Screening the PRISM library against *Staphylococcus aureus* reveals a sesquiterpene lactone from *Liriodendron tulipifera* with inhibitory activity

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Spectroscopic data for epi-tulipinolide, tulipinolide, and honokiol

Epi-tulipinolide: colorless oil; $[\alpha]^{23}_{D}$ +39.7 (*c* 0.1, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.30 (1H, d, *J* = 3.5), 5.72 (1H, m), 5.59 (1H, d, *J* = 3.0), 5.11 (1H, t, *J* = 9.2), 4.87 (1H, m), 4.76 (1H, d, *J* = 9.8), 2.90 (1H, m), 2.82 (1H, dd, *J* = 14.6, 5.2), 2.40-2.20 (4H, ovlp), 2.09 (1H, m), 2.06 (3H, s), 1.75 (3H, s), 1.50 (3H, s). HRESIMS *m/z* 313.1419 ($[M+Na]^+$ (calcd for $C_{17}H_{22}O_4Na$, 313.1416).

Tulipinolide: colorless oil; $[\alpha]^{23}_{D}$ +30.9 (*c* 0.1, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.35 (1H, m), 5.82 (1H, m), 5.03 (1H, m), 4.92 (1H, m), 4.84 (1H, m), 4.76 (1H, m), 3.03 (1H, m), 2.53 (1H, m), 2.43 (1H, m), 2.32 (1H, m), 2.21 (1H, m), 2.09 (3H, s), 1.71 (3H, s), 1.57 (ovlp). HRESIMS *m/z* 313.1418 ($[M+Na]^+$ (calcd for $C_{17}H_{22}O_4Na$, 313.1416).

Honokiol: white solids; ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.20 (2H, s), 7.02 (2H, m), 6.90 (2H, t), 6.06 (1H, m), 5.97 (1H, m), 5.20 (2H, m), 5.05 (2H, m) 3.44 (2H, m), 3.33 (2H, m); *m/z* 267 $[M+H]^+$.

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Figure S10. HRESIMS of laurenobiolide (*m/z* 313.1418) recorded on SCIEX Triple TOF mass spectrometer.

Figure S11. ^{1}H NMR of peak 2.2 (tulipinolide) (500 MHz, $CDCl_3$).

Figure S12. Cytotoxicity of isolated compounds from *L. tulipifera* (laurenobiolide, epi-tulipinolide, and tulipinolide) on human keratinocyte skin cells. Bars represent mean viability values compared to DMSO control (n=4) with error bars indicating standard deviation.

Figure S13. HPLC-DAD evaluation of epi-tulipinolide (E), laurenobiolide (L), and tulipinolide (T) in different parts of *L. tulipifera*.

Figure S14. HPLC-DAD evaluation of epi-tulipinolide (1), laurenobiolide (2.1), and tulipinolide (2.2) in different parts of *L. tulipifera* twigs (outer twig bark covering and inner twig material).

Figure S15. Figure S15. The agar dish shows the inhibitory effect of a twig poultice using jojoba oil as the carrier while a stripped twig without outer bark does not show any inhibitory effects. Controls were (+) gentamicin sulfate and (-) jojoba oil.

Figure S16. Confirmation of costunolide in *L. tulipifera*, *M. acuminata*, and *M. virginiana*.

Figure S17. Confirmation of dehydrocostus lactone in *M. macrophylla* and *M. virginiana*.

Figure S18. LC-MS/MS analysis of the *M. denudata* branch CH_3OH extract.

Figure S19. Species identification of plant specimen (URI12) used in the current report.

Table S1. Potential sesquiterpene lactone biosynthetic enzyme amino acid sequences

Annotation	Sequence
Farnesyl pyrophosphate synthase-like	MAAATSNGKSSGLRSVFLQVYARLKSELLQDPAFDWTEDSRQWIDRMLEYNVPGGKLNRGLSVIDSYKLLKDGQELSEDE IFLSCSLGWCIEWLQAYFLVLDDIMDGSHTRRGQPCWFRVPKVDMIAINDGILLRNHIPRILRKNFRERPYYVDLLDLFNEV EFQTASGQMLDLITTHEGEKDLSKYTMPVYCRIVQYKTAYYSFYMPVACALLMSGENLDNFTDVKNILIEMGTYFQVQDD YLDCFGDPKVIGKIGTDIEDFKCSWLVVQALERADENQRKILSENYGKSDAAHVAKVKQLYKDLDLESVYLEYESKSYEKLIA SIEVQPSKSVQEVLKSFLGKIYKRQK*
Cytochrome P450	MLSPLSPYIAYMRYPIHLSPLALSHKISNLHLKREKIQKRGKMALFLLMIACACIYLLYLQRRKQGLPPGNLGLPFIGETLQLVS AYKTDNPEPFIDARVRRYGSLFTTHVFGEPTVFSTDPEANRFVLQNEGKLFESSYPSSISNLLGRHSLLLMKGNLHKRMHSLT MSFANSSIIRDHLLVDIDRLVRFNLQRWDGLILLQDETKKITFELTVKQLMSFDPGEWTESLRKEYLLLIEGFFSVPIPFFFTTY GRALQARTKVAAALRERVRERKERRRNRKGEEQKDMLGALLDEGEGGFSEEEAVDFLLALLVAGYETTSTIMTLAVKFLTE TPSALALLKEEHEGIRAKKKESEALDWSDYKSMPFTQCVSLLFPLIN*
Cytochrome P450, E-class, group IV	MLSPLSPYIAYMRYPIHLSPLALSHKISNLHLKREKIQKRGKMALFLLMIACACIYLLYLQRRKQGLPPGNLGLPFIGETLQLVS AYKTDNPEPFIDARVRRYGSLFTTHVFGEPTVFSTDPEANRFVLQNEGKLFESSYPSSISNLLGRHSLLLMKGNLHKRMHSLT MSFANSSIIRDHLLVDIDRLVRFNLQRWDGLILLQDETKKITFELTVKQLMSFDPGEWTESLRKEYLLLIEGFFSVPIPFFFTTY GRALQARTKVAAALRERVRERKERRRNRKGEEQKDMLGALLDEGEGGFSEEEAVDFLLALLVAGYETTSTIMTLAVKFLTE TPSALALLKEEHEGIRAKKKESEALDWSDYKSMPFTQCVINETLRVANIISGVFRRAVSDVNIKGYTIPKGWKVFASFRAVHL DQDLYKDARTFNPWRWQVLCLLFPFTEGTGIVPDMLCFNPLAHAL*
acetyl-CoA acetyltransferase, cytosolic	MAPAAASDSIKPRDVCVVGIARTPMGGFLGTLSSLSATKLGSIAIECALKRADIDPKLVQEVYFGNVLSANLGQAPARQAAL GAGIPNTVICTTINKVCASGMKATMLAAQSIQLGINDVVVAGGMESMSNAPKYLSEARKGSRLGHDTIVDGMLKDGLW DVYNDYGMGMCAELCADQHSITREEQDSYAIQSFELGIAARNGGAFAWEIVPVEVSGGRGKPSVLVDKDEGLEKFDPVK LRKLRPNFKENGGSVTAGNASSISDGAAALVLVSGEKALELGLQVIAKISGYADAAQAPELFTTAPALAIPKAISNAGLEASQI DYYEINEAFAVVSVANQKLLGIHPDKLNVHGGAVSLGHPLGCSGARILVTLLGVLRQRNGKYGVAGICNGGGGASALVLEL MPVIRAERSSL*
terpene cyclase	DLSFQLHLSVEMAHQGPPSLFSNLQATEIPKPGVIRPTAGFHPTAWGDHFLNYSGENKNVDAWTKKVEMLKEEVRRMLV NNKVPVQEMNLIDDIQRLGVAYHFEKEIDKALQHIYDEYQNVHYDDLYVVALRFRLLRQGGYNVSSDVFSKFKGEDGNFK ATLSRDVKGMLSLYEAAYFSIQGEDILDEAIVFTSGHLTTIMAHLHRPLAENARRALELPLHKRIPRLDARYYISLYEEDKSHN DVLLELARLDFNLLQLLHQRELRDLSRWWKDLGLATKYPFARDRLVETYFWILGVYFEPQYTRARAIITKIMKIASIIDDIYDA YATFDELEIFTNAVLRWELEAAEELPEYMKACYLALLNTIDEIESQMMPDEKFYRTNFIKREMKVLVHSYLDEAKWVKRRYV PTFGEHLDVSLLSCGFPLLTGVAYVGMGDLASKEVFDWLNTHPKFVMDLSIVCRLVDDIAGHHFEQEREHVASTVECYMK EHGVSEQEACTKLREMIATAWKDVNKACLRPTVIPLPLLLVRGANSTRVIEDLYIRGDGYTHSKYETKERVTVVLVDPVPIPL*
terpene cyclase	MAHQGPPSPYSTLQATEIKKPEVVRPTAGFHPSVWGDRFLDYSEEQKVVDEWTGKVEVLQEEVRWMLINNKGSVQEM NLIDDIQRLGVAYHFEKEIDEALHRIYDAYTNVHYDDLYAGALRFRLLRQSGYNVSSDVFSKFKDEDGNFKETLSSDVRGML SLYEAAYLGIQGEDILDEAIVFTSGHLKTIVAQLHHPLVEKVRHALVLPLHKRVPRLEARYYISLYQEEESHNDVLLELARLDFN LLQSLHQRELRDLSRWWKDLGLVTKYPYARDRLVEAYYWILGVYFEPQYSRARVILTKIFKLTSIIDDTYDAYATLDEVQIFTN AIHRWELEAAEGLPDYMRACYLALLRTVDEIEDQMMADEKFYRTNWLKREMKVLVQAYFDEAKWMNSGHVPTLKEHL DVSLISAGYIFVYGVAFVGMGDEASKEIFDWMTAYPKFIMDLSIIARVGDDIGGHKFEQEREHVASTVECYMKEHGVSDKE ACIKLQEMITTAWKDLNKACLRPTVIPLPLLLRGLNLARVMEELYKQGDGYTHSNNETKEKIMAVLVDPILIRG*
terpene cyclase	CLEKTHSACLLLLSSFHSIMALILGGGHSDGPTNQGKNGKKEIGRASANYHPSVWEDRFIAASPDDKELDPYTKQRADMLK EEVKKMLCKVNNSVQKLNSIDAIQRLGVAYHFETDIEKELHRMYDGYNDDGDNLHVVALRFRLLRQHGYNVSSDVFRKFK DNKGKFKATMSSDIRGLLSLYEAAYLSIHGDDILDEAITFTTMHLKSAMLHLTSSLAKLVELALEVPLRKCVERLQSRYYISIYE EEKERSDILLEFAKLDFNLLQSLHRSELRDISRWWKENDFAVKLPFIRDRVVECYFWILGVYFEPHYSRARHMMTTIISLTSI MDDIYDVHGTLEELELYTAALESWDRGAVDRLPEYMKVHFVALLDAVDGFEDELSQEGKSYRISYLKEVYKVLARAYLQEA RWASSEYVPTYNEYMEVAQISSAYPLLTVISLVGMGDIVTKEAFEWAVNSPKVVTACSVICRLKDDITSSELEQQRSHVASA MQCYMREHSASYTDTCEKFEEMVAIAWKEVNKECLKPTHVPMSVIMRTVNLARVIEFLYGHQDGYTNSTRETKERIQMV MVDPVPV*
terpene cyclase	MALILGNGHSDIPTKNQVETKGRKEIGRGCANYHPSVWGDQFVTLSPDEMKIDVQTKQRAEILKEELKRMLLNVSDSLQE LTLINEIQRLGVAYHFEKEIKDALYRMYDAHSNGGNDVSDDLHAVALWFRLLRQQGYNVSSNVFRRFKDENGEFKATLKD

