

Received: 06 June 2026

Accepted: 09 October 2026

Published: 15 October 2026

Chemical Characterization of *Salvia officinalis* (Sage) Extract and Preliminary Antibacterial Assessment Against *Staphylococcus epidermidis*

Maryam Latif Abdullah

College of Science, Al-Farabi University, Iraq; maryam-
latif490@gmail.com

Latif Abdullah Hamad

College of Science, Al-Farabi University, Iraq

Cite this article:

Maryam Latif Abdullah and Latif Abdullah Hamad (2026). Chemical Characterization of *Salvia officinalis* (Sage) Extract and Preliminary Antibacterial Assessment Against *Staphylococcus epidermidis*. Revista Cultura Científica, (24), pp. 2058–2092. <https://doi.org/10.70517/revcc2624171>

Abstract

Salvia officinalis L. (common sage) is an aromatic medicinal plant whose extract composition and potential antibacterial properties depend on the preparation and testing conditions. This descriptive laboratory study reports elemental measurements, infrared spectral observations, gas chromatography–mass spectrometry (GC–MS) output and a preliminary agar-diffusion assessment against a bacterium identified in the source manuscript as *Staphylococcus epidermidis*. The reported elemental concentrations were nitrogen 1.30%, phosphorus 0.50%, potassium 1.68%, calcium 0.126% and sulfur 0.0591%, equivalent to 13,000, 5,000, 16,800, 1,260 and 591 parts per million, respectively, on the same stated percentage basis. An infrared spectrum and GC–MS chromatographic and library-search images were supplied, providing preliminary evidence of chemically heterogeneous extract material but not a validated quantitative identification of individual bioactive

compounds. The antimicrobial plate supplied with the study showed no clearly discernible inhibition halos around the tested preparations. This observation should be understood as an absence of visible agar-diffusion inhibition under the photographed conditions, not proof that the plant lacks antibacterial activity or clinical efficacy. Important reporting limitations include inconsistent solvent names, incomplete units for the stated concentration series, an unsubstantiated tabulated p-value, and absent details of bacterial strain verification, assay replication, instrument settings and chromatographic identification thresholds. The findings therefore constitute an initial analytical and microbiological description requiring standardized replication before firm chemical, antibacterial or therapeutic conclusions can be drawn.

Keywords: *Salvia officinalis*; sage; *Staphylococcus epidermidis*; elemental analysis; FTIR; GC–MS; agar diffusion

1. INTRODUCTION

Salvia officinalis L., commonly known as sage, is an aromatic perennial species in the family Lamiaceae. Its leaves have long been used in traditional preparations, culinary products and herbal formulations. Historical uses include preparations intended for digestive complaints, throat discomfort and other conditions; however, a history of medicinal use should be distinguished from contemporary demonstrations of safety and therapeutic efficacy. The biological characteristics of sage preparations have attracted interest partly because the plant contains diverse volatile and non-volatile constituents, and because composition can vary with geographical origin, plant material, cultivation conditions, extraction method and analytical procedure [1–3].

Previous investigations have characterized sage essential oils and related plant fractions using chromatographic and microbiological techniques. Kačániová et al. reported constituents and bioactivity in sage essential oil, including variation in effects across microorganisms [1]. Khalil and Li investigated essential oil obtained from sage collected in Syria and evaluated its activity against selected bacteria [2]. Rădulescu, Chiliment and Oprea characterized volatile and semi-volatile constituents of sage preparations by gas chromatography–mass spectrometry (GC–MS) [3]. These studies establish the relevance of chemical characterization, but their findings cannot simply be transferred to a different crude extract, solvent, extraction procedure or bacterial species. Essential oils, polar herbal infusions and solvent extracts may differ substantially in chemical profile and solubility.

Antimicrobial interest in sage is also supported by investigations of particular constituents. Horiuchi et al. identified oleanolic acid in a sage extract and evaluated its activity against vancomycin-resistant enterococci and other test organisms [4]. In a separate study, Mokhtari et al. examined methanolic sage, thyme and mixed extracts and reported antimicrobial activity against selected species, including *Staphylococcus aureus* [5]. Those results depend on the extracts, organisms and laboratory protocols used. Importantly, activity against *S. aureus* or enterococci does not establish activity against *S. epidermidis*.

Staphylococcus epidermidis is a common member of the human skin microbiota but can act as an opportunistic pathogen, notably in infections involving indwelling medical devices and biofilm formation. Its capacity to adhere to surfaces and develop biofilms creates challenges for infection prevention and management [6]. Although interest in plant-derived antimicrobial agents is considerable, a negative or positive screening result can be interpreted only when the plant preparation, microbial strain, inoculum, exposure procedure, controls and response measurements are adequately defined. Standardized diffusion-based susceptibility procedures highlight the importance of these experimental details; plant-extract screening additionally requires attention to extract solubility and diffusion through agar [7].

Chemical and biological observations also serve different evidentiary purposes. Elemental analysis can describe selected inorganic components, infrared spectroscopy can indicate classes of chemical bonds, and GC–MS can generate chromatographic peaks and tentative spectral-library matches. None of these methods alone demonstrates antibacterial efficacy, pharmacological safety or benefits in human disease. Accordingly, analytical observations and microbiological outcomes should be interpreted together without equating the presence of a chemical feature with an established biological mechanism [3, 8].

The present study reports measurements and laboratory images from an investigation of sage preparations and their activity against a bacterium described as *S. epidermidis*. Its objectives are to present the reported concentrations of five elements, discuss the infrared and GC–MS outputs within their methodological limits, describe the observed agar-diffusion outcome, and compare these observations critically with relevant published evidence. The study is presented as a preliminary descriptive investigation, not as a validated identification of all chemical compounds or a demonstration of medical efficacy. This distinction is necessary for interpreting both the observable results and the additional experimental work required before definitive conclusions can be made.

2. MATERIALS AND METHODS

2.1. STUDY MATERIALS AND EXTRACTION

The submitted laboratory report describes analysis of *S. officinalis* (sage) material using a Soxhlet extraction procedure, followed by elemental, infrared and GC–MS investigations. The extraction solvent is named “hexanol” in the manuscript; however, the GC–MS chromatogram supplied in the supporting PDF is labeled as a hexane extract. Because these refer to chemically different solvents, the identity and grade of the solvent must be confirmed from the original laboratory record before the extraction can be reproduced. Neither the mass of sage material, solvent volume, Soxhlet temperature or duration, extract yield, nor storage conditions were documented in the materials supplied for editing. Consequently, these parameters cannot responsibly be added as though they had been measured.

2.2. DETERMINATION OF SELECTED ELEMENTS

The reported analytical scheme included determinations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sulfur (S). Nitrogen was described as being measured by a distillation and titration procedure using sodium hydroxide and sulfuric acid. The source text gave incompatible concentration descriptions for the alkaline reagent (“10 M” and “50.0 N”); the correct reagent preparation requires confirmation. Phosphorus was measured using an ammonium-molybdate-based spectrophotometric procedure at 882 nm. Potassium was estimated by flame photometry, calcium by an ethylenediaminetetraacetic acid (EDTA) titrimetric method with an indicator, and sulfate by a turbidity method. The original procedures alternately refer to the extract and to “water samples.” The actual test matrix, sample digestion, standards, instrument calibration and conversion basis for the final percentages need documentation.

2.3. INFRARED AND GC-MS MEASUREMENTS

An infrared spectrum was provided for the sage sample and is reproduced as Figure 1. The supporting PDF also contains one GC-MS chromatographic output, followed by a series of mass-spectral library-search images. The analytical method was described as GC-MS, although the original source inconsistently used the abbreviation “GSMS.” The supplied materials did not give the GC column, temperature program, carrier-gas conditions, injection mode, mass-spectral ionization parameters, identification-score threshold or retention-index comparison. Individual library hits therefore cannot be treated as independently confirmed molecular identifications. The figure images are retained to allow readers to inspect the original analytical outputs.

2.4. PRELIMINARY ANTIBACTERIAL ASSESSMENT

For antibacterial screening, the original report describes aqueous preparations obtained using boiled distilled water and warm distilled water, together with an alcohol-based extract. A concentration series was recorded as 0.1, 0.2, 1, 2 and 5, without units or an explanation of whether these were percentages, mass concentrations or dilution factors. Separately, the photographed plate is labeled 1%, 2%, 5%, 10% and “D.W.” (apparently a distilled-water control). These discrepancies are retained as reporting limitations and must be resolved against the laboratory notes; no unit conversion is inferred here.

Mueller-Hinton agar was reported to have been prepared according to the medium manufacturer’s directions, sterilized at 121 °C for 15 minutes and poured into sterile Petri dishes. The source describes a diffusion-based screening procedure but does not specify whether discs or wells were used, the size of those carriers, the applied extract volume, the inoculum density, incubation conditions, positive-control antibiotic, number of independent replicates or measurement method. The isolate was described as *S. epidermidis*, but its identification and susceptibility profile were not supplied. These omissions preclude retrospective verification of assay sensitivity or compliance with a standardized diffusion protocol [7].

2.5. DATA PRESENTATION AND ANALYTICAL BOUNDARIES

Reported elemental percentages were converted to parts per million using the relationship 1% = 10,000 ppm, assuming an identical mass-fraction basis. This conversion is mathematical and is not a second independent measurement. The submitted results do not contain replicate measurements, measures of dispersion, comparison groups or a reproducible statistical test. Accordingly, the original nitrogen p-value is reproduced for transparency but explicitly identified as unverified. No independent statistical inference is made from the available observations.

3. RESULTS AND DISCUSSION

3.1. REPORTED ELEMENTAL COMPOSITION

Table 1 presents the original five-element measurements. Potassium had the largest stated percentage (1.68%), followed by nitrogen (1.30%), phosphorus (0.50%), calcium (0.126%) and sulfur (0.0591%). Corresponding converted values were 16,800, 13,000, 5,000, 1,260 and 591 ppm, respectively. These measurements indicate the reported quantities of selected elements in the analysed material; they do not demonstrate bioavailability, nutritional adequacy, medicinal suitability or therapeutic activity. The sample basis and method validation must be confirmed before the concentrations are compared with food-composition or pharmacological benchmarks.

3.2. INFRARED SPECTRAL DESCRIPTION

Figure 1 reproduces the original infrared spectrum. The accompanying laboratory narrative lists numerous reference absorption bands covering hydrocarbons, unsaturated and aromatic structures, oxygen-containing functions, nitrogen-containing groups and sulfur-containing groups. These band assignments should be treated as possible interpretive categories rather than direct evidence that all named functional groups or compound classes occurred in this one extract.

A defensible FTIR identification requires reporting the actual observed peak positions, signal quality, sample preparation and comparison with suitable reference spectra [8].

Table 1. Reported concentrations of selected elements in the sage sample (original measurements; analytical mass basis not specified)

Element	Concentration (%)	Equivalent (ppm)	Original reported p-value
Nitrogen (N)	1.30	13,000	< 0.001 ^a
Phosphorus (P)	0.50	5,000	Not reported
Potassium (K)	1.68	16,800	Not reported
Calcium (Ca)	0.126	1,260	Not reported
Sulfur (S)	0.0591	591	Not reported

^a The source reported $p < 0.001$ for nitrogen but provided no sample size, comparator, replicate data or test; statistical significance cannot be authenticated. ppm values are calculated directly from the percentages.

The original narrative described C–H stretching in the general range $3000\text{--}2840\text{ cm}^{-1}$, methyl-related regions near 2962 and 2872 cm^{-1} , and methylene-related regions near 2926 and 2853 cm^{-1} . It also mentioned characteristic absorptions attributed in the source to branched and cyclic hydrocarbons: approximately $1385\text{--}1380$ and $1370\text{--}1365\text{ cm}^{-1}$ for isopropyl-related deformation, $2990\text{--}3100\text{ cm}^{-1}$ for selected cyclic C–H environments, and reference positions around 1452 , 1468 , 1455 and 1442 cm^{-1} for various cycloalkane or alkane-related vibrations. These values are not a complete set of independently measured peaks in Figure 1; several are generic reference frequencies that overlap between functional groups.

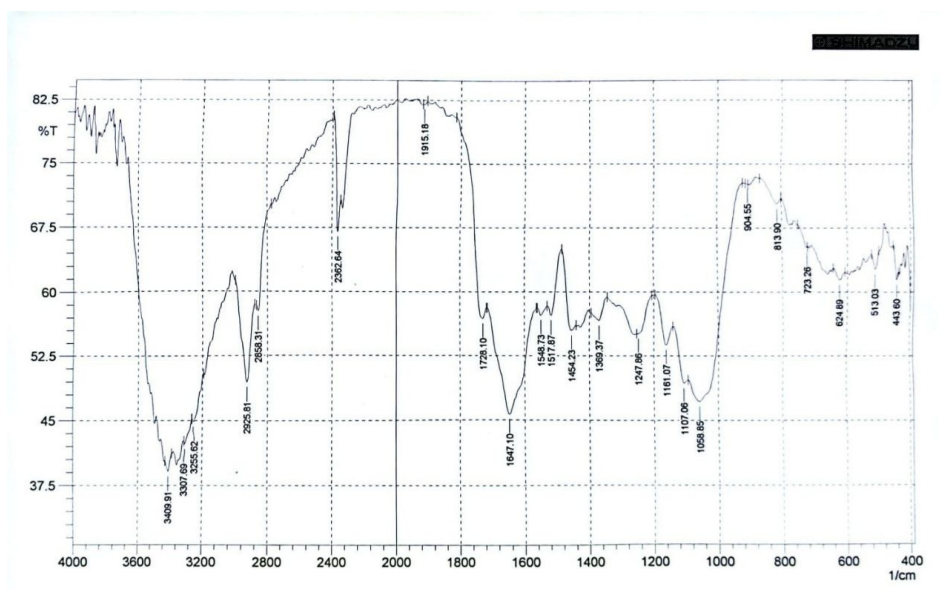


Figure 1. Infrared spectrum of the sage sample, reproduced from the author-supplied PDF. Functional-group assignments remain provisional.

For unsaturated and aromatic structures, the source provided approximate reference absorptions around $1667\text{--}1670\text{ cm}^{-1}$ for some substituted alkenes, 1640 cm^{-1} for vinyl-containing structures, 1670 cm^{-1} for more highly substituted alkenes, and $2000\text{--}1900\text{ cm}^{-1}$ for allene-related vibration. Regions around $900\text{--}675$ and $710\text{--}675\text{ cm}^{-1}$ were associated with aromatic or substituted-benzene out-of-plane motions, while bands near 1000 , 909 and 813 cm^{-1} were described as possible alkene-related fingerprint features. Such assignments remain tentative in mixtures without corroborating bands or purified standards.

The source further listed phenolic or O–H-related bands near $1390\text{--}1330$ and $1420\text{--}1330\text{ cm}^{-1}$, C–O–C features around $1170\text{--}1114$ and $1272\text{--}1200\text{ cm}^{-1}$, and numerous possible carbonyl absorptions over the broad range $1870\text{--}1540\text{ cm}^{-1}$. Specific reference intervals included $1655\text{--}1690\text{ cm}^{-1}$ for quinone-like carbonyls, $1720\text{--}1740\text{ cm}^{-1}$ for some aldehydes, $1370\text{--}1210$ and $1440\text{--}1395\text{ cm}^{-1}$ for carboxylic-acid-associated motions, and $1730\text{--}1715\text{ cm}^{-1}$ for selected unsaturated esters or related carbonyl compounds. The original text also listed $1140\text{--}1190\text{ cm}^{-1}$ for ester-associated fingerprint absorptions and $1655\text{--}1620\text{ cm}^{-1}$ for an amide-associated band; none of these ranges alone is sufficient to identify a particular compound.

The nitrogen- and sulfur-related reference ranges in the original manuscript included $1650\text{--}1580$ and $1342\text{--}1266\text{ cm}^{-1}$ for amine-related vibrations, $3100\text{--}2600\text{ cm}^{-1}$ for some protonated amine signatures, $2273\text{--}2000\text{ cm}^{-1}$ for nitrile/isocyanate-like or cumulated-bond regions, $1661\text{--}1499$ and $1389\text{--}1259\text{ cm}^{-1}$ for asymmetric and symmetric nitro-group vibrations, $700\text{--}600\text{ cm}^{-1}$ for some C–S vibrations, $500\text{--}600\text{ cm}^{-1}$ for weak S–S vibrations, $1250\text{--}1020\text{ cm}^{-1}$ for possible thiocarbonyl or related features, $1070\text{--}1030\text{ cm}^{-1}$ for sulfoxides, and $1410\text{--}1380$, $1204\text{--}1117$, $1370\text{--}1335$

and 1170–1155 cm^{-1} for certain sulfonyl/sulfonamide-related modes. The source additionally described organohalogen-associated regions of 600–500 and 1400–730 cm^{-1} . Because many of these signals overlap with unrelated vibrations, they do not establish the presence of isocyanates, halogenated drugs, sulfur pharmacophores or any other specified bioactive compound without validation.

