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Isolation And Identification Of Essential Oils From *Cymbopogan Citratus* (Stapf) Dc Using Gc-Ms And Ft-Ir

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Abstract

Citral is present in the oils of several plants, including lemon myrtle (90-98%), *Litsea citrata* (Blume.) (90%), *Litsea cubeba* (Louv.) (70-85%), lemongrass *Cymbopogon Citratus* (stapf) (65-85%), lemon tea-tree (70-80%), *Ocimum gratissimum* (L) (66.5%), *Lindera citriodora* (Siebold and Zucc) (about 65%), *Calypranthes parriculata* (about 62%), petit grain (36%), lemon verbena (30-35%), lemon ironbark (26%), lemon balm (11%), lime (6-9%), lemon (2-5%), and orange. Citral, present in lemon grass, is used as a flavor and for fortifying lemon oil.

This study involved isolation of essential oil from samples of lemon grass grown in Thika, Kiambu County, using hydro distillation and in comparison with correlation charts, functional groups present determined using FT-IR in the fingerprint region. The results obtained from hydro distillation indicated that the percentage yield of oil extracted is higher on dried leaf samples as compared to freshly cut leaves. The spectra obtained from GC-MS was used for relative quantification of various chemical constituents of the oil.

Key words: correlation charts, functional groups, FT-IR, GC-MS,

1.0 Introduction

Cymbopogon (lemon grass) is a genus of about 55 species of grasses, (of which the type species is *Cymbopogon citratus*) native to warm temperate and tropical regions of the Old World and Oceania. It is a tall perennial grass. Common names include lemongrass, barbed wire grass, silky heads, citronella grass, and fever grass. Lemongrass is native to the Philippines and it is widely used as a herb in Asian cuisine. It has a citrus flavor and can be dried and powdered, or used fresh. Lemongrass oil is used as a pesticide and a preservative . Research shows that lemongrass oil has anti-fungal, antibacterial, and anticancer properties.

Chemical investigations of essential oils in the nineteenth century revealed that many of the compounds responsible for the pleasant odors contained exactly ten carbon atoms. These ten carbon compounds came to be known as terpenes, if they were hydrocarbons and terpenoids if they contained oxygen and were alcohols, ketones, or aldehydes. Eventually, it was found that there are also minor and less volatile plant constituents with fifteen, twenty, thirty, and forty carbon atoms. Because compounds of ten carbons were originally called terpenes, they came to be called terpenoids if they contained oxygen and were alcohols, ketones, and aldehydes (Pavia *et al*, 2005).

Kenya needs to explore the medicinally important plants by use of sophisticated modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods.

1.1 Essential oils

The essence or aromas of plants are due to volatile or essential oils, many of which have been valued since antiquity for their characteristic odors. Essential oils are used for their pleasant odors in perfumes and incense. They are also used for their taste appeal as spices and flavoring agents in food. A few are valued for antibacterial and antifungal action. Some are used medicinally (camphor and eucalyptus) and others as insect repellant (citronella). Essential oil components are often found in the glands or intercellular spaces in plant tissue. They may exist in all parts of plant but are often concentrated in the seeds or flowers (Pavia *et al*, 2005).

Essential oils and aroma chemicals constitute a major group of industrial products. They are adjuncts of cosmetics, soaps, pharmaceuticals, perfumery, confectionery, ice-creams, aerated waters disinfectants, tobacco, agar bath and a host of related products. Aromatherapy involves the use of essential oils and aromatics derived from plants to cure diseases. Some of the essential oils are reported to be in many ways better than antibiotics due to their safety and wide spectrum of activity.

1.2 Quantitative composition of *C. Citratus* essential oil

The major terpenes in C *citratus* include citral-α or geranial (10%–48%) and citral-β or neral (3%–43%), borneol (5%), geraniol (2.6%–40%), geranyl acetate (0.1%–3.0%), linalool (1.2%–3.4%), and nerol (0.8%–4.5%). There are traces of camphene, camphor, α-camphorene, Δ-3-carene, caryophyllene, caryophyllene oxide, 1,8-cineole, citronellal, citronellol, *n*-decyldehyde, α,β-dihydropseudoionone, dipentene, β elemene, elemol, farnesal, farnesol, fenchone, furfural, iso-pulegol, iso-valeraldehyde, limonene, linalyl acetate, menthol, menthone, methyl heptenol, ocimene, α-oxobisabolene, β-phellandrene, α-pinene, β-pinene, terpineol, terpinolene, 2 undecanone, neral, nerolic acid, and geranic acid (Akhila *et al*, 2010).