	DIRGLLSLYEAAYLGTREDNILDEAINFTTEQLKSAMSHLSSPLSTLVQLALAVPLHRRVERLQSRYYISIYQQEKERNDVLLEF AKLDFNMLQSLHKKELSDISRWWKENDFSRKLPFIRDRIVELYFWVLEVYFEPQYARARRMMTTIISLTSILDDIYDVYGTLE ELELYTVAIESWDWAAMDQLPDYIKPHYTALLNAVEKFEDELSQEGKSYRIPYLKKALTVLAKGYLEEARWTSAEHTPTLEE YMKIALITNGYPMLTIASMVGMGDIVTKEAFEWAINVPKVVEASAAICRLRDDITSNEFEQERTHVASGIQVYMKEYNTTY EEACNIFLQKTANAWKDANMECMEPTPVPREVIKRPINLGRVIELLYQHKDSYTNSAFETKEHITMVLVDPIPL*
terpene cyclase	RRKLRSSREAVPKDHTRHAYTLSSLPPFISSFHSIMALIFGSGHSYSPTTQGKNGKKEIGRSCANYHPSVWGDSFIATSPHDK ELDPSTMRRVEKLKEEIKKMLCDVDNLVEKLNLIDAIQRLGIAYHFETDIEKELHKVYDGYNDNGDNLHVIALQFRLVRQQG YNVSSDVFRKFKDNEGKFKAKLSSDIRGLLSLYEAAYLSTHGDDILDEAIIFTSEHLKSALPHLTSPLTKLVQLALEVPLWRRVE RLQSRYYISIYEEEEERSDVLLEFAKLDFNLLQSLHRRELRDISKWWKKNDFAAKLPFIRDRVVECYFWILGVYFEPHYSRARR MMTTIISLTSIMDDIYDVYGTLEELELYTSVIESWDRGAVDKLPEYMKGHFVALLDAVDGFEDELSREGKSYRISYLKEAYNG VARAYLQEARWASSEYVPTYEEYMEVAQISSAYPMLIVISQVGMGDIVTKEALEWAINIPKVVTACSVICRLKDDITSSKLE QARGHVASAMQCYMREHGNSYTDTCEKFQEMVAMAWKEVNKECLKPTHVPMPVIMRAVNLARVIELLYVHQDGYTN STCETKERIAMVMVDPLVV*
terpene cyclase	SFPTRRSSDLFQLHPSIEMAHQGPPSLCSTLQANKIRKSEVVRQTAGFHPTVWGDHFLNYSVEDKNVDAWTRKVEVLKEE VRKMLVNAKGSVQEMILINDIQRLGVAYQFEKEIDEALSSIYDAYTNVHYDDLLAVALRFQLLREAGFNVSSDVFRKFKDDD GNFKATLSSDVRGMLCLYEAAYFGIQGEEILDEAIVFTSGHLNSIMPHLHHPLVAKVQRALELPMRKRILRLEARYYISLYQD EESHNDVLLELARLDFNILQSLHQTELKDLCRWWKDLGLATKYPFARDRLVEGYFWVLGVYFEPQYTCARAILTKIFKILSIM DDIYDTYATLDELETFTNAIHRWELEAAEGLPDYMKACYLALLNTLNEIEGQMMPDEKVYRTNYIKREMKAMVQGYLDEA KWANKRHVPTLGEHLDVSLVTAGNRLLVGVTYAGMGDLASKEVFDWLNTHPKFIMDLNIIGRLVDDIVGHQFEQERMH AASTVECYMKDHGVSEQEACAKLQEMVATAWKDLNKACLRPTVIPLPLLLPAIGLVRVVEDLYIHGDGYTDSRNETKEKVI MVLVDPIPIPMRK*
terpene cyclase	MTTCFIVSPEAMKRCNFRFQTDAIFDLILFLYMMLMQRLNSHKRRGEELKEVVRNMLCTIDDPVLKMNLIDAIQRLGVAY HFEMDIDKALRRMYDDNINGNDDGFDLQALALQFRLLRQQGYNVSSSVFTKFKDDEGNFNAILSSDTRSLLSLYEAAFLGI HGDDILDEAITFTTAHLKSTLSHLTPPLKKLVELALEIPLQRCFERLQTRYYISIYEEDNERNDVLLEFAKLEFHIFQSLHQRELR DMSLWWKEMNLIAKLPFARDRVVEGYFWTVGVYFEPHYSLARMIMAKMIALTTVMDDIYDIYGTLEELELLTATIQRWD RGDMDQLPDSMKVFFIALLDTVDAFEDELTREEKSYRMYYLKEAIKDQAKVYLLEARWASSGYVPTSEEYMKVAVISAAYP MLFVAFLIGMGEVVTKEVLEWAKHVPMMMRTCSTMVRLMDDIQSSKLERERQHVSSAVECYMKEHGSSYQETIQKLRE MVASGWKDINKECLKPTPAPTAVINVILNFTRVLELIYRYRDGYTDSTVETKEQIALVLVDPVPL*

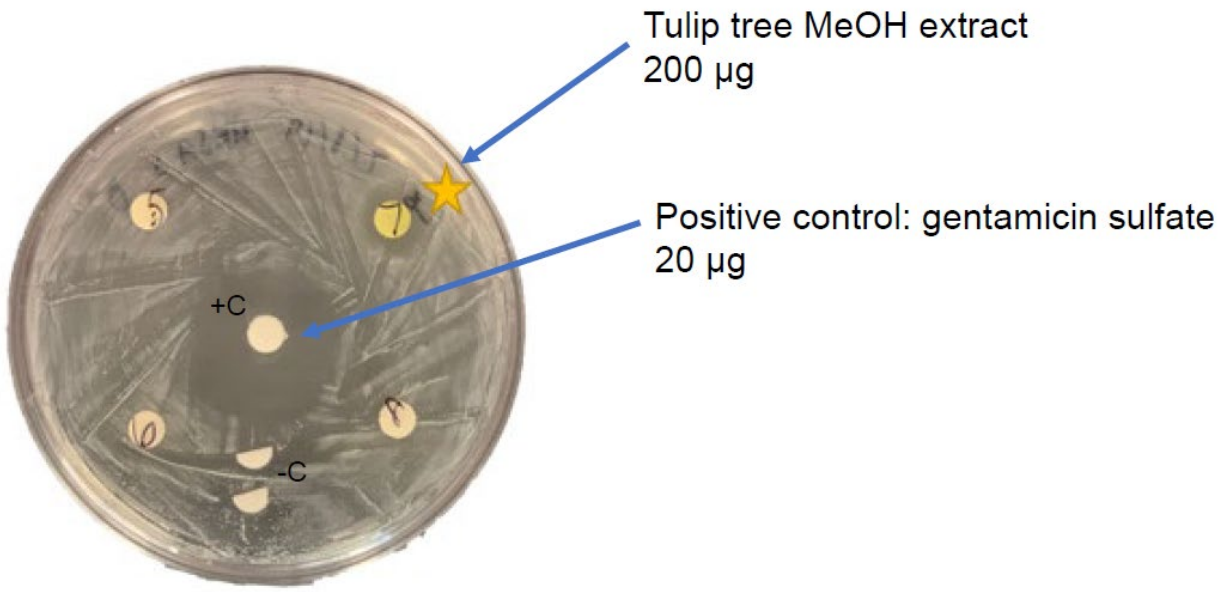

Figure S1. Evaluation of tulip tree (*Liriodendron tulipifera*) extract against MSSA. A zone of inhibition was observed around the disk containing the tulip tree extract.