It would be particularly inappropriate to infer antioxidant, free-radical-scavenging or antimicrobial effects merely from possible FTIR absorption bands. The figure does not contain an antioxidant assay, and the study did not provide functional testing that would justify therapeutic claims. The appropriate conclusion is that infrared analysis offers a qualitative description of spectral features that may inform targeted follow-up characterization.

3.3. GC-MS OUTPUT AND TENTATIVE COMPOUND INFORMATION

The total-ion chromatogram and associated peak report are reproduced in Figure 2.

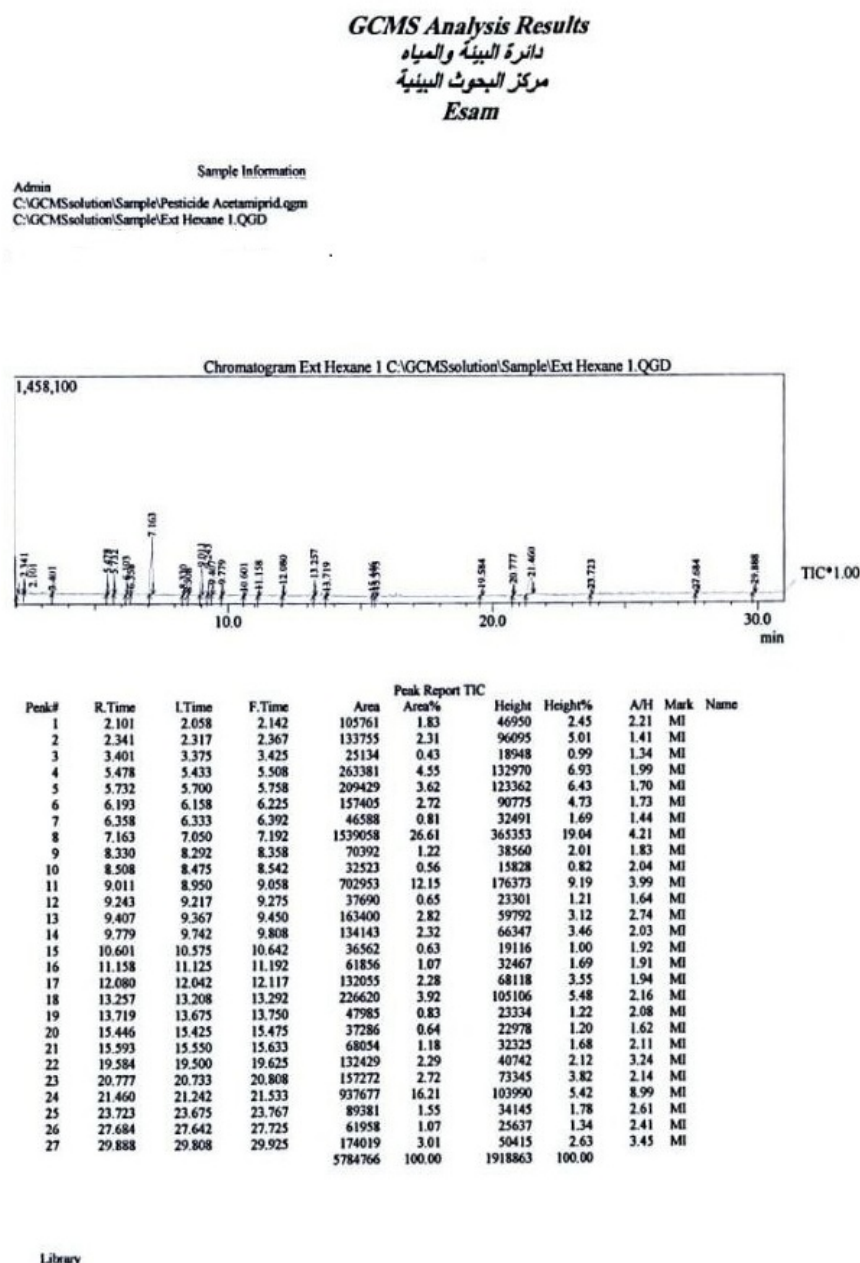


Figure 2. GC-MS total-ion chromatogram and peak report for the extract, reproduced from the supplied analytical output; solvent annotation requires verification.

The supporting PDF then presents a sequence of mass spectra and candidate library comparisons. Figures 3, 4, 5, 6, 7, 8 and 9 reproduce the early part of this sequence. Figures 10, 11, 12, 13, 14, 15 and 16 contain subsequent mass-spectral panels. Figures 17, 18, 19, 20, 21, 22 and 23 document the next set of library-search displays. The final set of panels is

shown in Figures 24, 25, 26, 27, 28 and 29. Thus, all supplied GC–MS output images are retained and separately identified.

These images show multiple chromatographic and mass-spectral features, but the supplied source does not provide a complete validated peak table containing retention times, integrated areas, reference-library scores, retention indices and confirmed compounds. In addition, the solvent label and reported extraction method disagree. The graphics can therefore substantiate that the source contains chromatographic output and candidate library-matching information, but cannot establish a reliable percentage composition for named phytochemicals. Published analyses of sage extracts show that different methods recover different volatile and semi-volatile substances, reinforcing the need to report the specific experimental conditions [1–3].

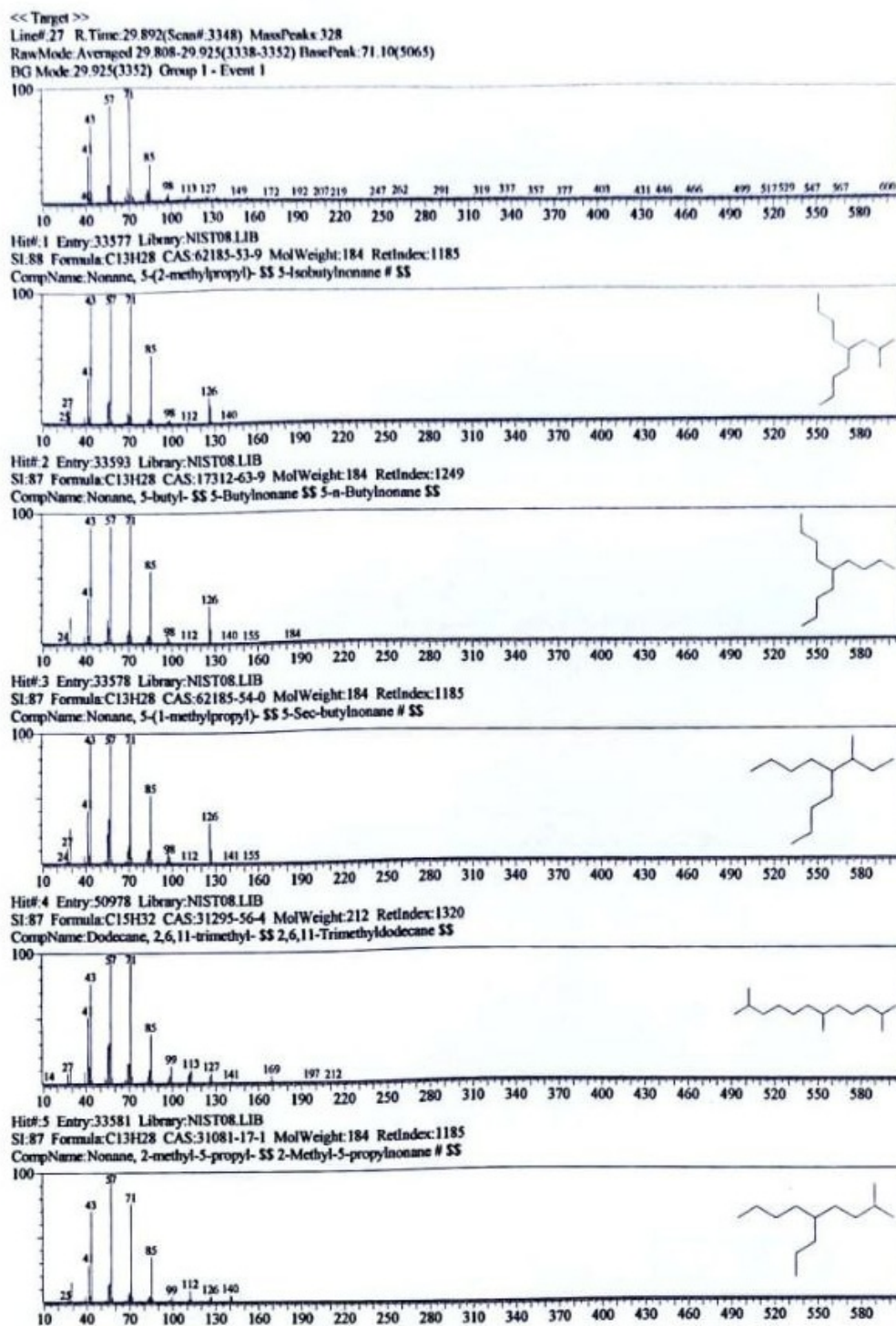


Figure 3. GC–MS mass-spectral library-search output, panel 1 of 27; candidate matches are tentative and not confirmed compound identities.

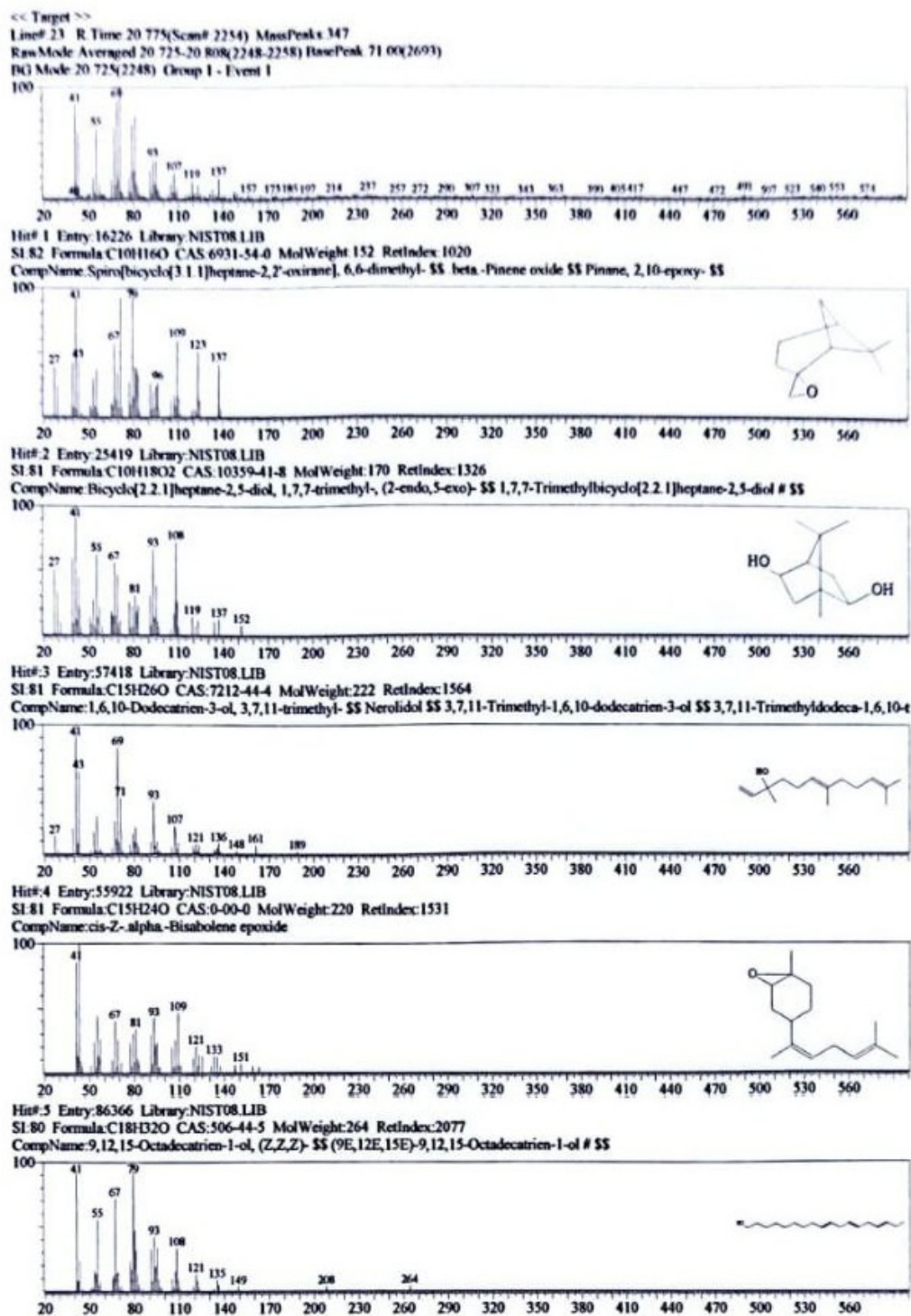


Figure 4. GC-MS mass-spectral library-search output, panel 2 of 27; candidate matches are tentative and not confirmed compound identities.

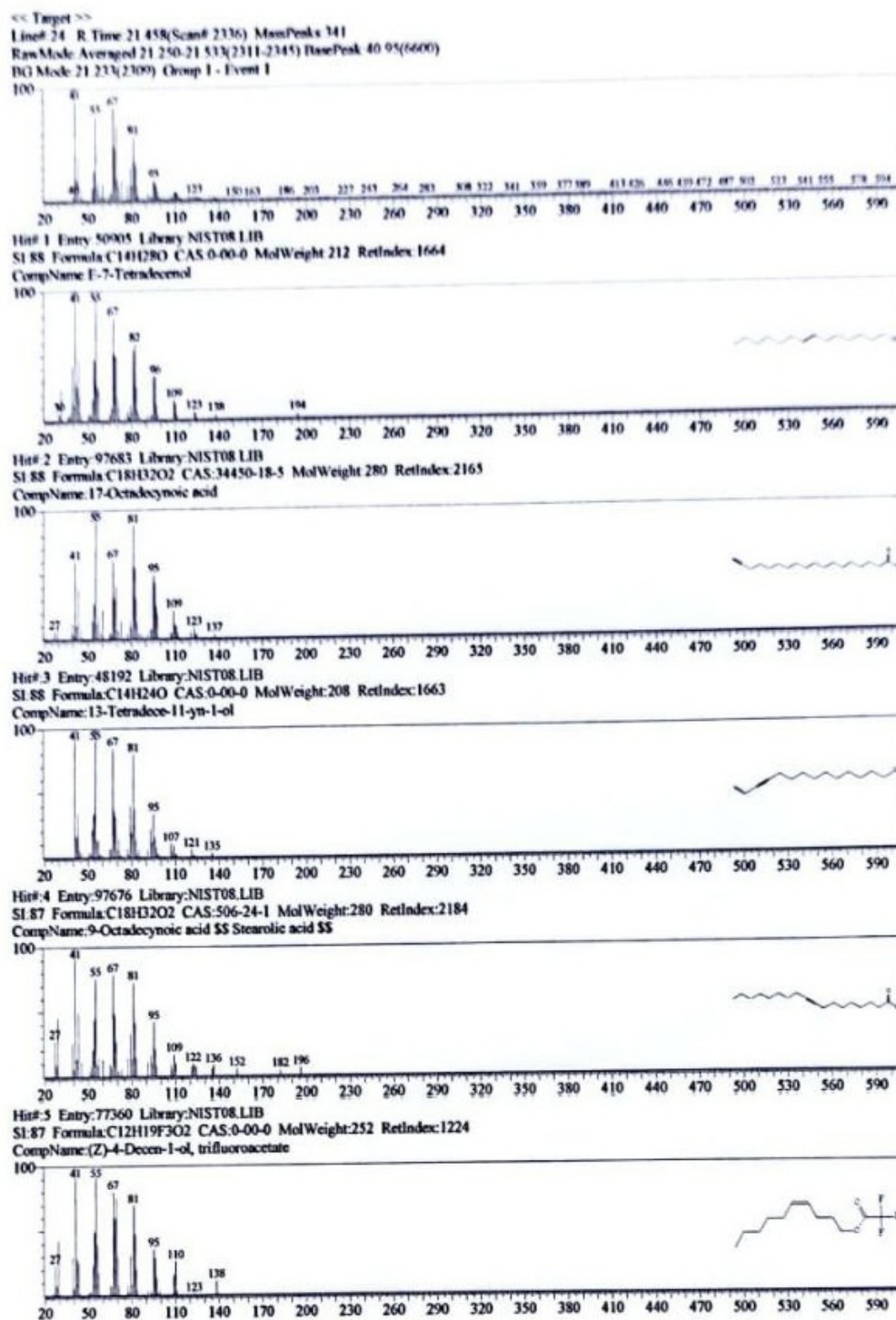


Figure 5. GC-MS mass-spectral library-search output, panel 3 of 27; candidate matches are tentative and not confirmed compound identities.

