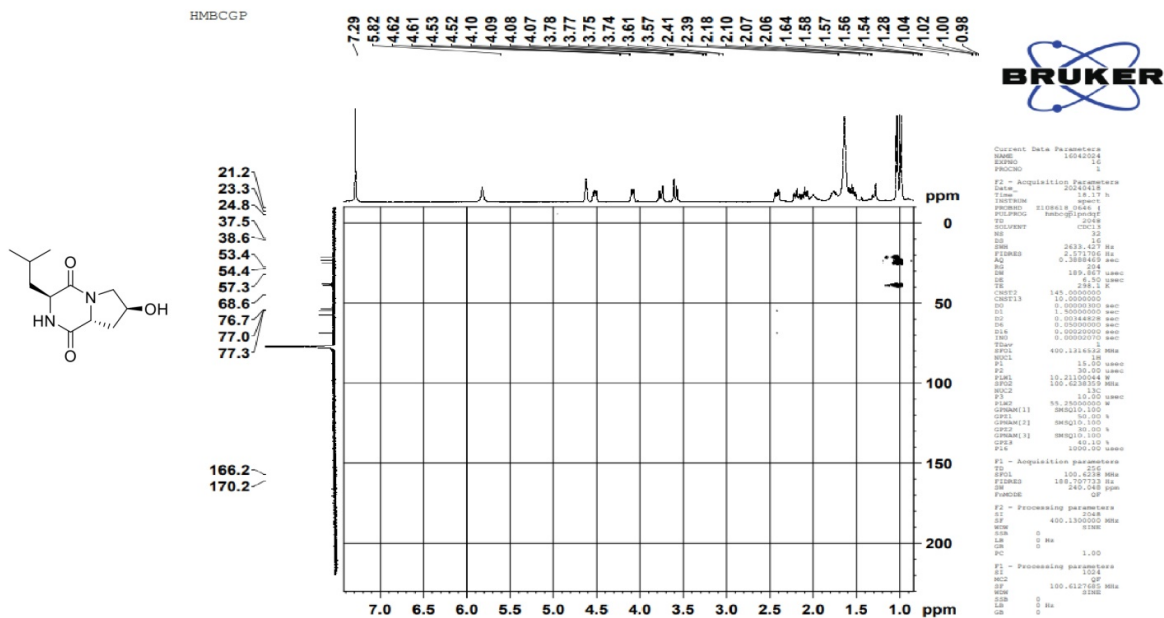


Figure S10 HMBC of compound DSM-05

F11-14-40\FRACTION-CDCL3-NOESYGPPH

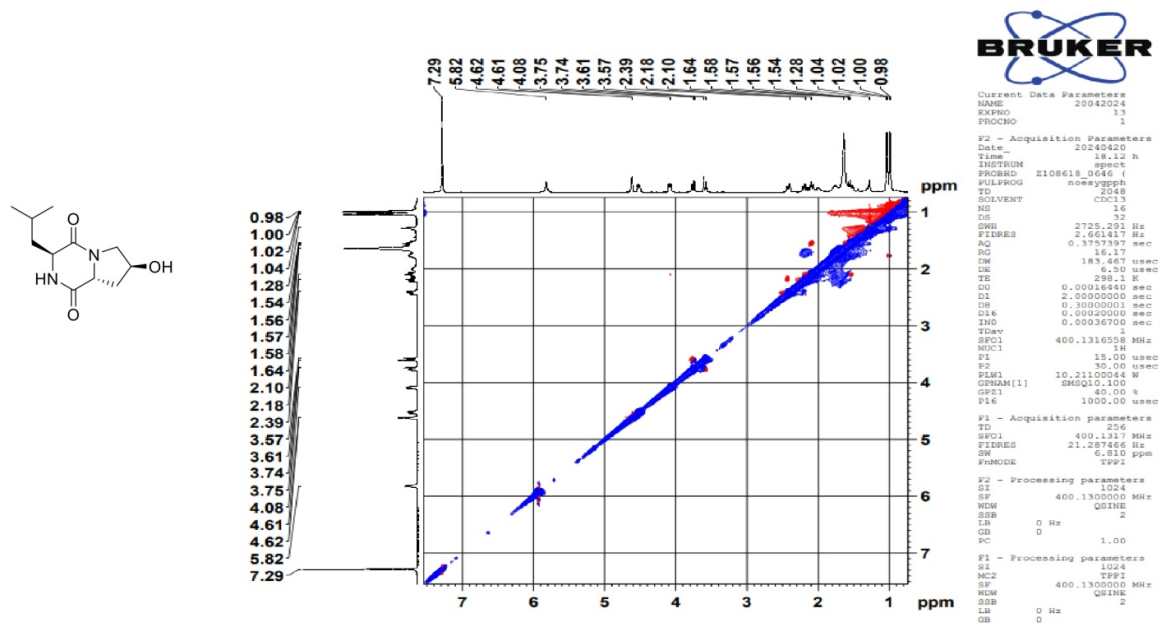


Figure S11 NOESY of compound DSM-05

| Table S1 Column chromatography protocol | | | | |
|---|------|---|------------|--|
| REQUIREMENTS | | PROPERTIES | | |
| Silica Gel | | 100-200 mesh | | |
| Cleaning solvent | | Water-acetone-hexane | | |
| Amount | | 20 times more than the sample weight | | |
| Drug adsorption | | 0.65 proportionate quantity of silica gel | | |
| Eluent composition | S.NO | Eluent Composition | Fractions | Inference |
| | 1 | 100% Hexane | F-1 to 33 | Clear oil to brown sticky material |
| | 2 | Ethyl acetate: Hexane(5:95) | F-34 to 44 | Brown sticky material to No residue with a distinct odour |
| | 3 | Ethyl acetate: Hexane(20:80) | F-45 to 58 | Creamy solid residue to Clear crystals with a distinct odour |
| Washing | | 100% Ethyl acetate | | |
| TLC Plate mobile phase | | 10% Ethyl acetate : Chloroform | | |
| Visualization | | For 254 nm and 366 nm UV-active compounds | | |

Table S3 Performing a similarity search for isolate DS5 using the 16S rRNA gene sequence and employing BLAST at the National Centre for Biotechnological Information

| NCBI-BLASTn hits (top 5-10) | | | | | | | | |