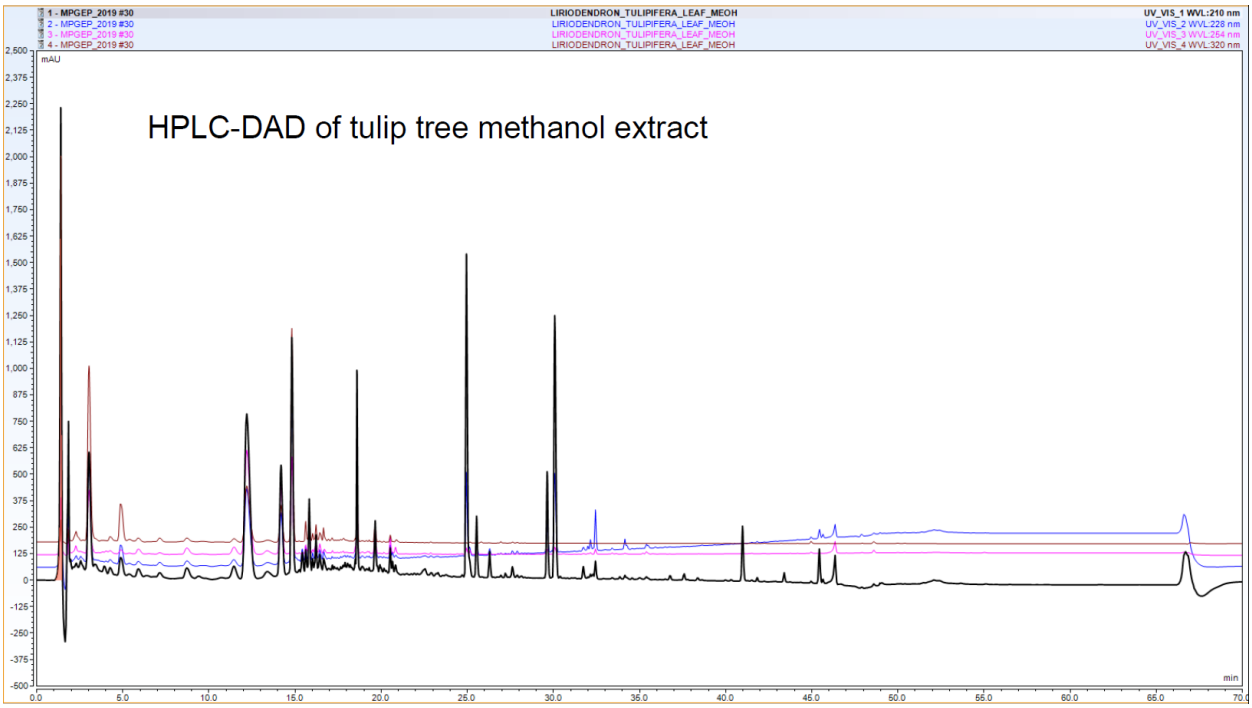

Figure S2. HPLC-DAD of the tulip tree extract. UV monitoring at 210 (black), 228 (blue), 254 (pink), and 320 (brown).

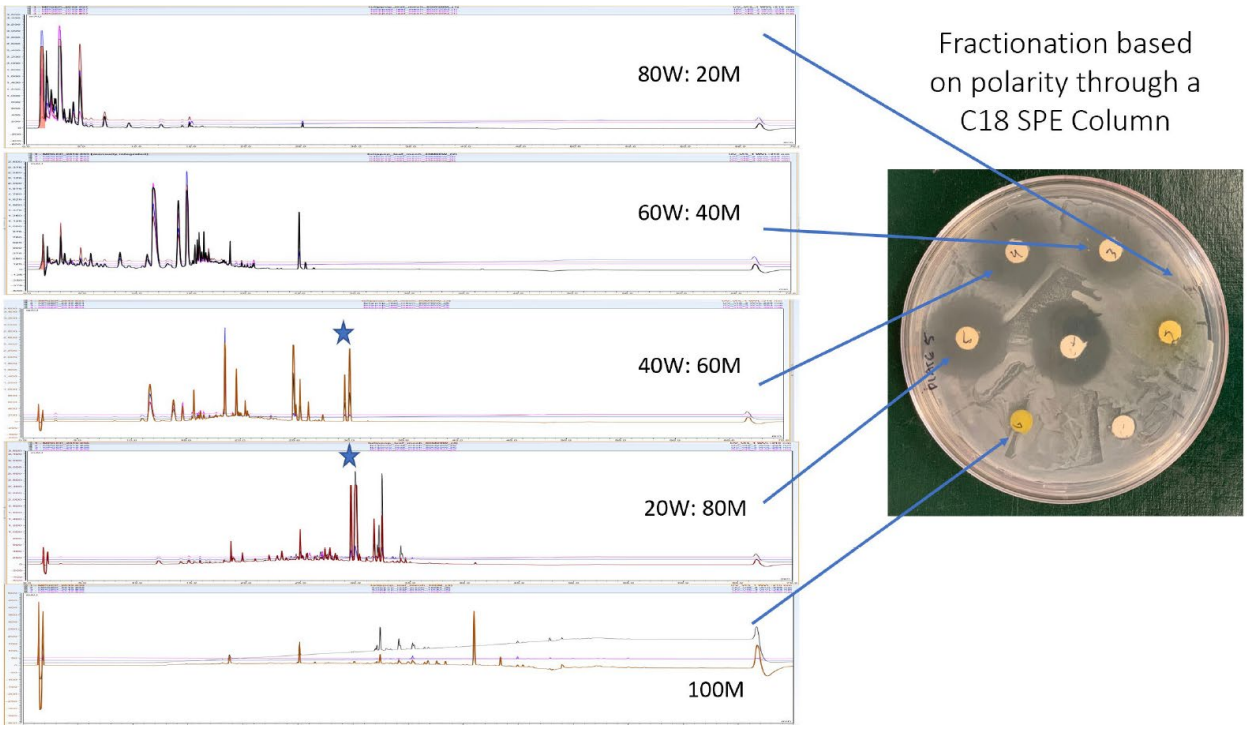

Figure S3. Antibacterial activity of *L. tulipifera* chromatography fractions on methicillin-susceptible *S. aureus*. Samples were prepared at 10 mg/mL and disks contained 200 µg of each fraction. Zones of inhibition were evaluated after 24 h after incubation at 37°C. Controls were (+) gentamicin sulfate (center) and (-) blank. Stars indicate common peaks of interest from the most active fractions.