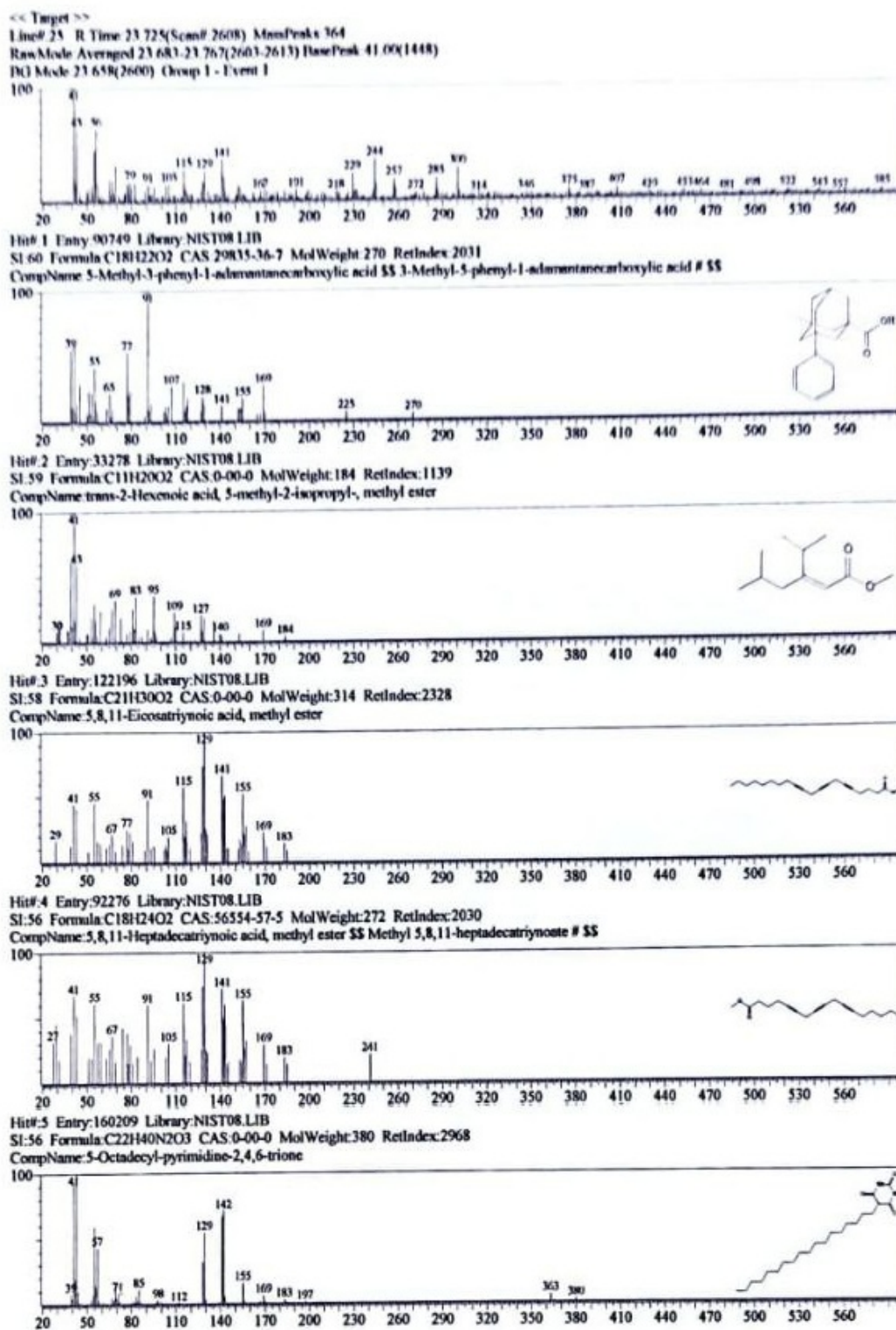


Figure 6. GC-MS mass-spectral library-search output, panel 4 of 27; candidate matches are tentative and not confirmed compound identities.

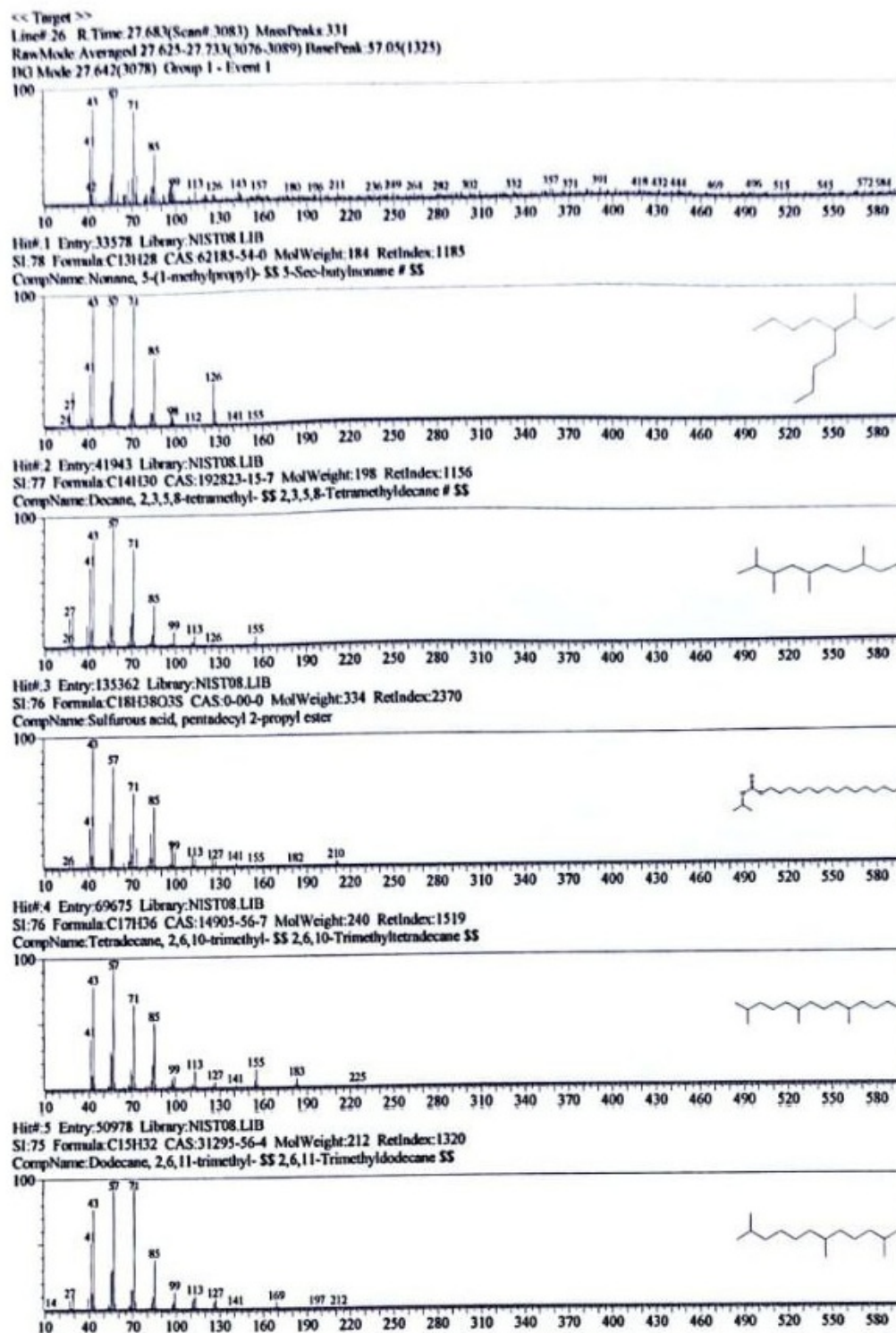


Figure 7. GC-MS mass-spectral library-search output, panel 5 of 27; candidate matches are tentative and not confirmed compound identities.

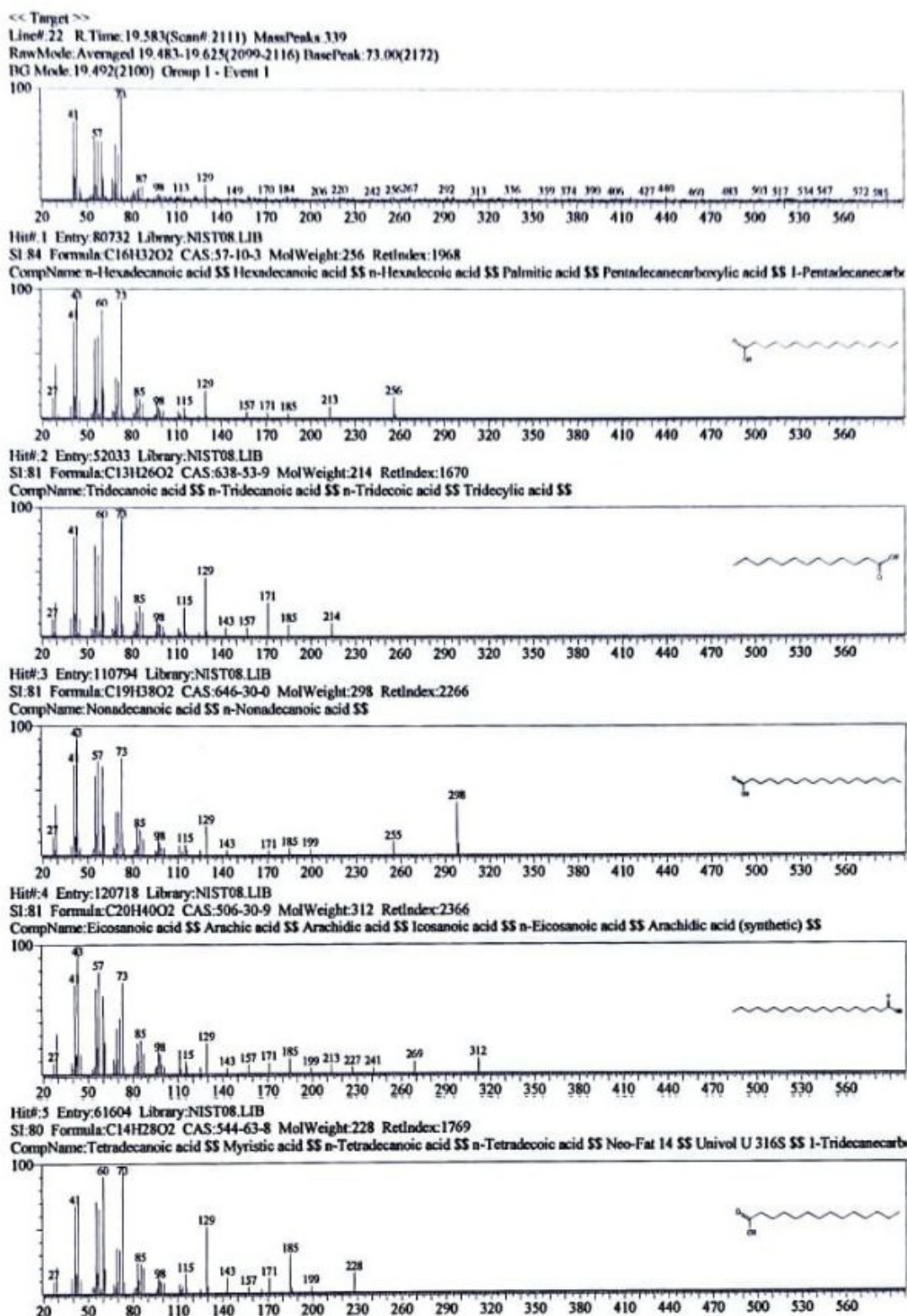


Figure 8. GC-MS mass-spectral library-search output, panel 6 of 27; candidate matches are tentative and not confirmed compound identities.

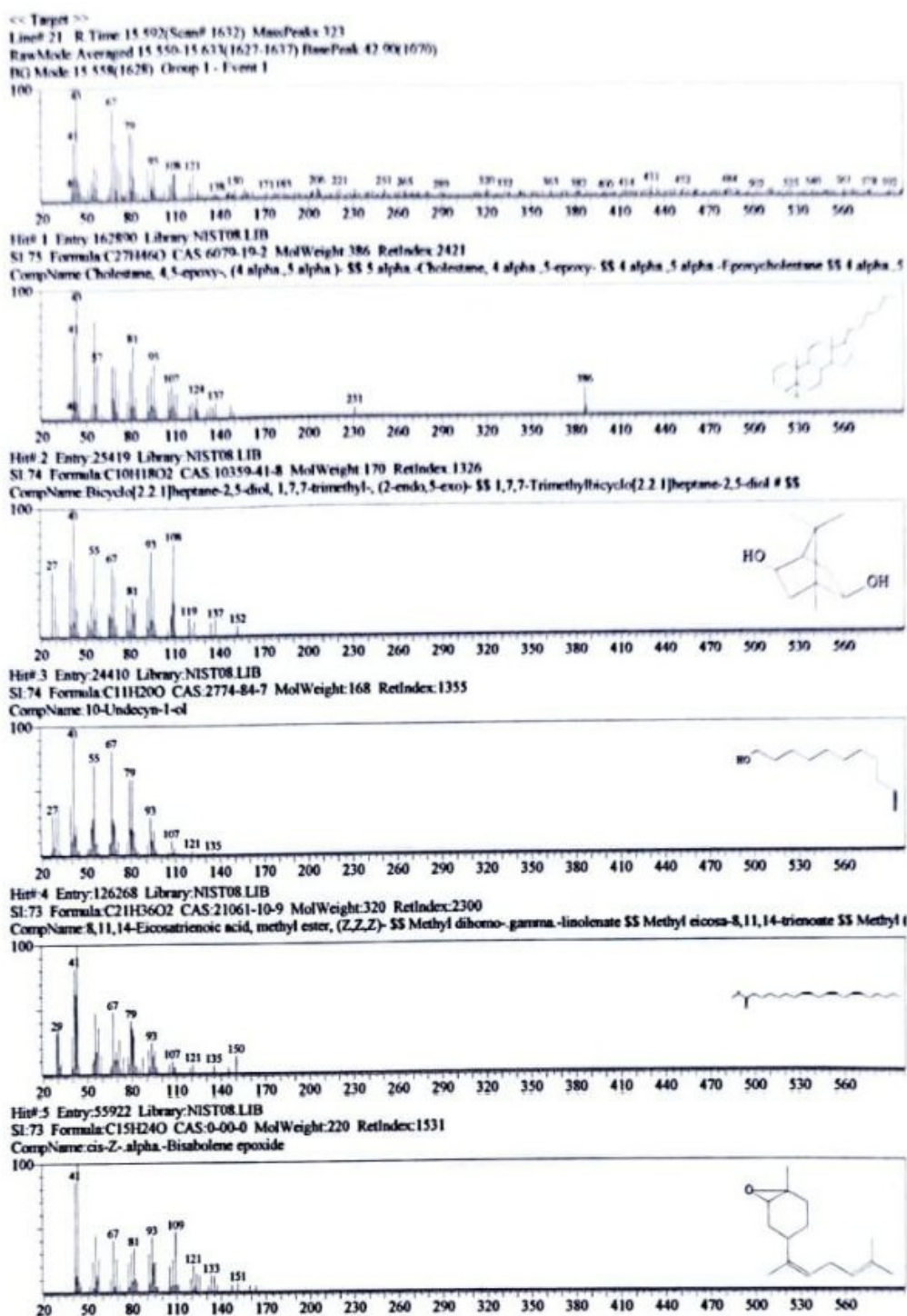


Figure 9. GC-MS mass-spectral library-search output, panel 7 of 27; candidate matches are tentative and not confirmed compound identities.