|--|---------------------------------|-----------|-------------|-------------|---------|------------|----------|-------------|
| Description | Scientific Name | Max Score | Total score | Query cover | E value | Per. Ident | Acc. Len | Accession |
| <i>Micrococcus aloeverae strain AE-616S ribosomalRNA, partial sequence</i> | <i>Micrococcus aloeverae</i> | 1295 | 1295 | 100% | 0.0 | 100.00% | 1411 | NR_134088.1 |
| <i>Micrococcus luteus strain NCTC 2665 16S ribosomal RNA, partial sequence</i> | <i>Micrococcus luteus</i> | 1290 | 1290 | 100% | 0.0 | 99.86% | 1525 | NR_075062.2 |
| <i>Micrococcus yunnanensis strain YIM 65004 16S ribosomal RNA, partial sequence</i> | <i>Micrococcus yunnanensis</i> | 1290 | 1290 | 100% | 0.0 | 99.86% | 1426 | NR_116578.1 |
| <i>Micrococcus luteus strain DSM 20030 16S ribosomal RNA, partial sequence</i> | <i>Micrococcus luteus</i> | 1290 | 1290 | 100% | 0.0 | 99.86% | 1418 | NR_037113.1 |
| <i>Micrococcus luteus strain ATCC 4698 16S ribosomal RNA, partial sequence</i> | <i>Micrococcus luteus</i> | 1290 | 1290 | 100% | 0.0 | 99.86% | 1418 | NR_037113.1 |
| <i>Micrococcus endophyticus strain YIM 56238 16S ribosomal RNA, partial sequence</i> | <i>Micrococcus endophyticus</i> | 1267 | 1267 | 100% | 0.0 | 99.29% | 1438 | NR_044365.1 |