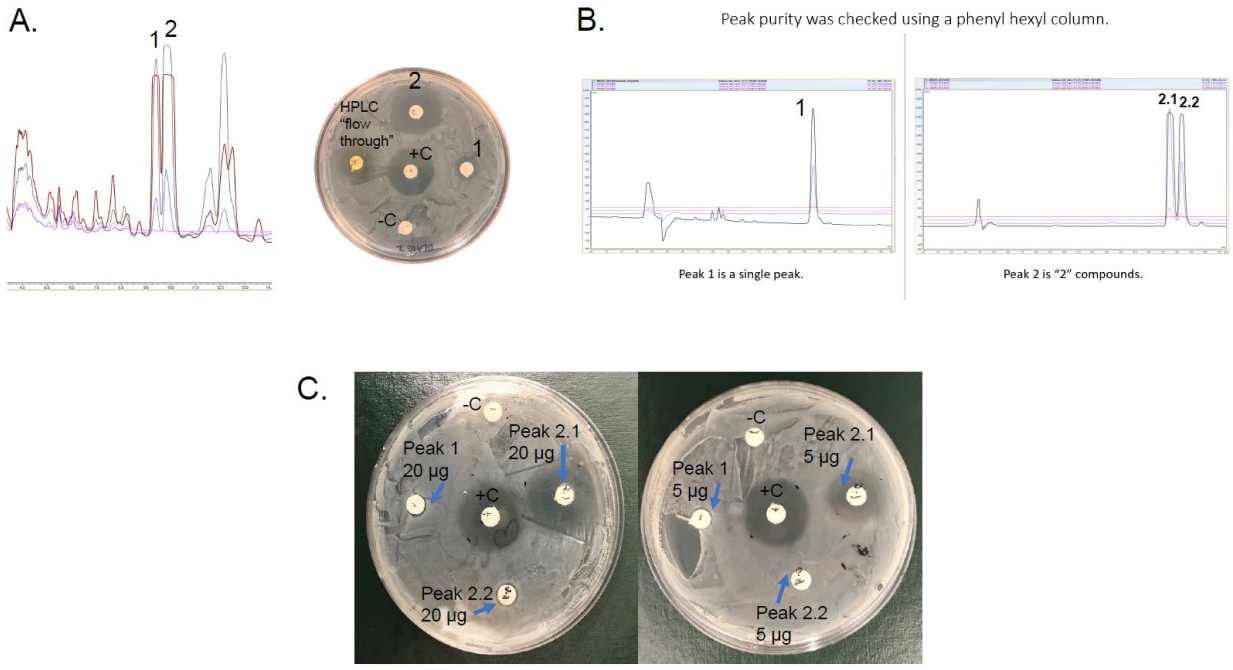

Figure S4. Antibacterial activity of *L. tulipifera* HPLC fractions on methicillin-susceptible *S. aureus.* A) Initial peaks collected and tested (1 and 2) with corresponding MSSA test results. B) Peak 2 was composed of two compounds following HPLC analysis using phenyl hexyl column (250 x 10 mm, 5 µm). C) Peak 2.1 (laurenobiolide), 2.2 (tulipinolide), and 1 (epi-tulipinolide) tested at 20 and 5 µg, respectively. Only 2.1 showed zones of inhibition. Controls were (+) gentamicin sulfate (20 µg) and (-) blank.

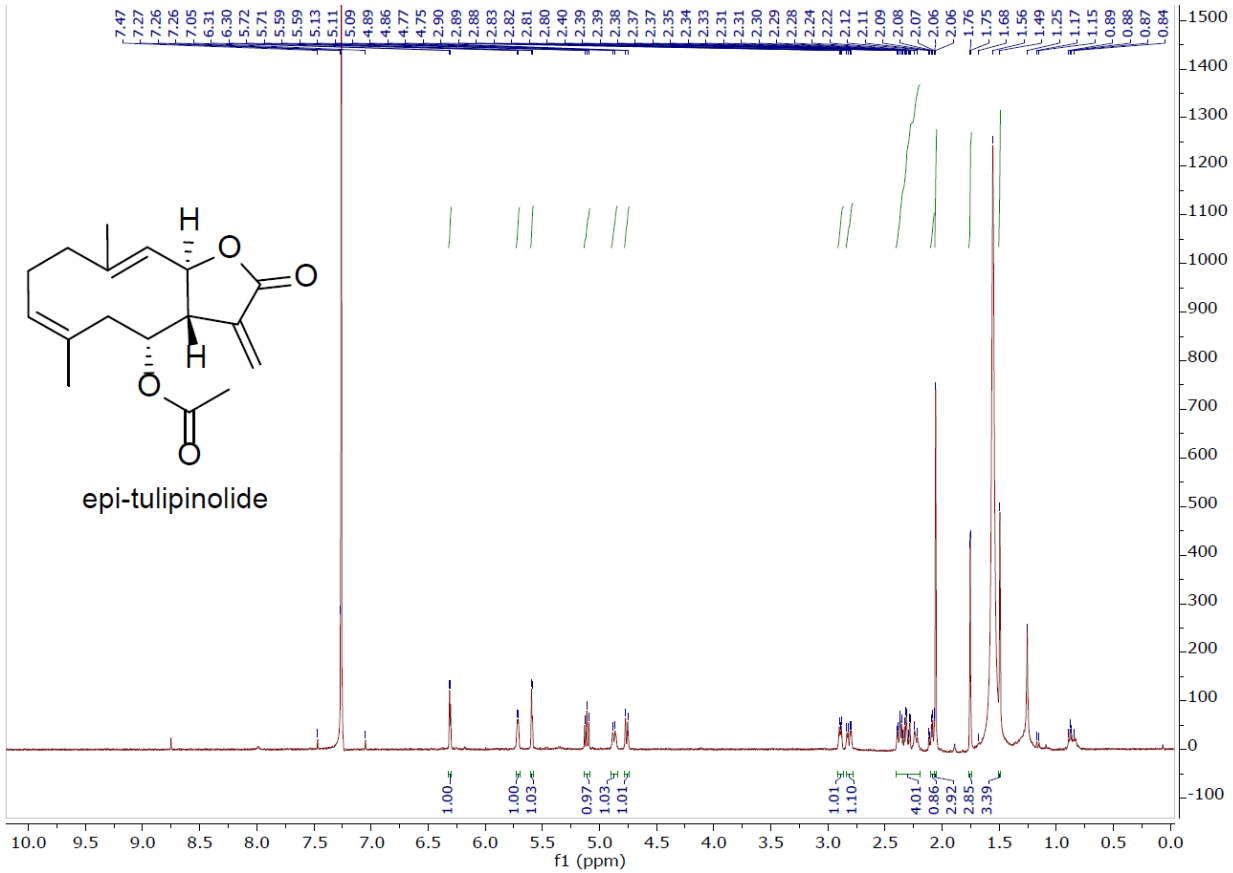

Figure S5. ^{1}H NMR of peak 1 (epi-tulipinolide) (500 MHz, $CDCl_3$).

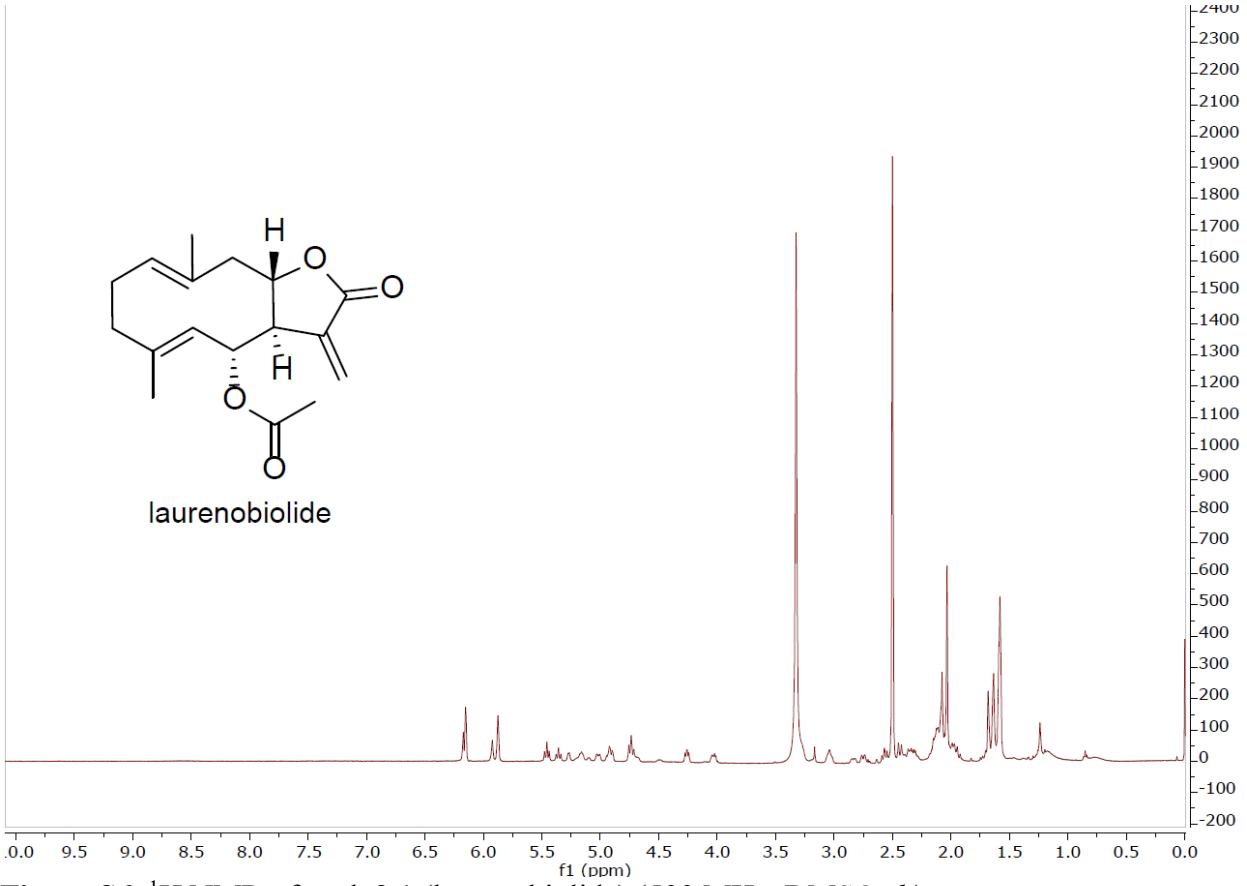

Figure S6. ^{1}H NMR of peak 2.1 (laurenobiolide) (500 MHz, DMSO-d_6).