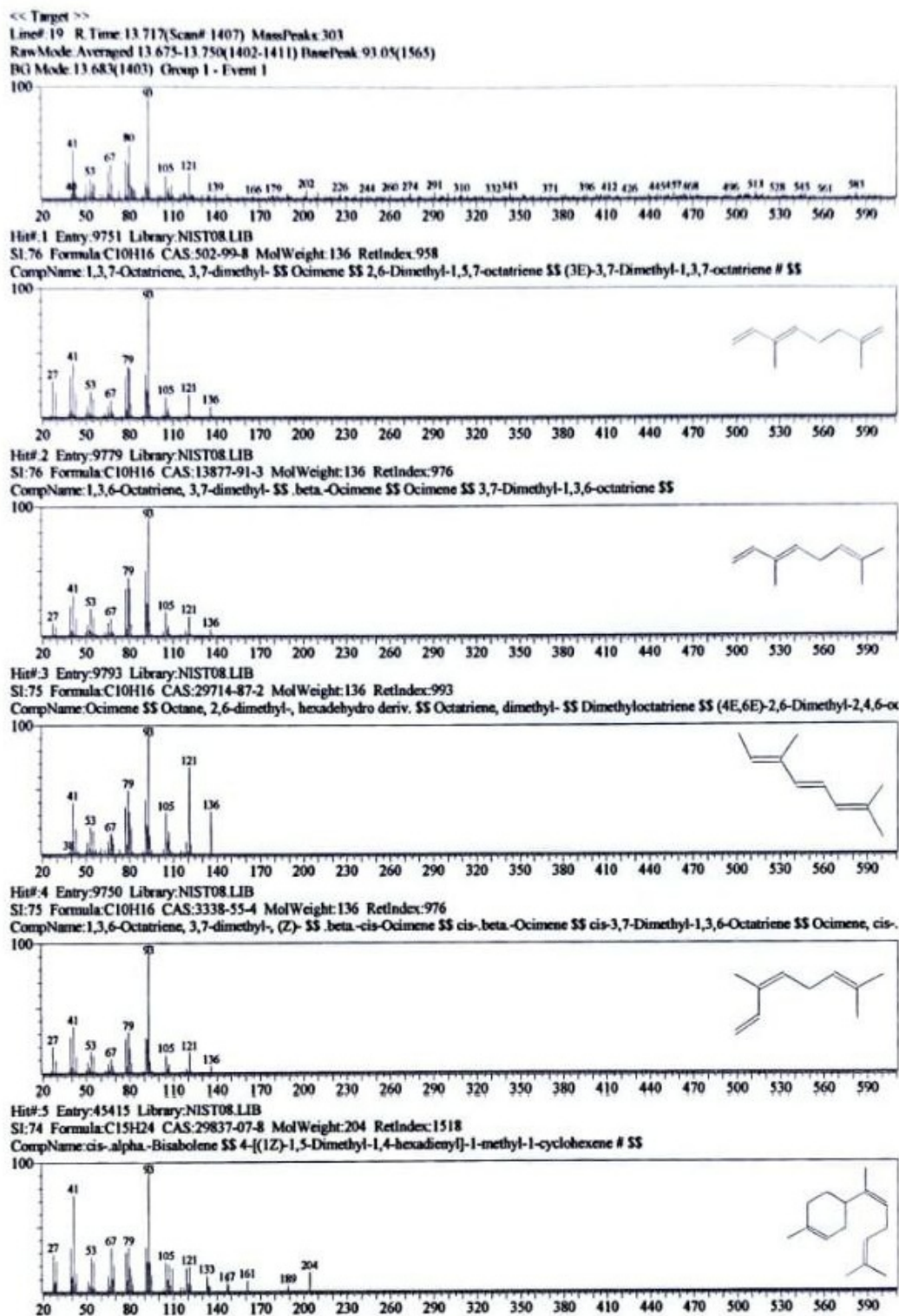


Figure 10. GC-MS mass-spectral library-search output, panel 8 of 27; candidate matches are tentative and not confirmed compound identities.

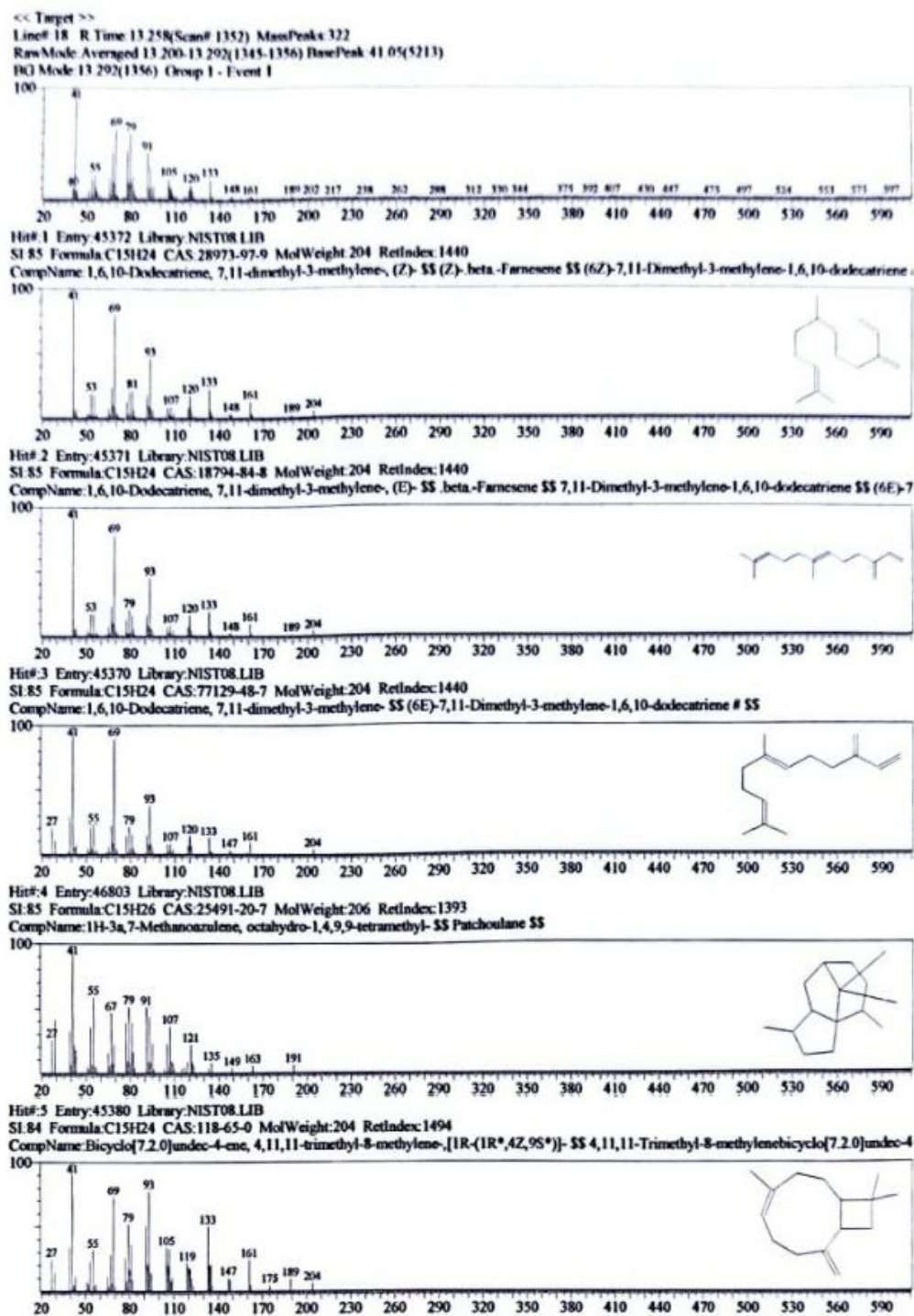


Figure 11. GC-MS mass-spectral library-search output, panel 9 of 27; candidate matches are tentative and not confirmed compound identities.

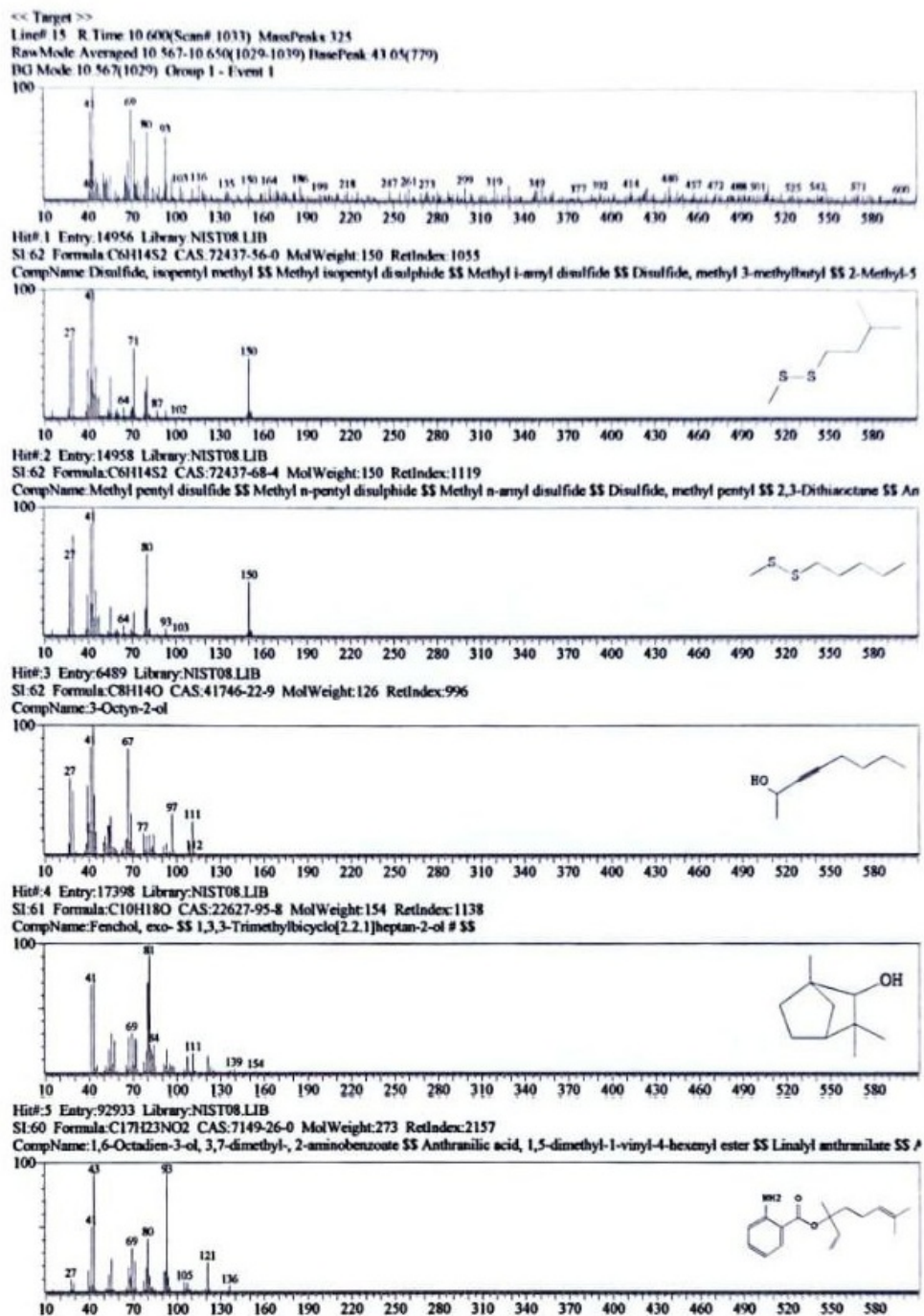


Figure 12. GC-MS mass-spectral library-search output, panel 10 of 27; candidate matches are tentative and not confirmed compound identities.

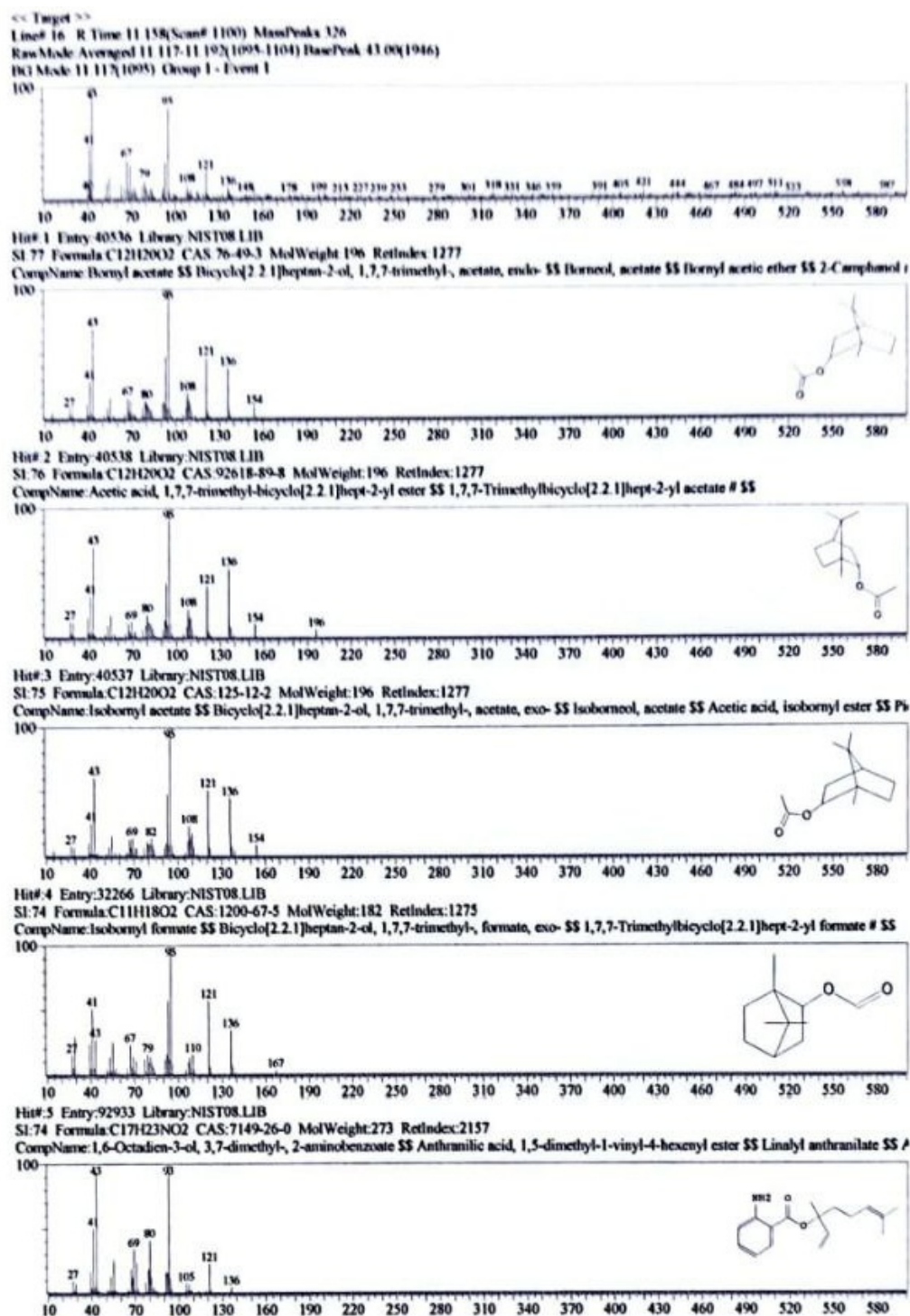


Figure 13. GC-MS mass-spectral library-search output, panel 11 of 27; candidate matches are tentative and not confirmed compound identities.