1.3 Qualitative and quantitative analysis of essential oils

Essential oils are analyzed for a variety of reasons such as (i) to determine the qualitative and/or quantitative composition of the product, (ii) to control the quality and authenticity of the product, or perhaps (iii) to detect the presence of adulteration or contamination. Essential oils and fragrances often exhibit overwhelming complexity in terms of number of components present in them. (Lourdes *et al* 2009). The main limitation of chromatography in isolation is its inability to provide an unequivocal identification of the components of a mixture even if they can be completely separated from each other. Identification is based on the comparison of the retention characteristics, simplistically the retention time, of an unknown with those of reference materials determined under identical experimental conditions. The combination of the separation capability of chromatography to allow 'pure' compounds to be introduced into the mass spectrometer with the identification capability of the mass spectrometer is advantageous, particularly as many compounds with similar or identical retention characteristics have quite different mass spectra and can therefore be differentiated. This extra specificity allows quantitation to be carried out which, with chromatography alone, would not be possible (Fiefield *et al*, 2000).

2.0 Materials and method

2.1 Equipment and apparatus

1000ml round bottom flask, 50ml burette, 50ml pipette, Graduated measuring cylinders, Separating funnel, Heating mantle, Collection flask, Clevenger apparatus, GC 8000 – electron impact MS voyager, Shimadzu FTIR 8400 CE series

2.2 Reagents

Methylene chloride, anhydrous sodium sulphate, KI , Boiling stones.

3.0 Methodology

3.1 Sample identification and collection

C. citratus (lemongrass) was collected from Thika in Kiambu County in the morning at around 09:00, during the month of October 2011 in the absence of rain. The samples were packed into two polythene bags and then transported to the laboratory for distillation and further analysis. One batch was used fresh, without drying, while the other was stored in the laboratory for a period of two weeks for them to dry up before being used.

3.2 Sample preparation

50 g dried and freshly cut material from each batch were taken and hydro distilled for 3 hours in 250ml distillation units.

3.4 Hydro-distillation

Before commencing the distillation process, the samples in the flask were wetted with hot water for a period of 15 minutes. The cooling water was turned on and the heating started. The extraction was performed for a maximum of 3 hours.

3.5 Extraction of the essential oil

The distillate was then transferred to a clean separating funnel and 50ml of methylene chloride added to extract the distillate. The methylene chloride layer was transferred to a clean Erlenmeyer flask and the extraction procedure repeated with fresh 30 ml portion of the reagent.

The methylene chloride solution was dried by adding 20 gm of anhydrous sodium sulfate to the flask and left to stand for 15 minutes. As the organic solution was drying, a clean, dry, beaker was weighed and the solution transferred to it, leaving the drying agent behind. The methylene chloride was evaporated from the solution in a water bath at 40 degrees in the hood. When the solvent had been removed, the beaker was reweighed and the percentage yield of the oil calculated from the original amount of the grass used and recorded.

3.6 Gas chromatography-mass spectrometry (GC-MS) analysis

Analysis of the neat essential oil sample was performed by gas chromatography- voyager EI mass spectrometry, with identification of constituents made by comparing the spectra obtained, with those of the equipments data bank. The GC-MS analysis was performed using a GC 8000 chromatograph coupled with an electron impact MS voyager (CE Finnigan UK) mass selective detector.

A Capillary DB5 fused silica column (15 m×0·25 mm; 0·25 µm film thickness) was used. The injector temperature was set 200°C and column temperature was set initially at 40°C and then programmed at 8°C/min to 240°C. The interface temperature was set at 250^0C while the source temperature 200^0C. This was an optimized temperature program.

Carrier gas used was helium, with a linear gas velocity of 1.0 ml/min; split less injection; injected volume was 2·0 µl and the inlet pressure 25 kbar. Mass spectra was taken at 70 eV; decomposition speed 1·000; decomposition interval 0·50; fragments from 45 to 450 Da were decomposed.

3.7 Infrared spectroscopy determination

The infrared spectrum of the oil was obtained using shimadzu FT-IR 8400CE series spectrophotometer and structure determined with the help of correlation charts. The samples were examined neat by placing them in between potassium bromide cells. The solvent spectrum was also obtained to aid in analyte identification.

4.0 Results And Discussions

4.1 Determination of moisture content

The samples were analyzed for their moisture content by weighing five fresh, 10g replicate samples, in weighed, dry, tarred crucible and placing them overnight in the oven set at 58^0C (Gentry *et al* 2002).

The moisture content was calculated for 10.05612g ± 0.5284 lemon grass samples. From our calculation, it was determined that the moisture content for the samples was 74.067765% ± 3.8194502 while the percent solids was 25.9322% ± 3.81945. Statistical evaluation of the data for the presence of outliers using 3SD test, Q test and Grubb's test revealed that the data obtained did not have outliers and that the suspect values were indeed not outliers as previously thought.