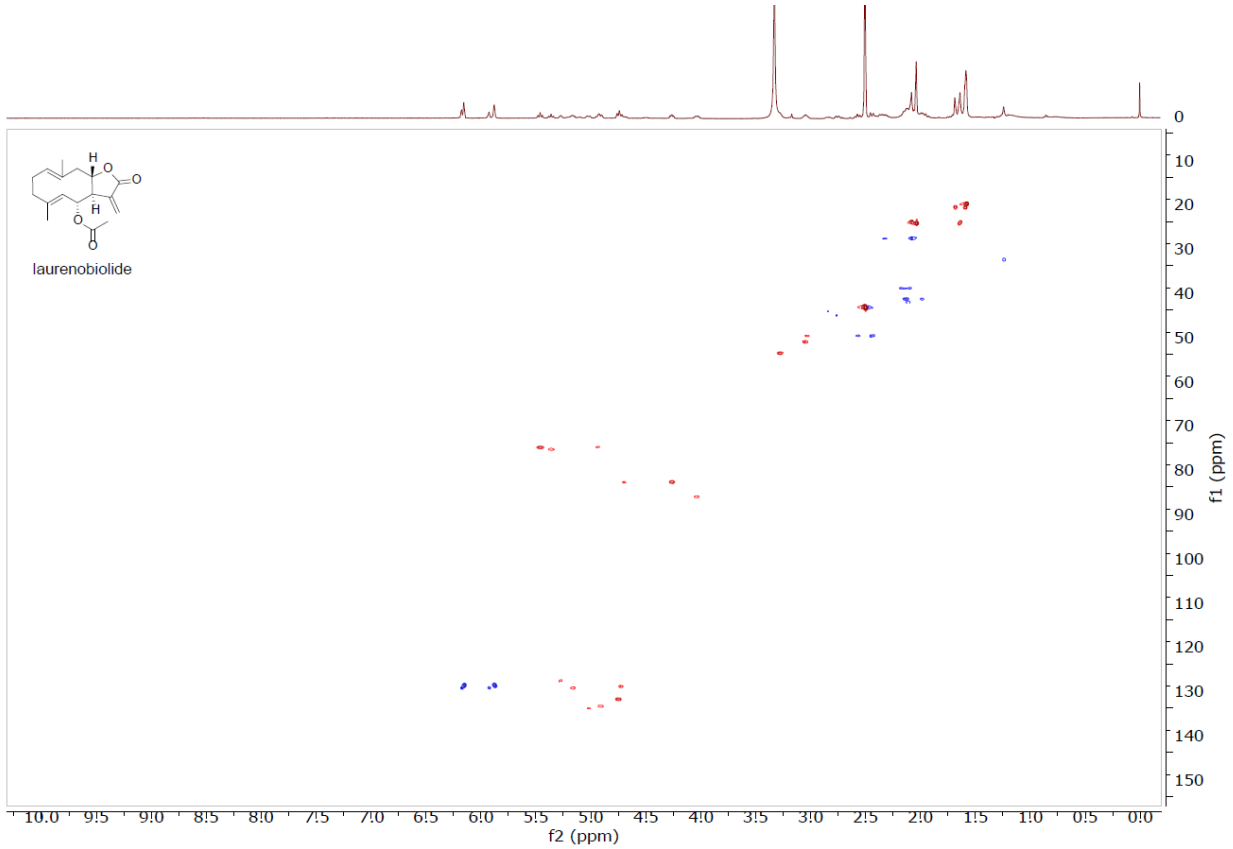

Figure S7. HSQC of laurenobiolide (DMSO-d_6).

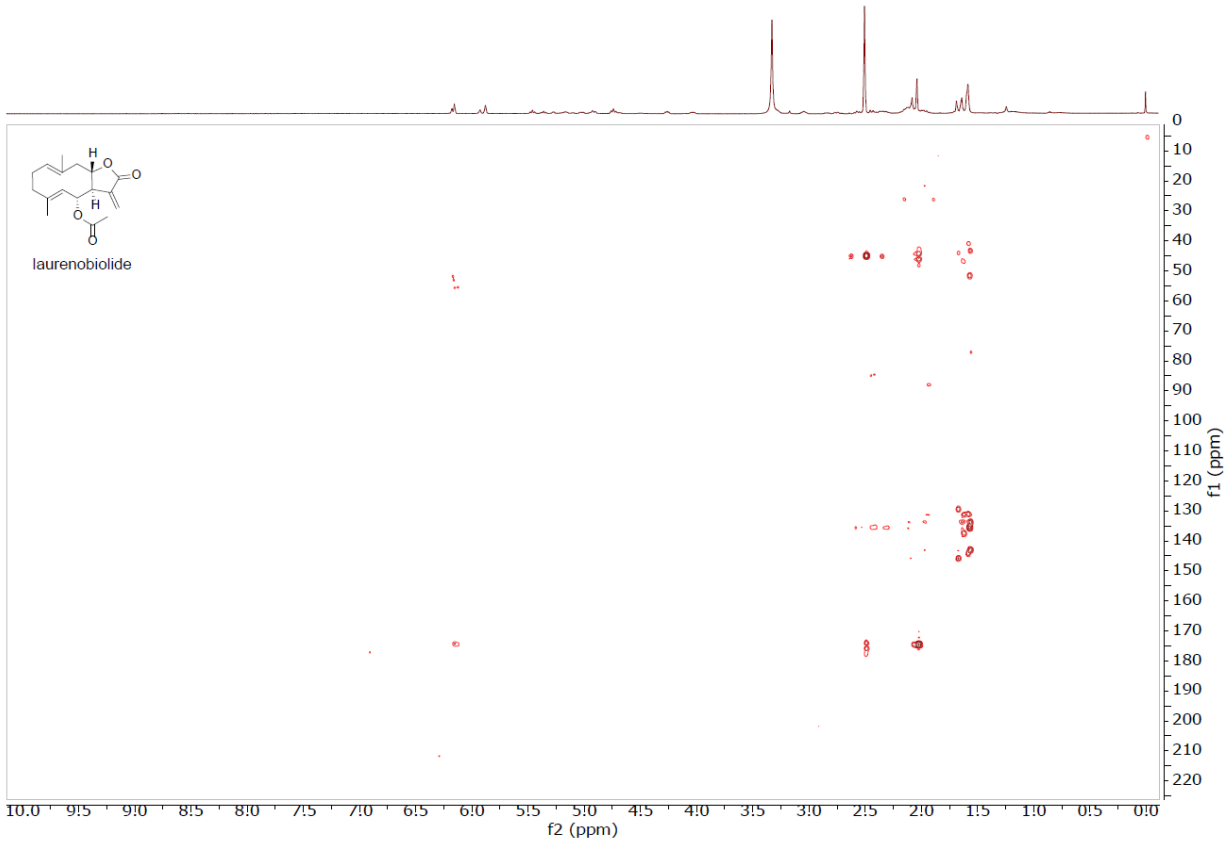

Figure S8. HMBC of laurenobiolide (DMSO-d_6).

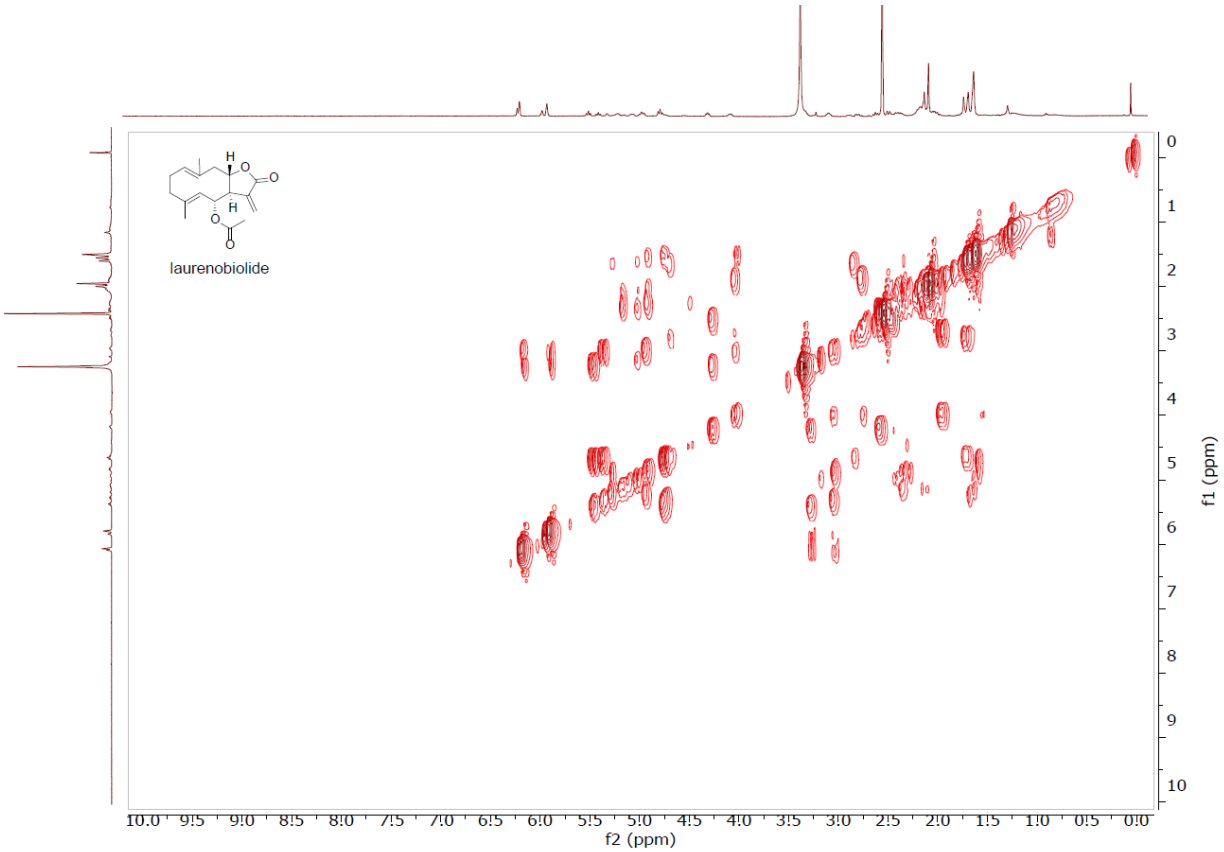

Figure S9. COSY of laurenobiolide (DMSO-d_6).