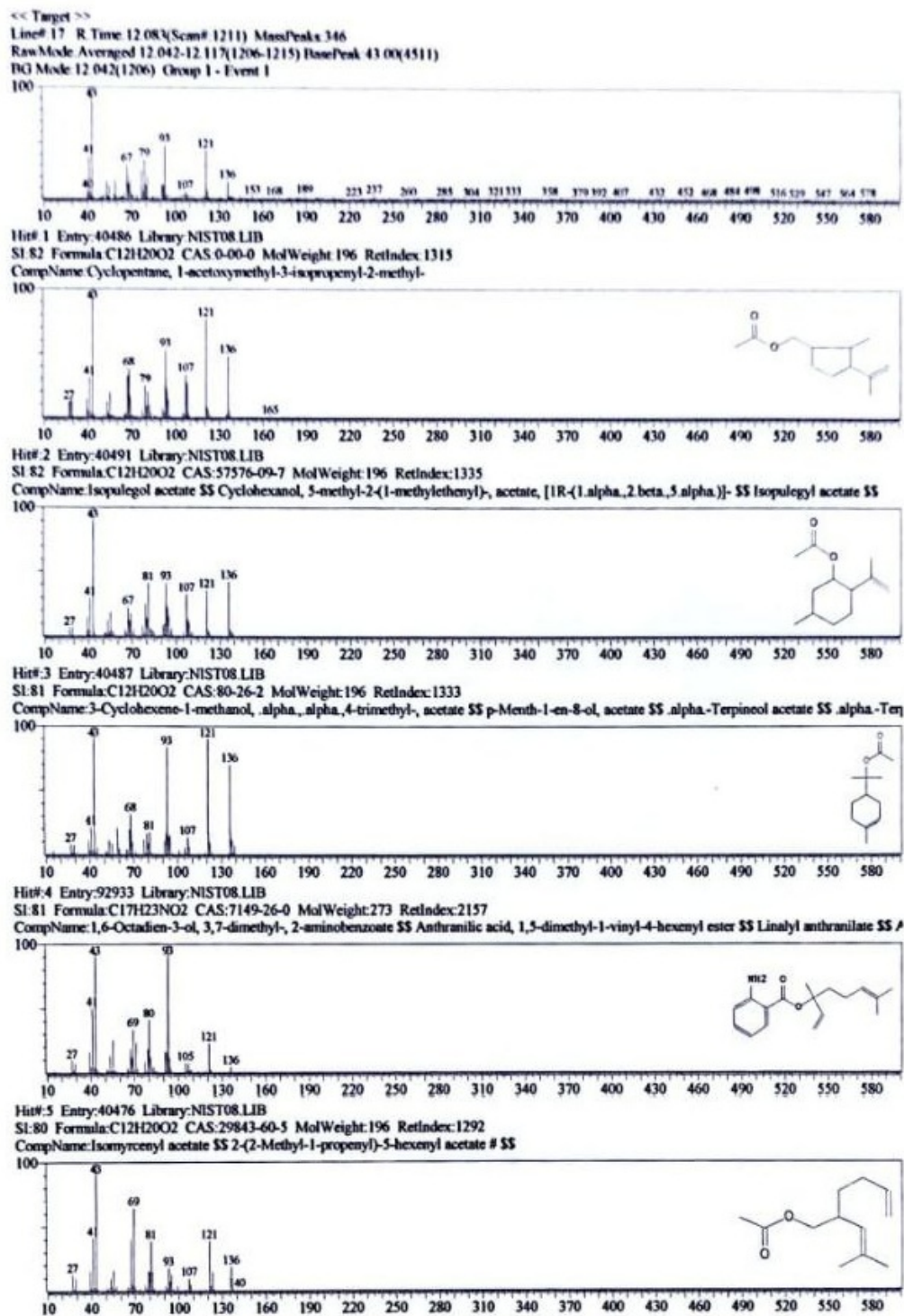


Figure 14. GC-MS mass-spectral library-search output, panel 12 of 27; candidate matches are tentative and not confirmed compound identities.

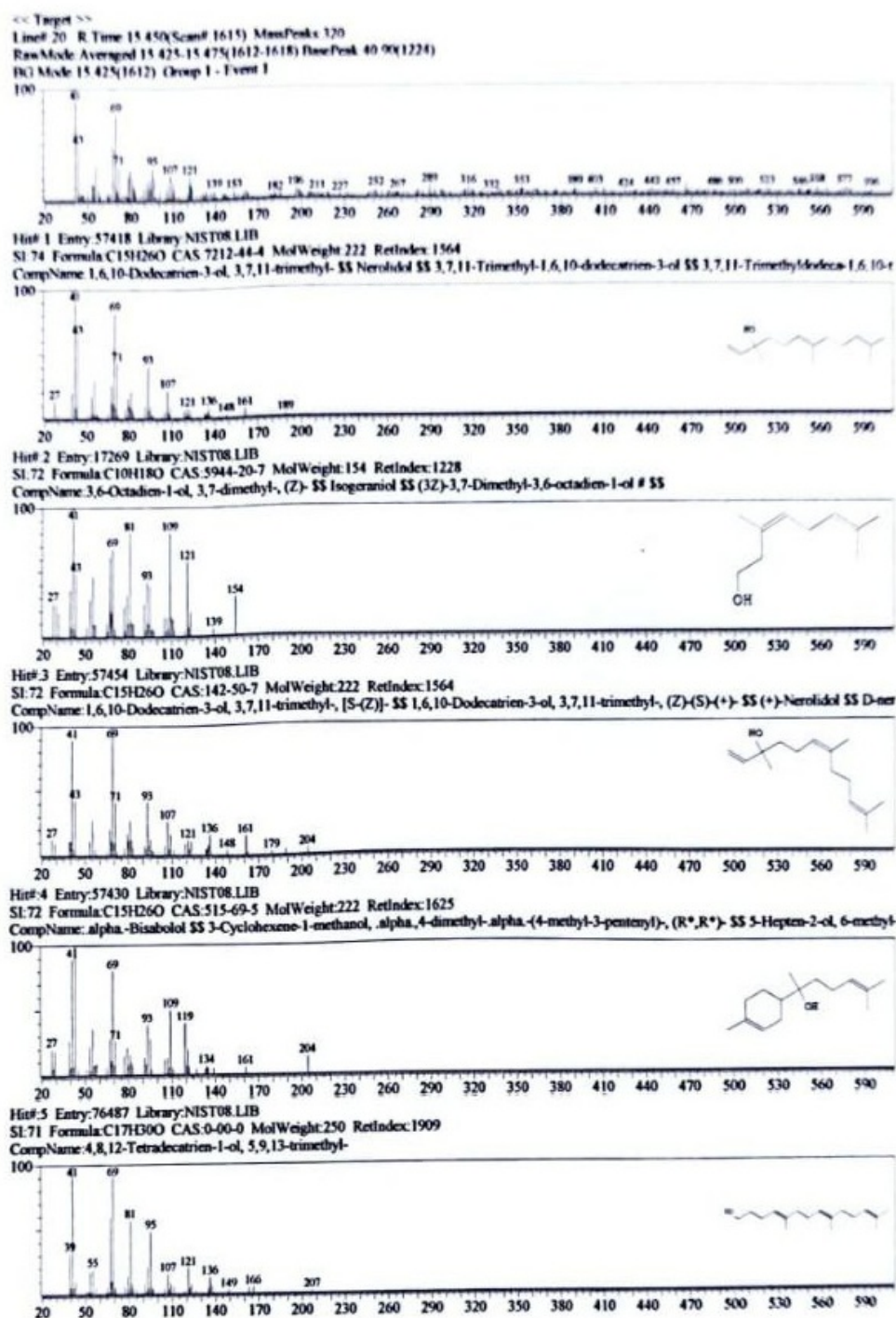


Figure 15. GC-MS mass-spectral library-search output, panel 13 of 27; candidate matches are tentative and not confirmed compound identities.

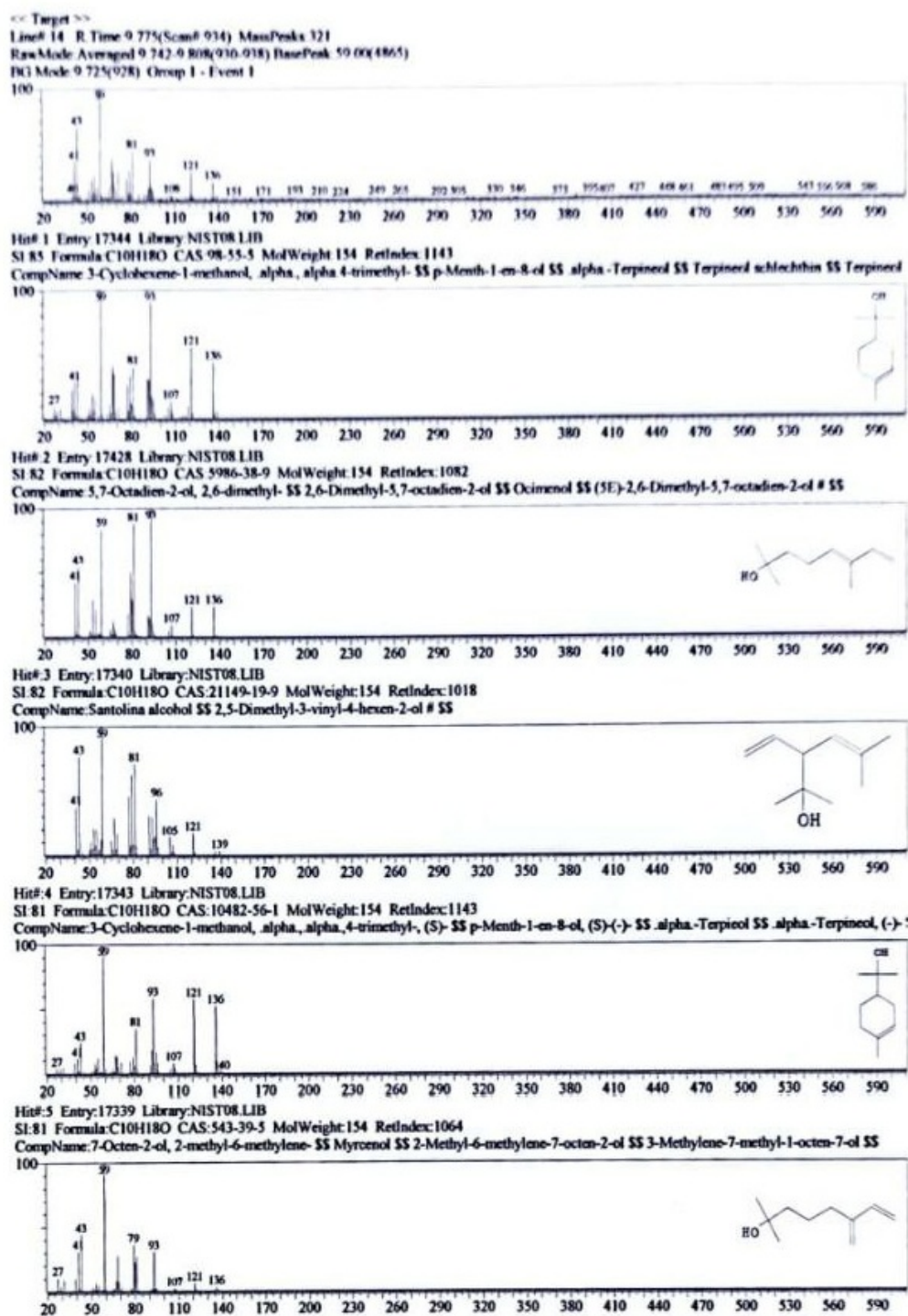


Figure 16. GC-MS mass-spectral library-search output, panel 14 of 27; candidate matches are tentative and not confirmed compound identities.

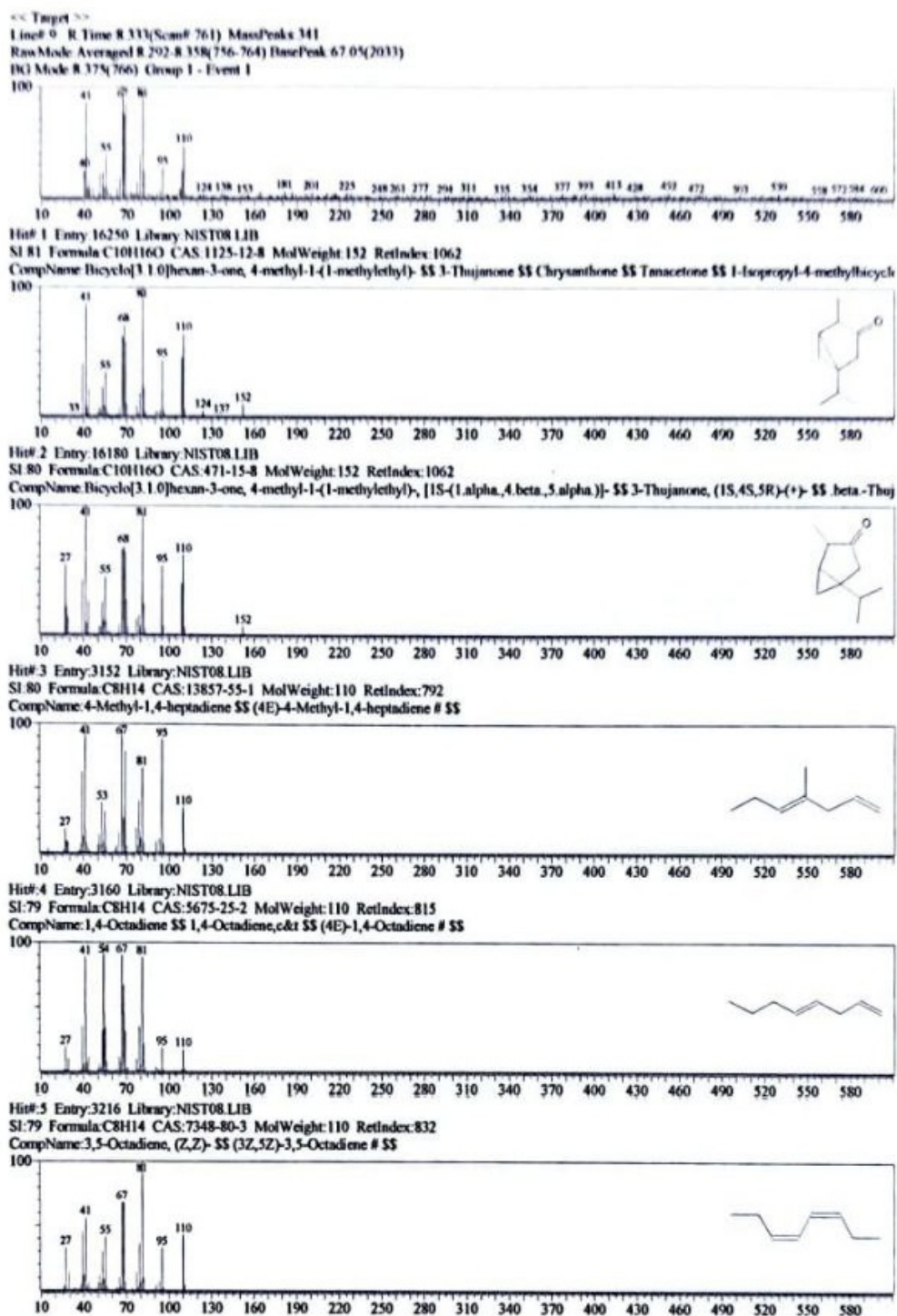


Figure 17. GC-MS mass-spectral library-search output, panel 15 of 27; candidate matches are tentative and not confirmed compound identities.

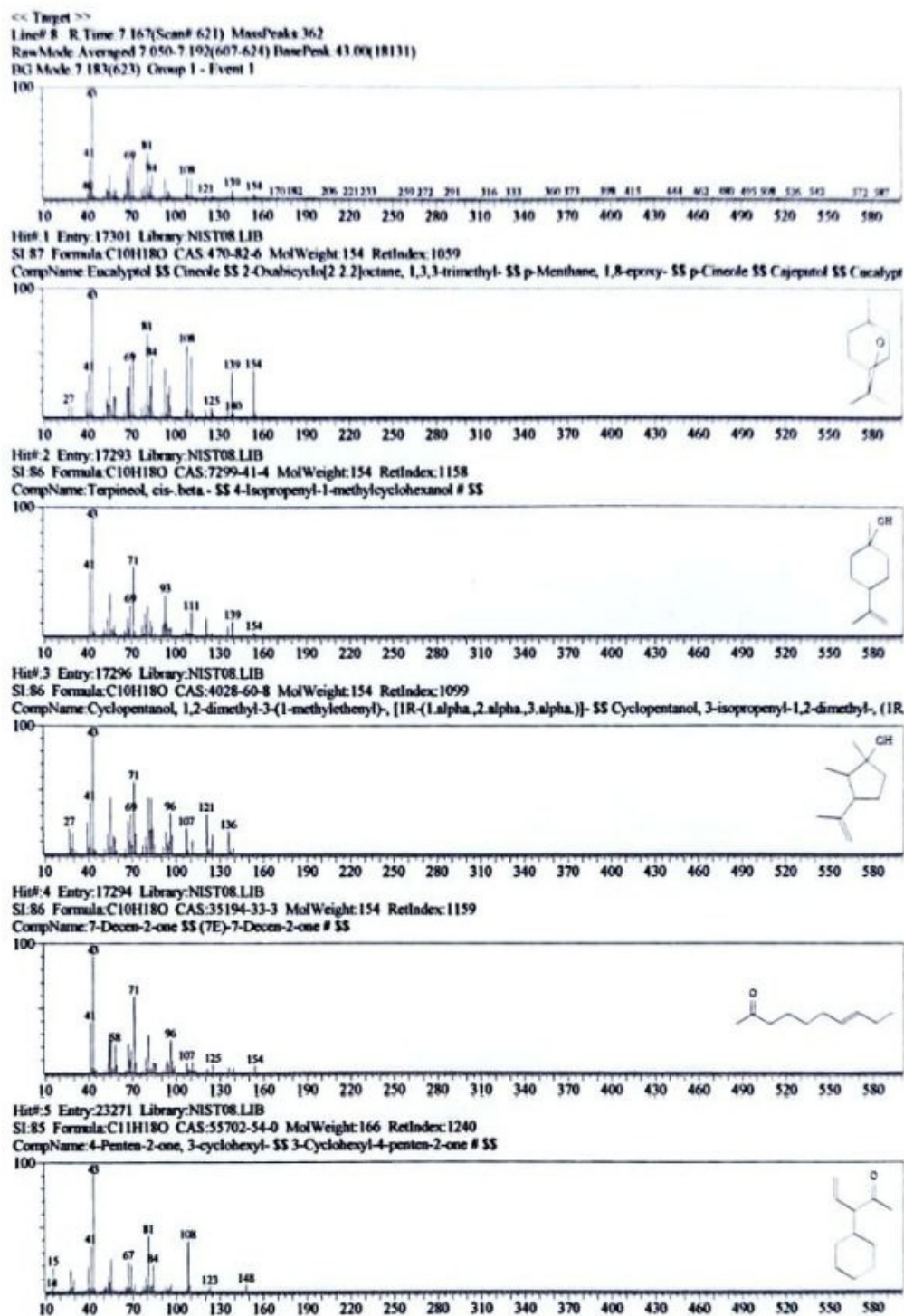


Figure 18. GC-MS mass-spectral library-search output, panel 16 of 27; candidate matches are tentative and not confirmed compound identities.

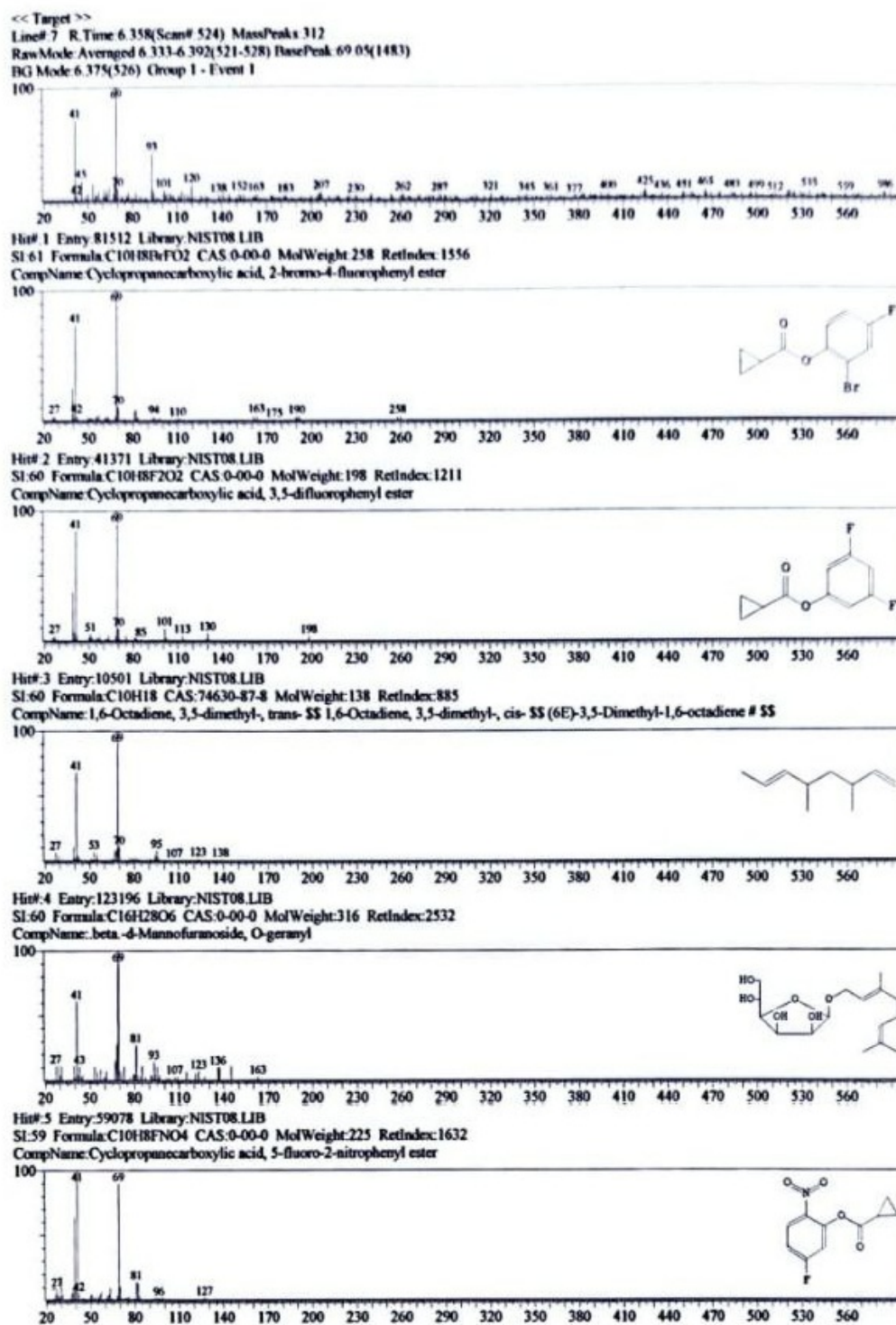


Figure 19. GC-MS mass-spectral library-search output, panel 17 of 27; candidate matches are tentative and not confirmed compound identities.

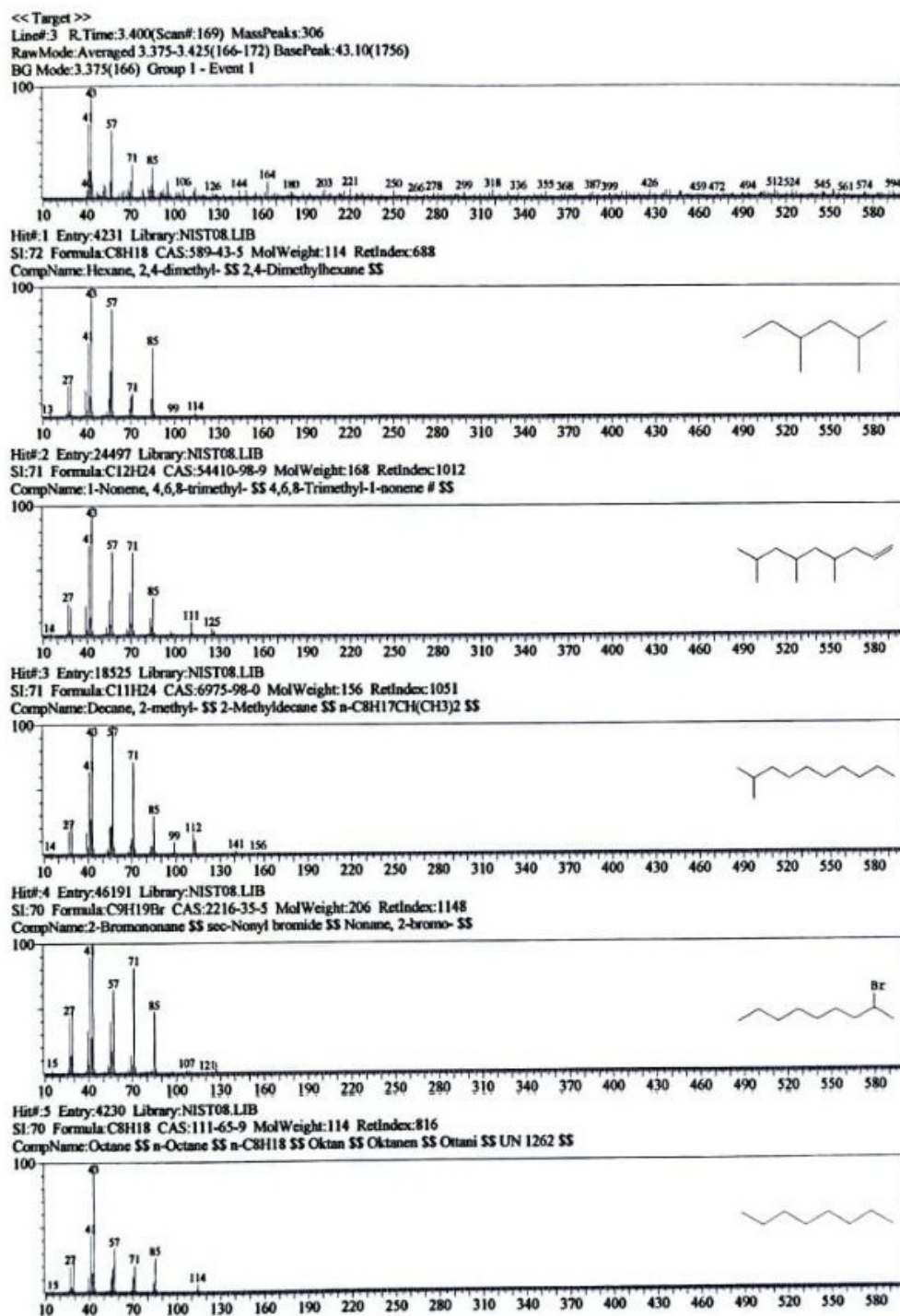


Figure 20. GC-MS mass-spectral library-search output, panel 18 of 27; candidate matches are tentative and not confirmed compound identities.

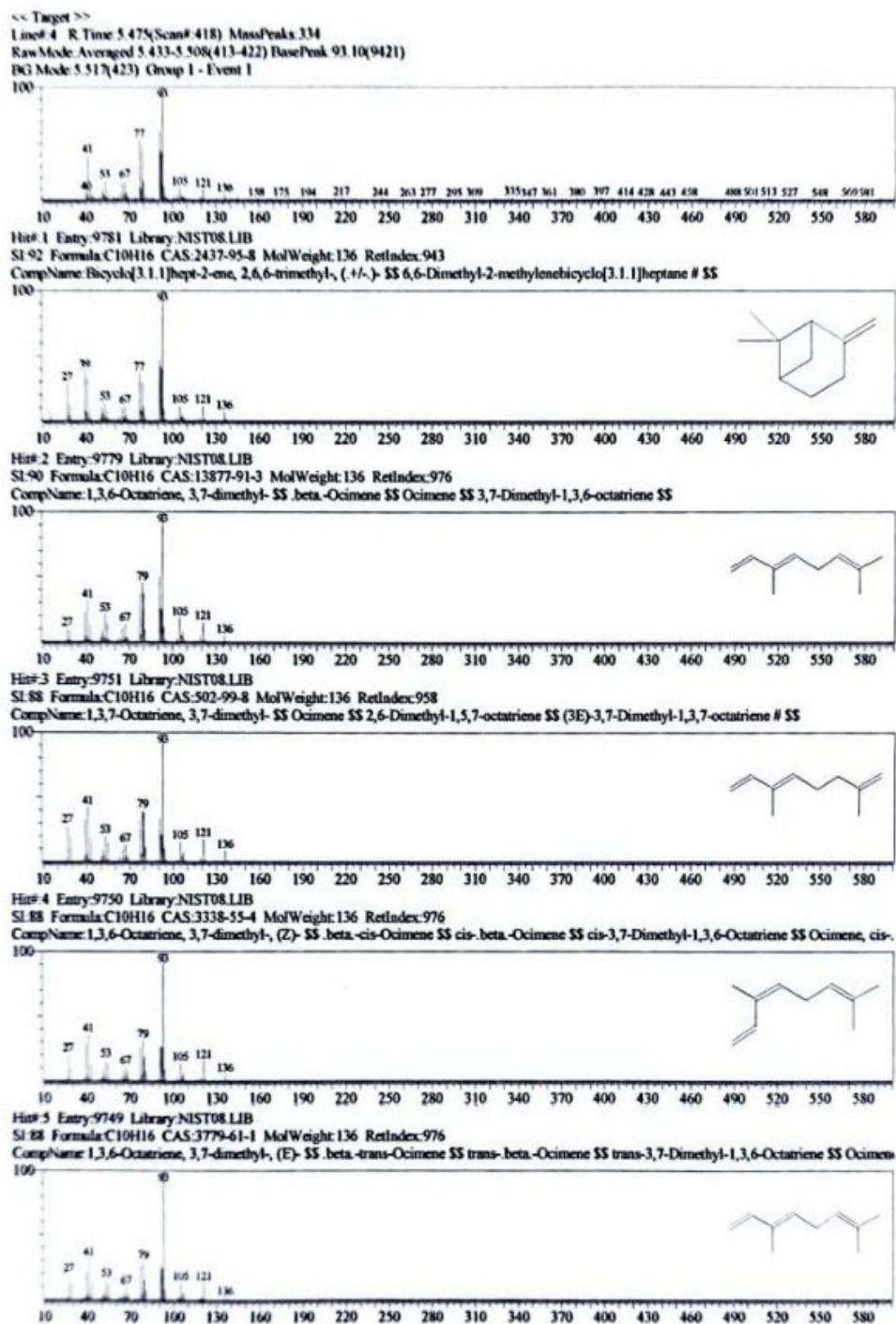


Figure 21. GC-MS mass-spectral library-search output, panel 19 of 27; candidate matches are tentative and not confirmed compound identities.

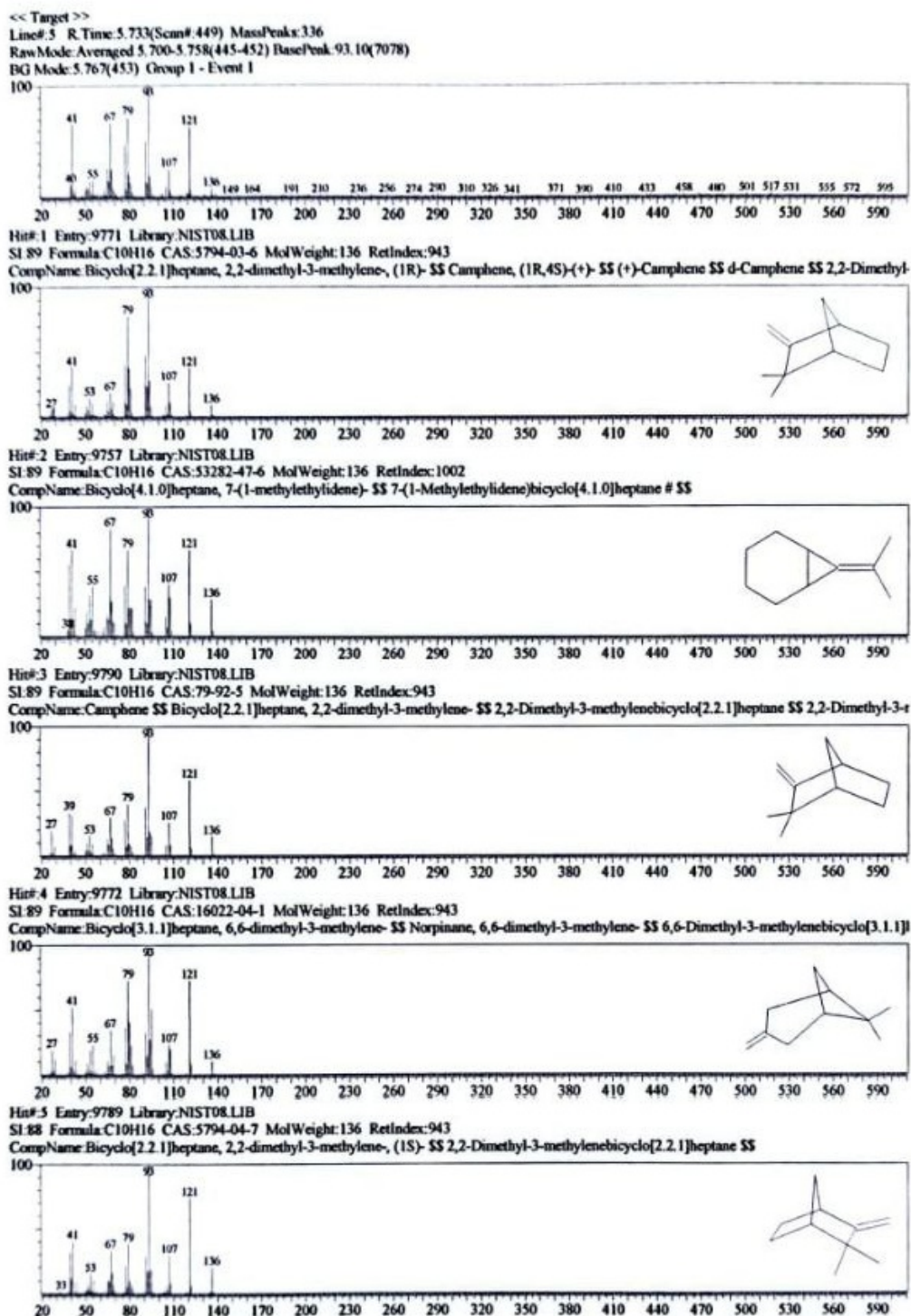


Figure 22. GC-MS mass-spectral library-search output, panel 20 of 27; candidate matches are tentative and not confirmed compound identities.