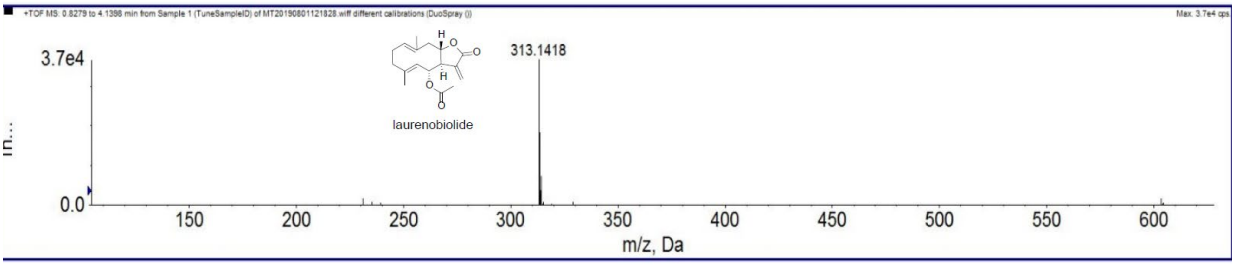

Figure S10. HRESIMS of laurenobiolide (*m/z* 313.1418) recorded on SCIEX Triple TOF mass spectrometer.

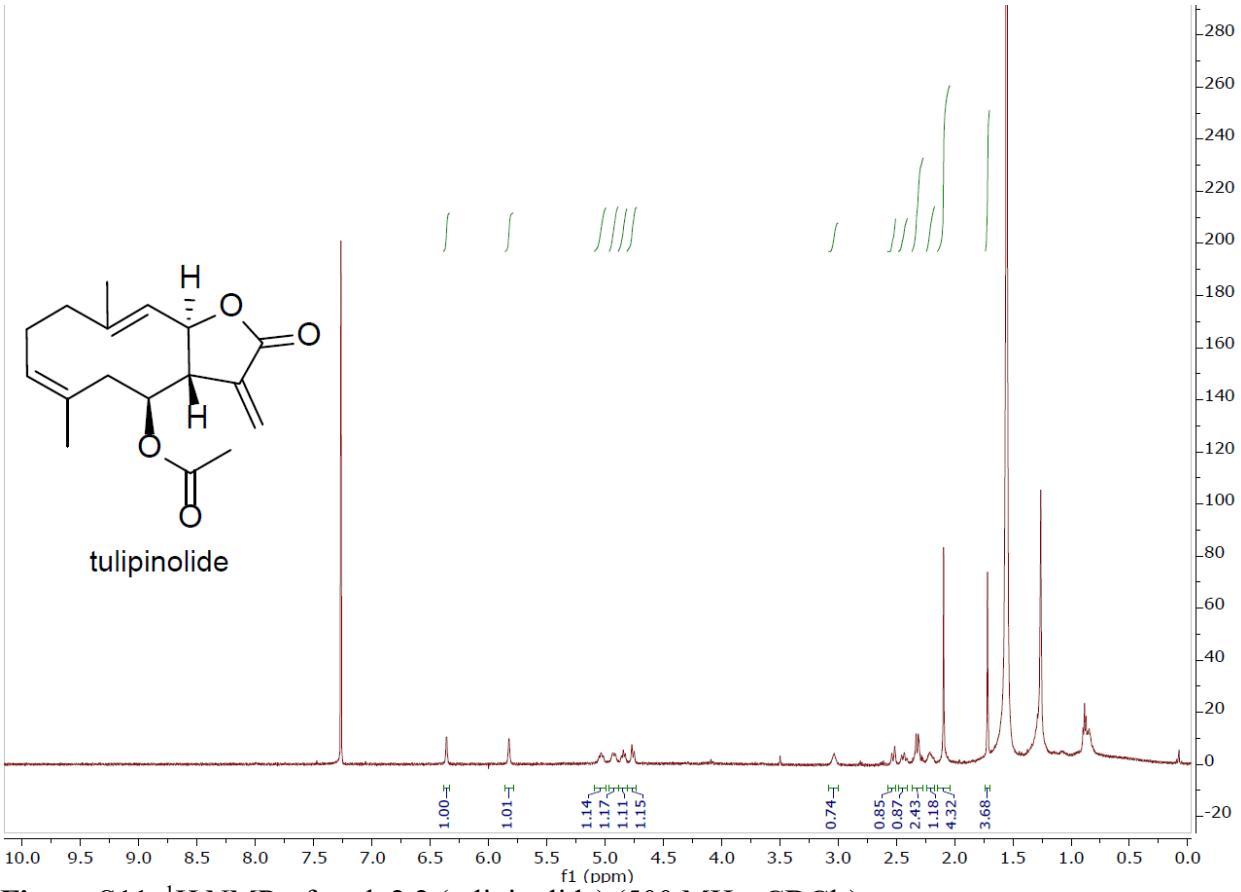

Figure S11. ^{1}H NMR of peak 2.2 (tulipinolide) (500 MHz, $CDCl_3$).

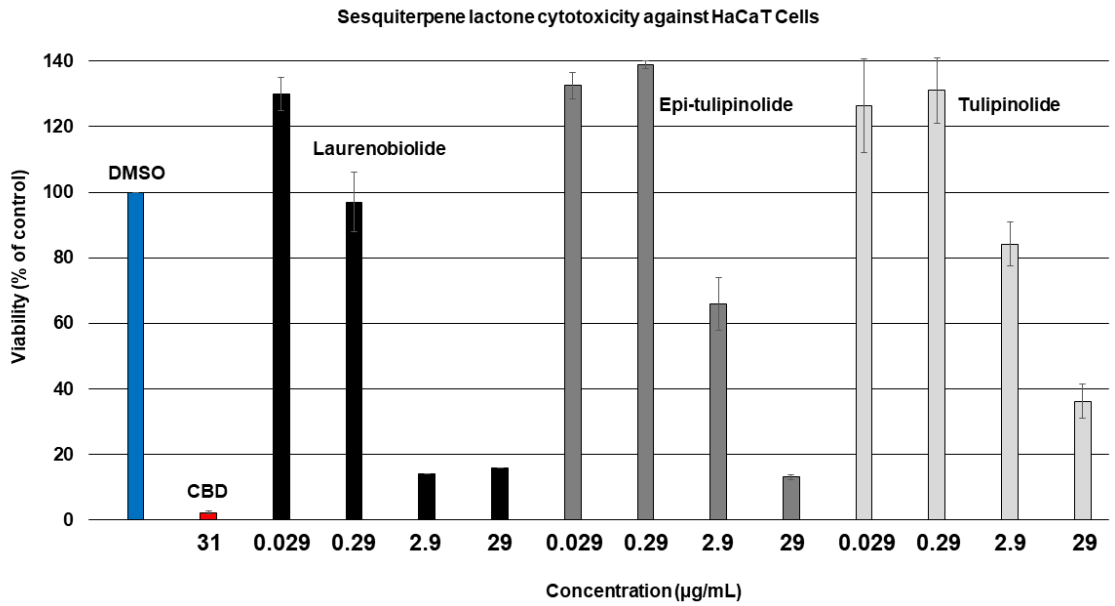

Figure S12. Cytotoxicity of isolated compounds from *L. tulipifera* (laurenobiolide – black bars, epi-tulipinolide – dark gray bars, and tulipinolide – light gray bars) on human keratinocyte skin cells. Bars represent mean viability values compared to DMSO control (blue bar) (n=4) with error bars indicating standard deviation.