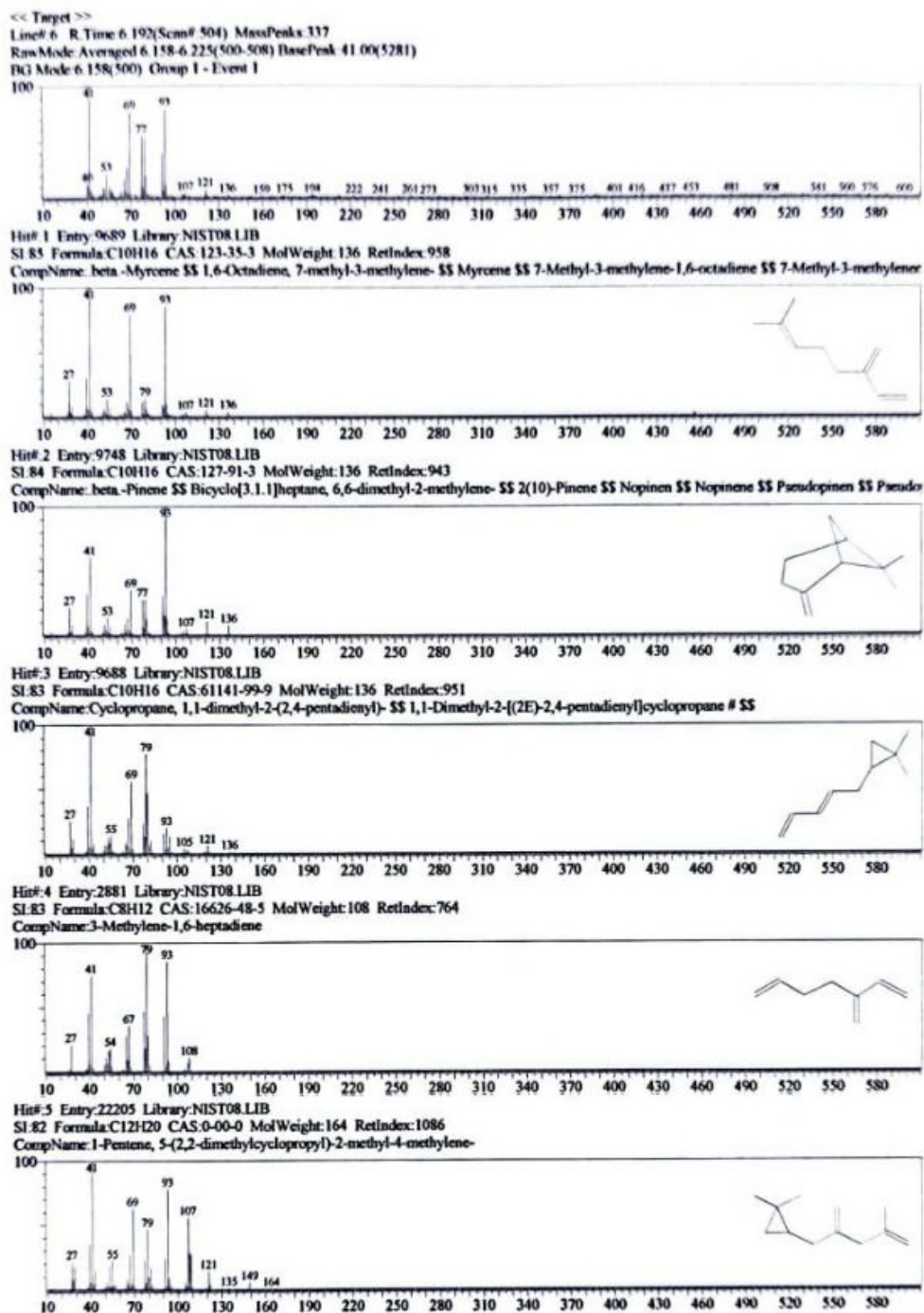


Figure 23. GC-MS mass-spectral library-search output, panel 21 of 27; candidate matches are tentative and not confirmed compound identities.

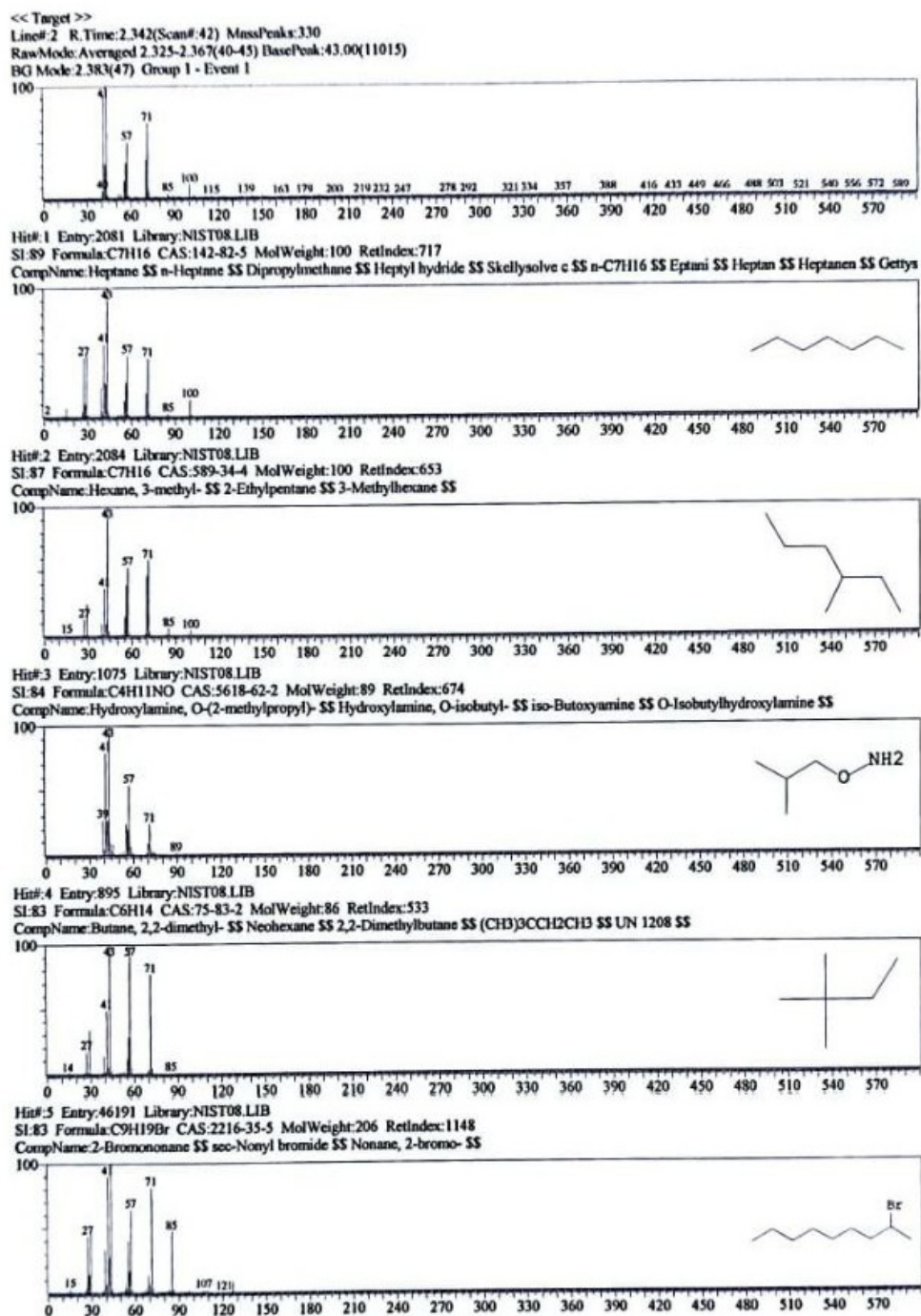


Figure 24. GC-MS mass-spectral library-search output, panel 22 of 27; candidate matches are tentative and not confirmed compound identities.

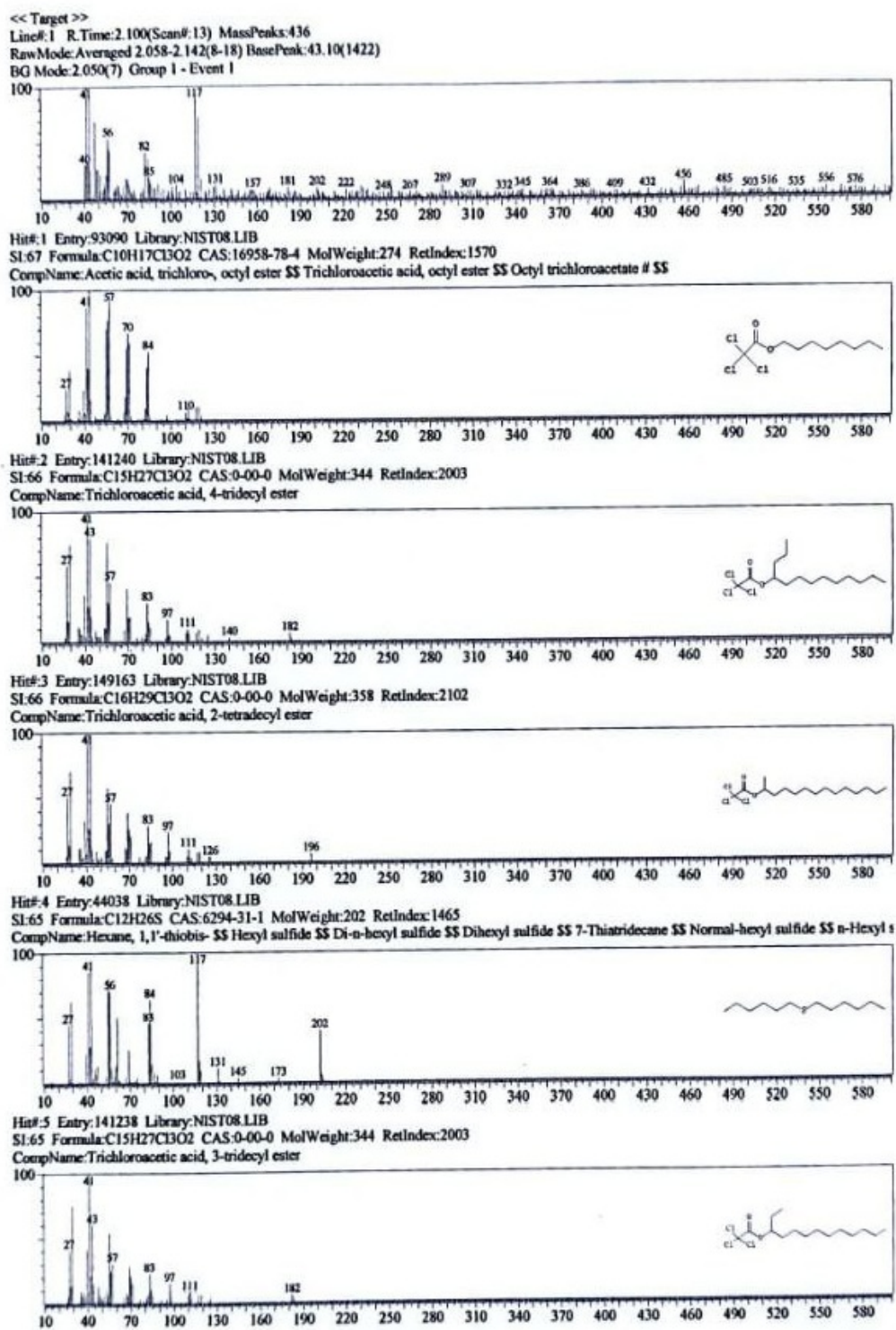


Figure 25. GC-MS mass-spectral library-search output, panel 23 of 27; candidate matches are tentative and not confirmed compound identities.

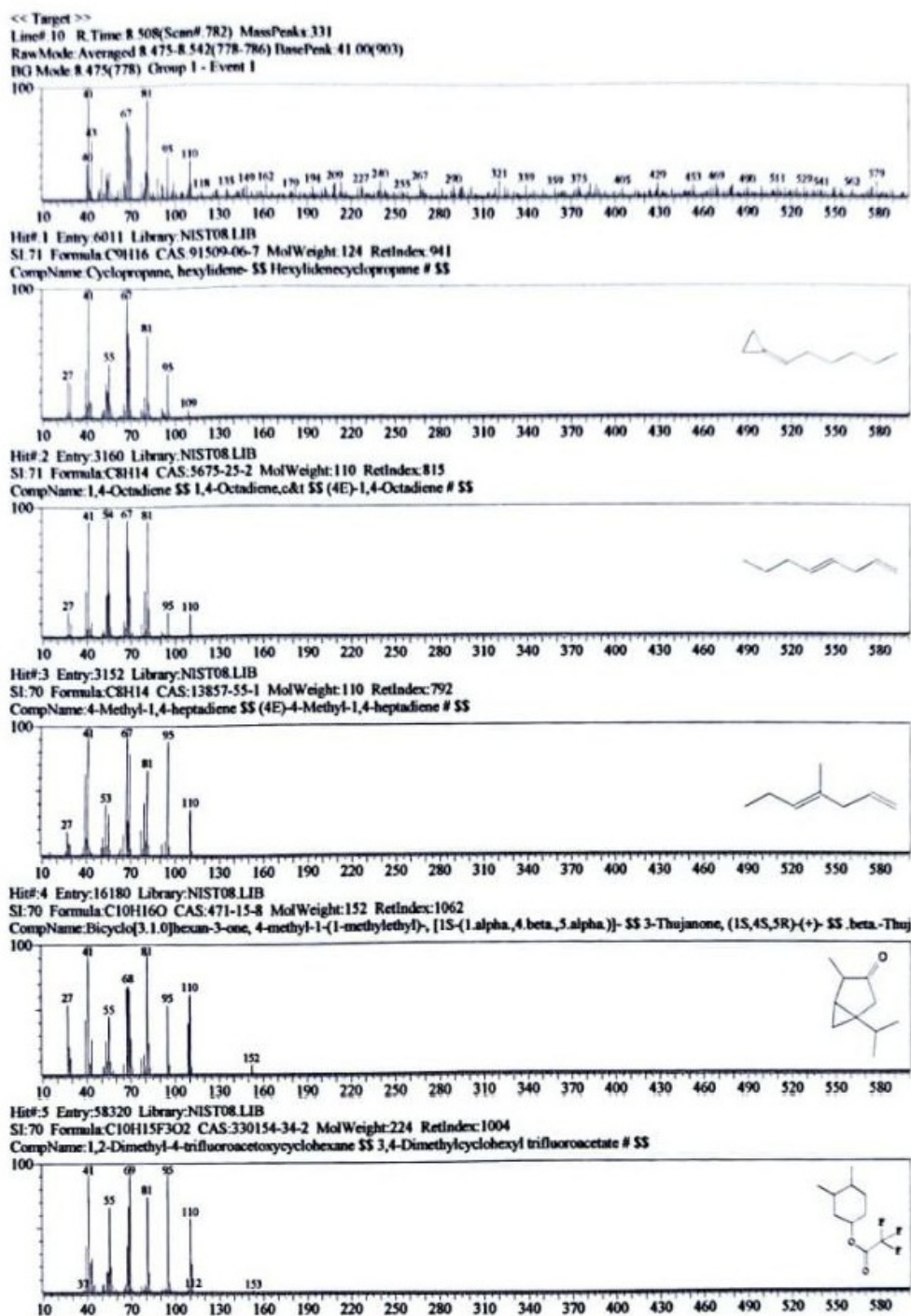


Figure 26. GC-MS mass-spectral library-search output, panel 24 of 27; candidate matches are tentative and not confirmed compound identities.