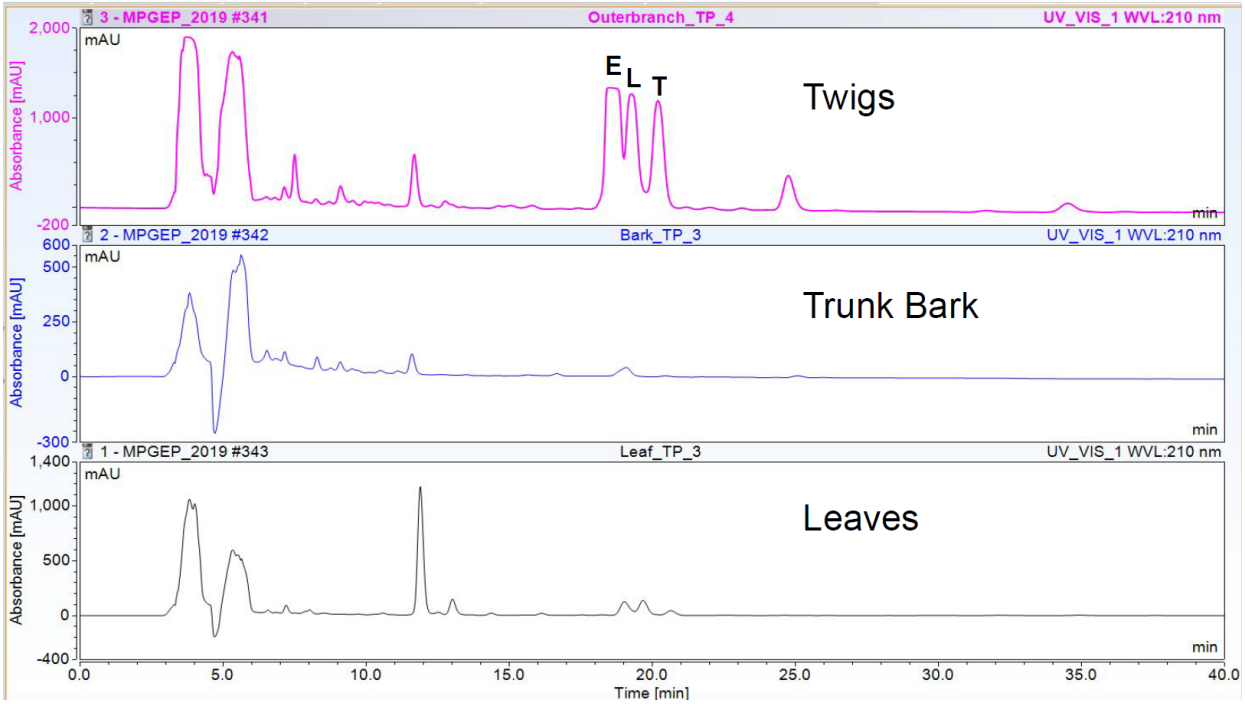

Figure S13. HPLC-DAD evaluation of epi-tulipinolide (1), laurenobiolide (2.1), and tulipinolide (2.2) in different parts of *L. tulipifera*.

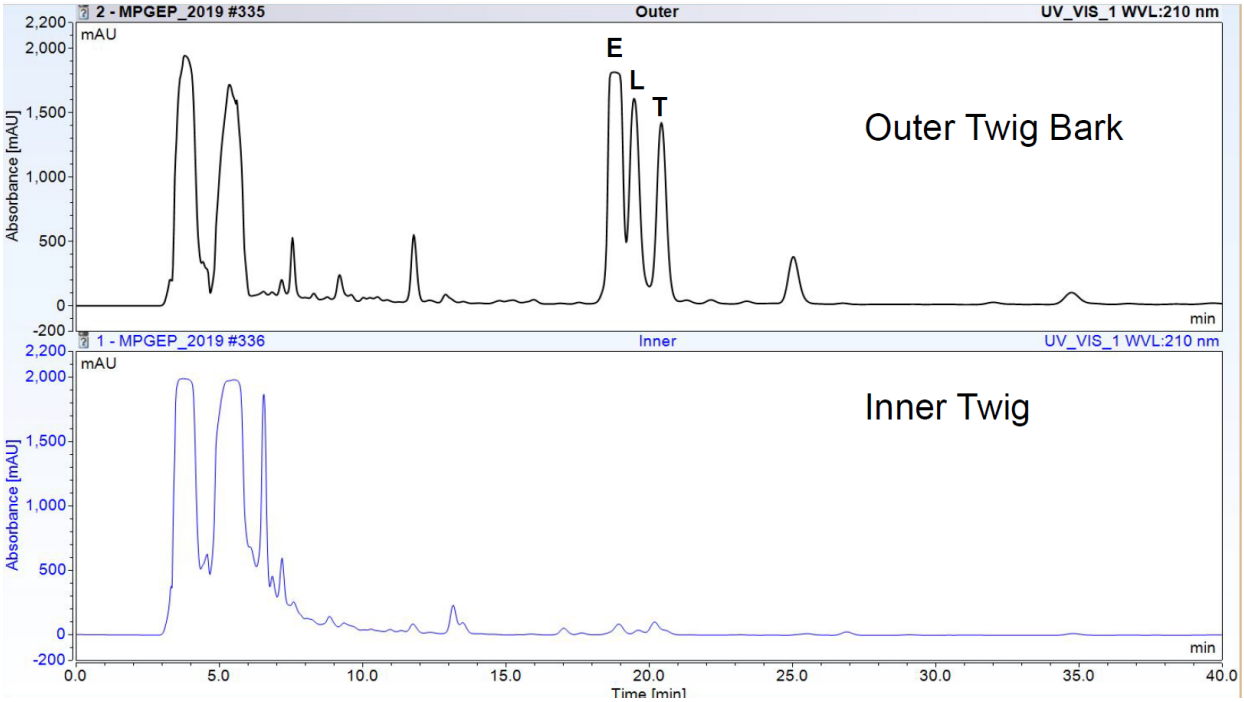

Figure S14. HPLC-DAD evaluation of epi-tulipinolide (1), laurenobiolide (2.1), and tulipinolide (2.2) in different parts of *L. tulipifera* twigs (outer twig bark covering and inner twig material).

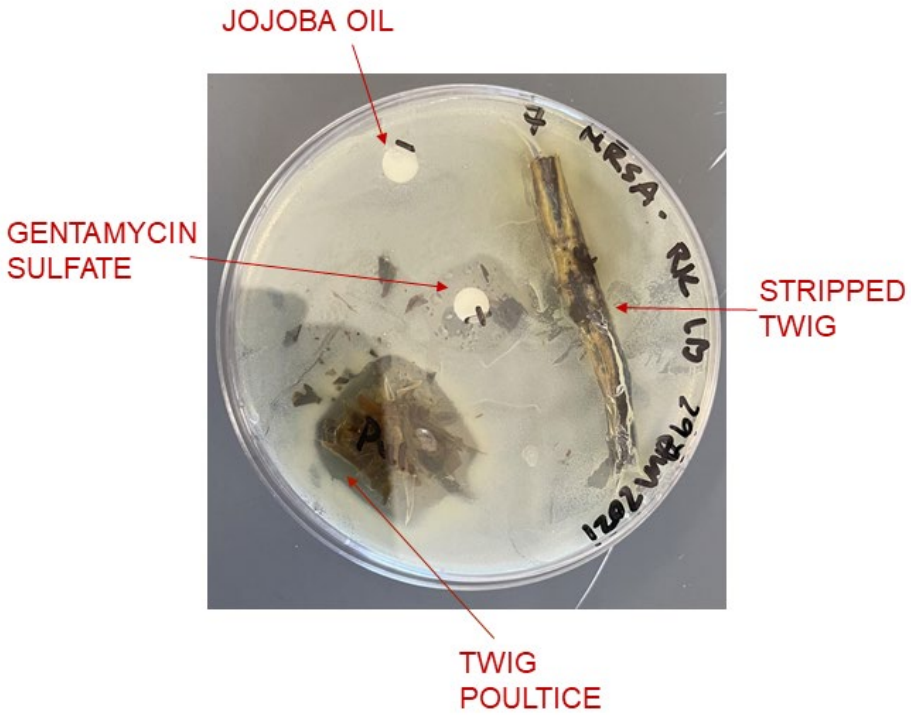

Figure S15. The agar dish shows the inhibitory effect of a twig poultice using jojoba oil as the carrier while a stripped twig without outer bark does not show any inhibitory effects. Controls were (+) gentamicin sulfate and (-) jojoba oil.