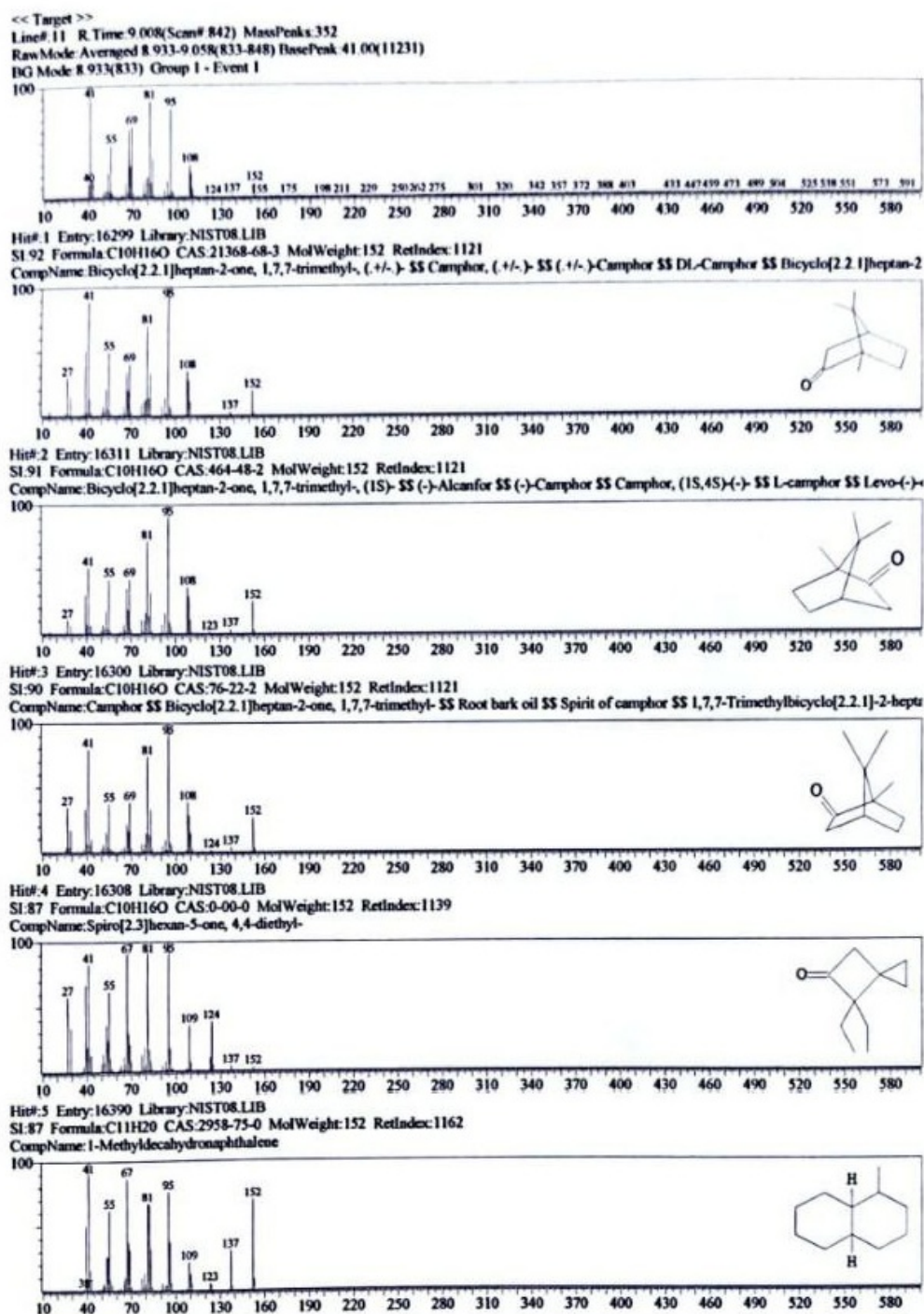


Figure 27. GC-MS mass-spectral library-search output, panel 25 of 27; candidate matches are tentative and not confirmed compound identities.

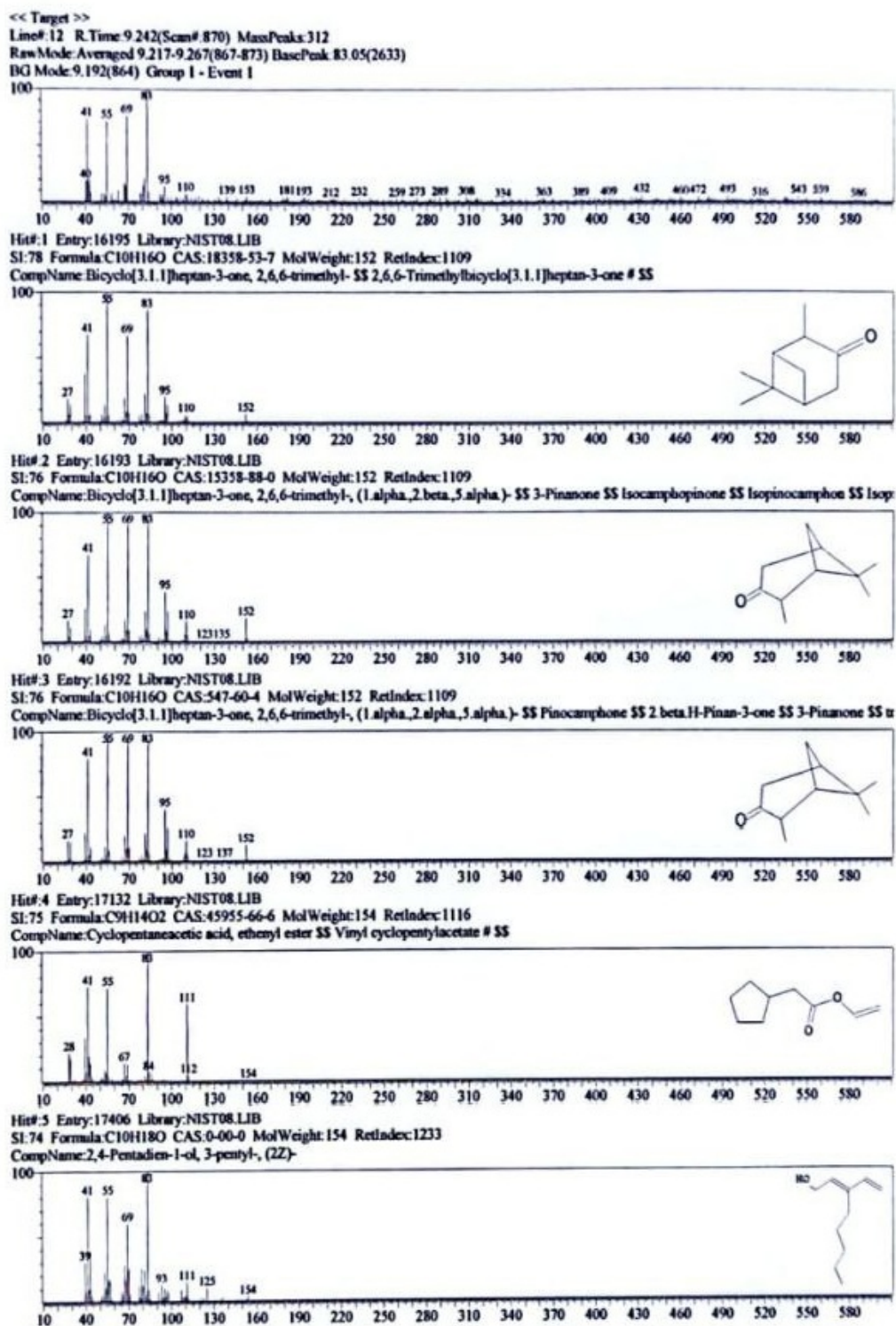


Figure 28. GC-MS mass-spectral library-search output, panel 26 of 27; candidate matches are tentative and not confirmed compound identities.

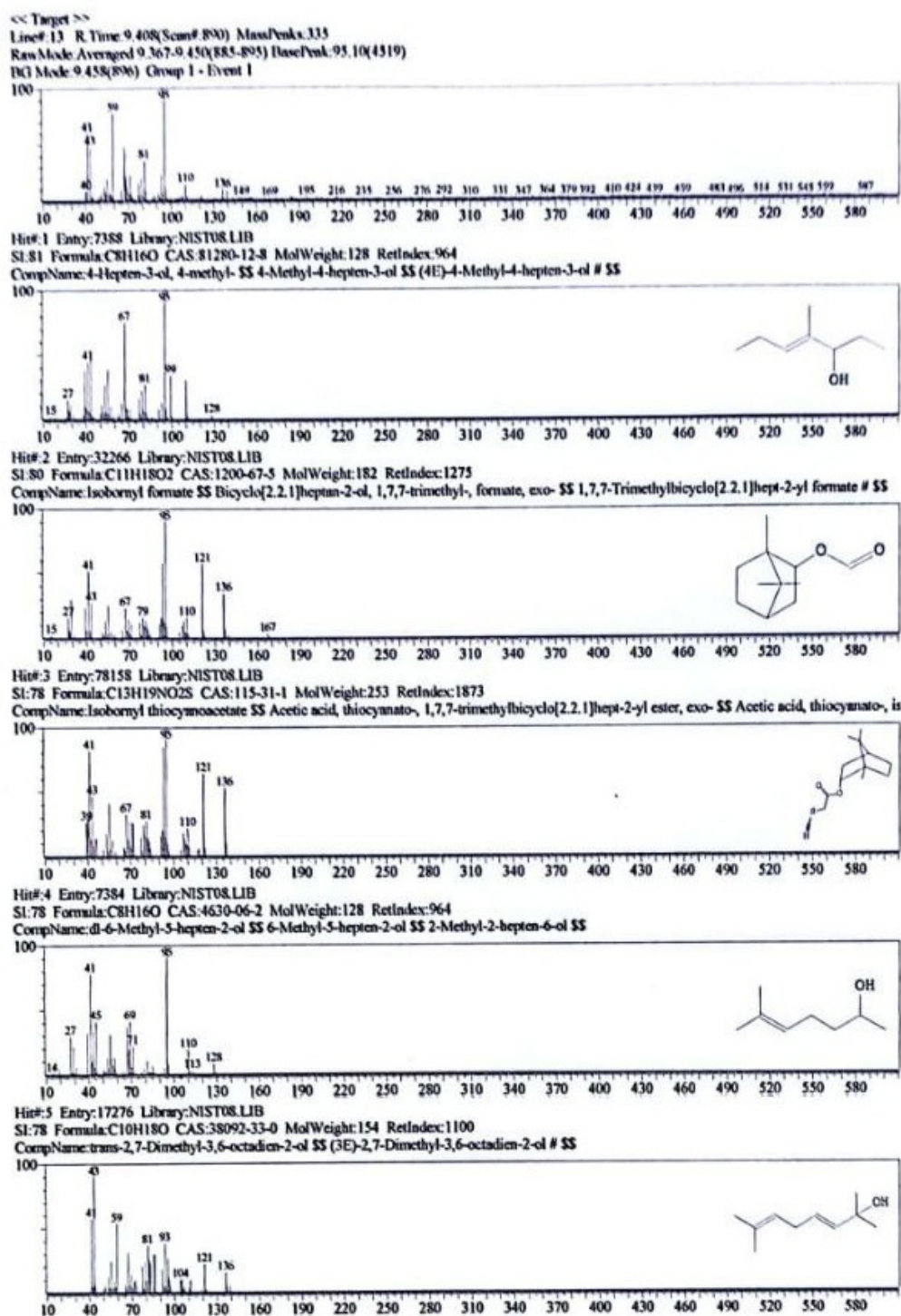


Figure 29. GC-MS mass-spectral library-search output, panel 27 of 27; candidate matches are tentative and not confirmed compound identities.

3.4. PRELIMINARY ANTIMICROBIAL OBSERVATION

Figure 30 presents the supplied diffusion-assay photograph. The principal plate contains carrier positions labeled 1%, 2%, 5%, 10% and "D.W." A clearly defined, measurable halo surrounding the tested positions is not apparent in the image, which is consistent with the laboratory narrative's report of no detectable inhibition zone. Because the underlying measurement sheet, test controls, extract quantities, organism identification and incubation record are unavailable, no numerical inhibition-zone diameters, minimum inhibitory concentrations or significance tests can be reported. The most supportable observation is the absence of a discernible inhibition zone in the photographed assay conditions.

This observation should not be generalized to all preparations of *S. officinalis*. Published experiments have reported activity of sage essential oil against some microorganisms and activity of specific sage-derived constituents against selected

resistant bacteria [2, 4]. Mokhtari et al. observed activity of methanolic plant preparations against organisms including *S. aureus*, but their work investigated different extracts, a different microbial panel and different extraction procedures [5]. Differences in solubility, agar diffusion, concentration, strain susceptibility and extraction chemistry may all influence a visible-zone assay. Consequently, the present photographic result is not a direct refutation of those studies, nor can it establish whether the tested material would act differently in a broth assay or against a different microorganism.

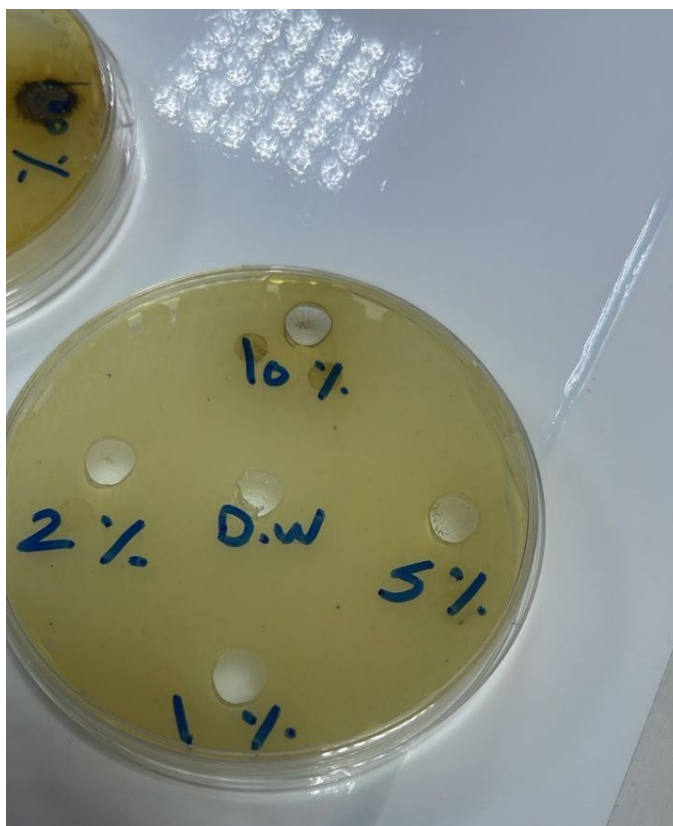


Figure 30. Supplied agar-diffusion photograph labeled 1%, 2%, 5%, 10% and D.W.; no clear inhibition halo is visible. Test conditions and controls require author confirmation.

4. LIMITATIONS AND REQUIREMENTS FOR REPRODUCIBILITY

This report is constrained by incomplete analytical and microbiological metadata. First, the identity of the Soxhlet solvent is unresolved, and essential details of sample collection, botanical authentication, drying, extraction conditions and yield were not supplied. Second, the elemental percentages lack information on the mass basis, calibration, analytical recovery and replicate variation. The tabulated nitrogen p-value cannot be evaluated without a test statistic and experimental design. Third, the infrared spectrum lacks an accompanying measured peak-position table, and the GC–MS library-search images are not a validated compound-identification report. Fourth, the microbiological method does not establish the strain's accession or identification procedure, control performance, inoculum standardization, replication or quantitative endpoint. The disagreement between the stated concentration series and the plate labels further limits interpretation.

These limitations are material rather than cosmetic. For reproducibility, future studies should specify an authenticated botanical specimen, the exact extraction solvent and procedure, method-specific sample preparation, calibrated standards, validated chromatographic assignments and appropriately controlled antimicrobial testing. Replicate measurements and uncertainty estimates would also allow descriptive observations to be distinguished from statistically supported findings. Finally, future work should distinguish exploratory inhibition-zone screening from biofilm inhibition, toxicity, resistance mechanisms and clinical therapeutic effectiveness, none of which was demonstrated by the current material.

5. CONCLUSIONS AND RECOMMENDATIONS

The source materials provide a preliminary description of selected elemental concentrations in a sage preparation, an infrared spectrum, GC–MS chromatographic and library-search outputs, and one diffusion-assay photograph. The reported elemental percentages were 1.30% N, 0.50% P, 1.68% K, 0.126% Ca and 0.0591% S. The spectral outputs indicate that chemical characterization was attempted, but the available evidence is insufficient to confirm the identities and concentrations of specific antimicrobial constituents. No clear inhibition zone was visible in the supplied *S. epidermidis* assay photograph under the reported conditions.

The findings should therefore be treated as preliminary and method-dependent. Future experiments should verify the extraction solvent, test a documented concentration series with appropriate controls and replicates, report quantitative inhibition-zone diameters or other suitable endpoints, and provide complete FTIR and GC–MS metadata. The currently supplied observations do not justify claims that sage is clinically effective against infections or that particular infrared bands confer therapeutic activity.

Author Contributions

Both authors contributed equally to this study.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

The authors reported receiving no external funding for this research.

Data Availability

Upon reasonable request, dataset are available from the corresponding author.

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