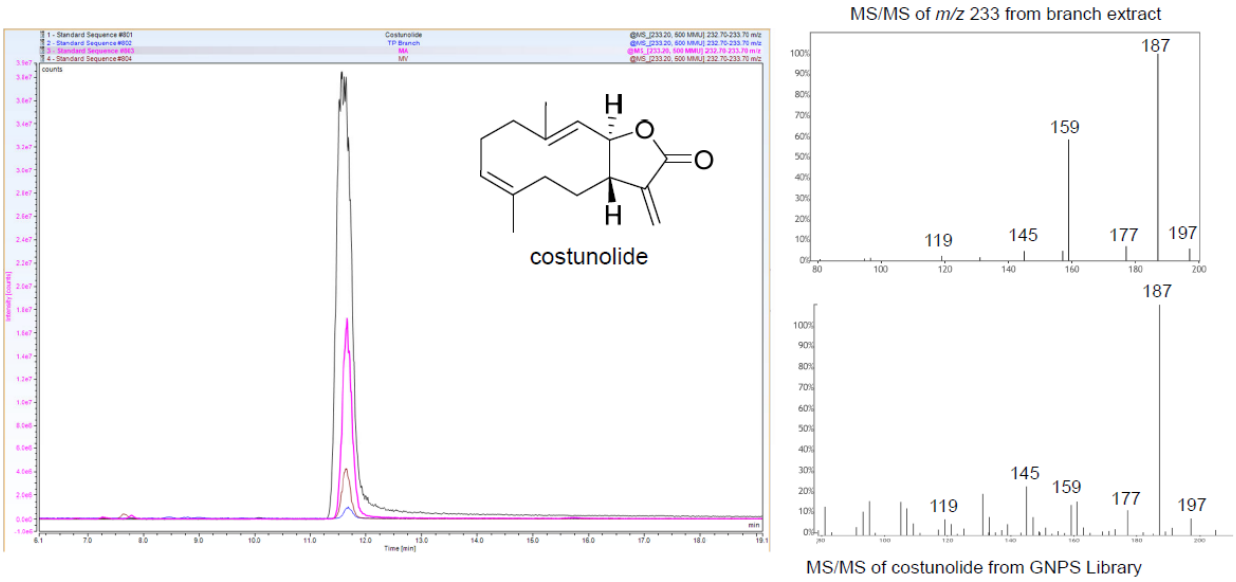

Figure S16. Confirmation of costunolide in *L. tulipifera*, *M. acuminata*, and *M. virginiana*. Constunolide standard is in black, while *L. tulipifera*, *M. acuminata*, and *M. virginiana* are in blue, pink, and brown, respectively. MS/MS fragmentation comparison of *m/z* 233 from the *L. tulipifera* extract with the MS/MS spectrum of constunolide available in the GNPS library is shown at right.

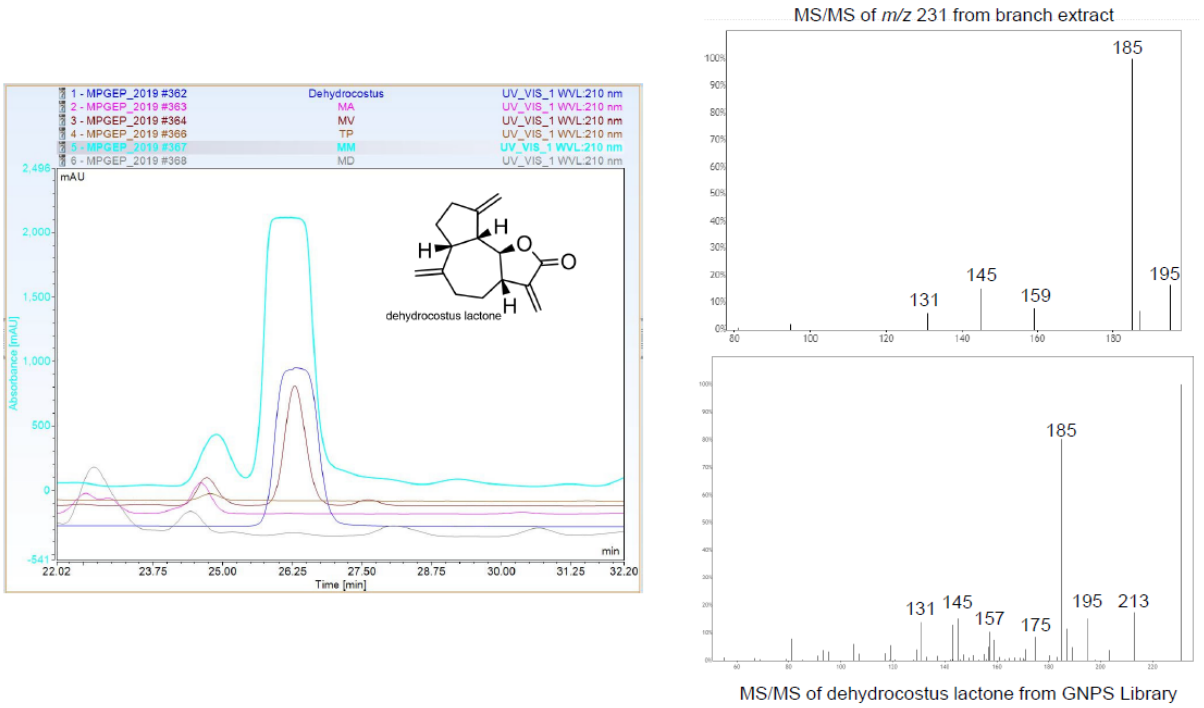

Figure S17. Confirmation of dehydrocostus lactone (blue) in *M. virginiana* (brown) and *M. macrophylla* (cyan).

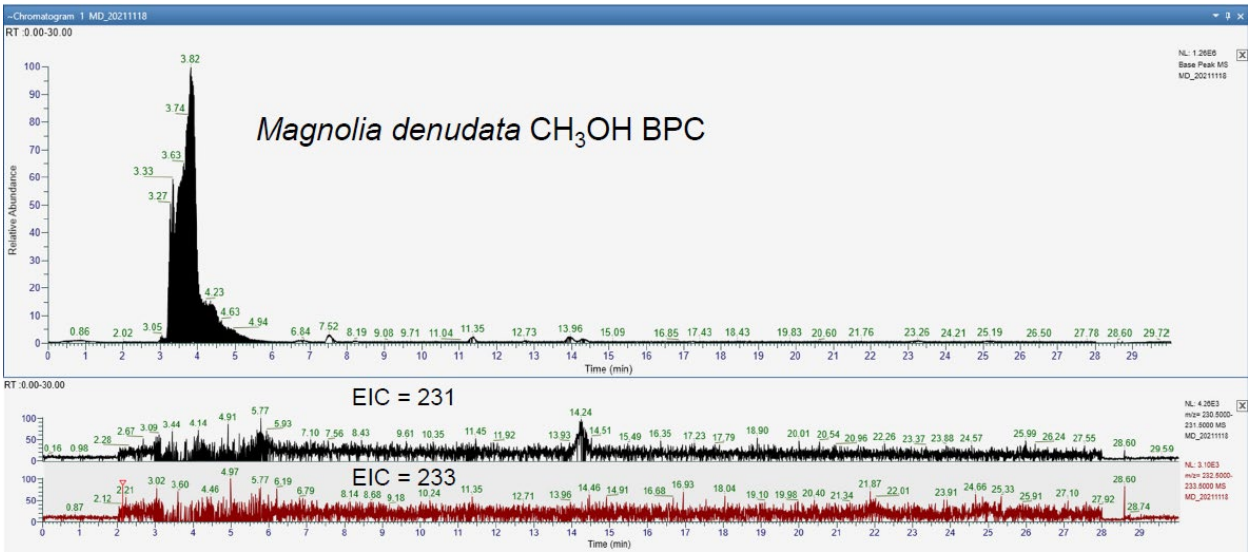

Figure S18. LC-MS/MS analysis of the *M. denudata* branch CH_3OH extract. The top panel is the base peak chromatogram. The middle panel shows an EIC for *m/z* 231 and the bottom panel shows an EIC for *m/z* 233.

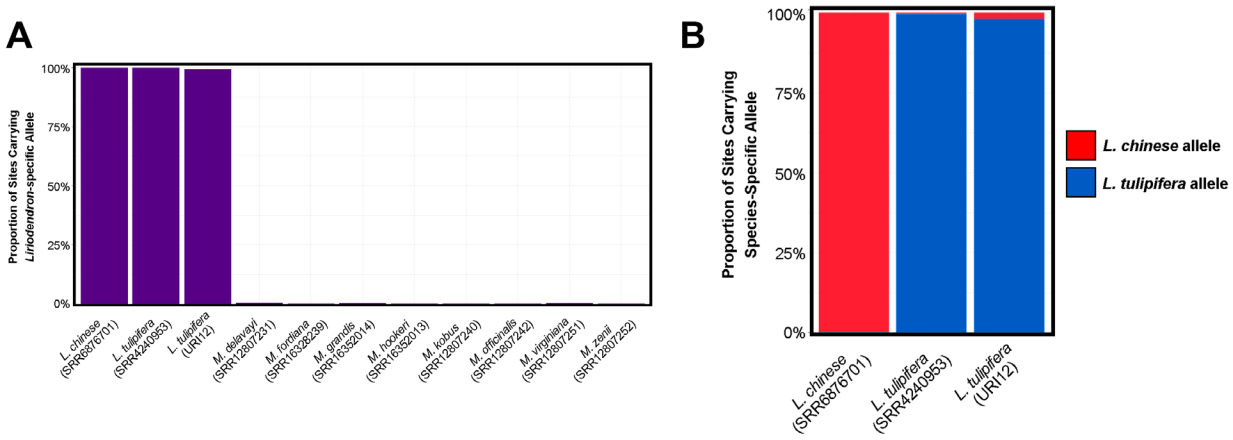

Figure S19. Species identification of plant specimen (UIR12) used in the current report. Proportion of sites carrying alleles matching either (A) the *Liriodendron* genus-specific alleles or (B) the *L. tulipifera* or *L. chinese* specific alleles was tabulated. This SNP analysis clearly positioned URI12 as *Liriodendron tulipifera*.