4.2 Determination of lemon grass oil yield in fresh and dried samples

The percentage yield of the oil for the fresh and dried samples was determined by placing the extracted solution from the separating funnel that constituted the analyte and the extracting solvent in a weighed beaker. The extracting solvent was then evaporated in a water bath maintained at 40^0C and after evaporation of the solvent, the beaker was reweighed and the weight recovery of the oil was calculated from the original amount of the sample used (Pavia *et al* 2005).
From our calculations, the percentage yield of essential oil from the dried samples was found to be 1.0378% ± 0.7039 while that of the fresh samples was found to be 0.4136% ±0.1815. Evaluation of the data obtained for the presence of outliers using the 3SD test, Q test and Grubb's test revealed that their were no outliers. Thus, the percentage essential oil yield for the dried samples was found to be higher than that of the fresh sample as expected

4.3 Determination of the functional groups present using FT-IR

The functional groups present were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer with those of an IR correlation chart. The vibration frequencies of the solvent was also obtained to aid in the determination of sample vibration frequencies.

The strong methylene/methyl band (1446.5 cm^{-1}) and a weak methyl band (1384.8 cm^{-1}), plus a band at 740 cm^{-1} (methylene rocking vibration) is indicative of a long-chain linear aliphatic structure (figure 1). The saturated hydrocarbon C- H stretching absorptions all occur below 3000 cm-1. Any band structures observed between 3150 and 3000 cm^{-1} are almost exclusively indicative of unsaturation (C = C- H) and/or aromatic rings and their absence in the IR spectrograph obtained is thus indicative of the absence of aromatic compounds. The position of the C = C stretching frequency does vary slightly as a function of orientation around the double bond, but it is less informative than the C - H information.

Carbonyl compounds are often the strongest band in the spectrum and will lie between 1825 and 1575 cm–1; its exact position being dependent upon its immediate substituent. For a double-bonded functionality, conjugation plays an important role in the observed carbonyl frequency. This includes connection to an aromatic ring or conjugation to a C = C or another C = O.

In aliphatic compounds, the C - X bond typically possesses a unique group frequency, which may be assigned to the halogen–carbon stretching. When a single halogen is present, the determination of this group is straightforward. However, if more than one halogen is present, the interpretation is usually more complex. The high electro negativity of the halogen atom can have a noticeable impact on the spectrum of neighboring group frequencies, including adjacent hydrogen atoms. In such cases, significant shifting of the C - H frequencies can occur – the direction of the shift being dependent on the location of the C - H, and whether the halogen adds or extracts electron density from the C - H bond – adding strengthens (higher frequency) and extracting weakens (lower frequency). Thus, as expected the vibration frequencies of methylene chloride are shifted to lower wave numbers 466.7 cm^{-1}.

4.4 GC-MS chromatogram

A total of nine components, with different retention times, were eluted from the GC column as indicated by the chromatogram (figure 2) and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in figure 3 to 6. The relative amount of individual components was calculated based on GC peak areas.

Comparison of the GC-MS spectrograph obtained with the instruments data bank together with NIST ms data demo version revealed that the essential oil of C. citratus contained a mixture of terpenes that eluted at different retention times depending on the boiling point of the eluted component. The GC chromatogram obtained revealed a high concentration of citral indicated by presence of two large peaks which eluted at 10.526 and 10.943 minutes with peak areas of 9870795776 and 3720576000 respectively (figure 2). The presence of these two peaks may be due to the two isomers of citral that is geranial and neral, which may be difficult to distinguish with GC-MS. The instruments data bank was also able to indentify the presence of p-mentha-1,(7) 3-diene (figure 3), p-mentha-1,(7) 8-diene (figure 4), pinene (figure 5), and citral (figure 6) with retention times of 4.576, 5.126, 8.56 and 10.526 minutes.

4.5 Fragmentation patterns of selected eluted components

With an electron beam of 9 – 15 eV the principle ion produced is the molecular ion, which is produced by the loss of a single electron. This gives a very simple spectrum with essentially the entire ion appear with the parent peak. With organic compounds, because of the small but observable natural abundance of carbon 13 and tritium there is a small

peak appearing one mass unit higher than the parent peak (M+1) and if two isotopes happen to be in the same molecule there is even a smaller peak at M+2. Halogens yield abnormally high M+2 peaks and the absence of this peak in our mass spectrographs is an indication of lack of halogen atom in our eluents even though methylene chloride was the extracting solvent. The base peak is the largest peak observed and all other peak heights are measured with respect to it. Thus, ion abundances are given in terms of ions produced and the relative strength of these ions is related to the strength and chemical nature of the bonds which held the fragments to the rest of the molecule. Cleavage is usually favored at branched carbon atoms as a consequence of the stability of the carbonion ions produced that is tertiary > secondary > primary. It is also favored by the formation of small stable molecule like water and CO.

5.0 Conclusion

The percentage essential oil yield in dried samples is higher as compared to fresh samples. The essential oil of C. *citratus* is mainly composed of monoterpenes hydrocarbons, of which, citral is the main component as indicated by the strong intense vibration frequency in the FT-IR spectrograph and two large peak areas at around 10.526 and 10.943 in the GC-MS spectrograph obtained.

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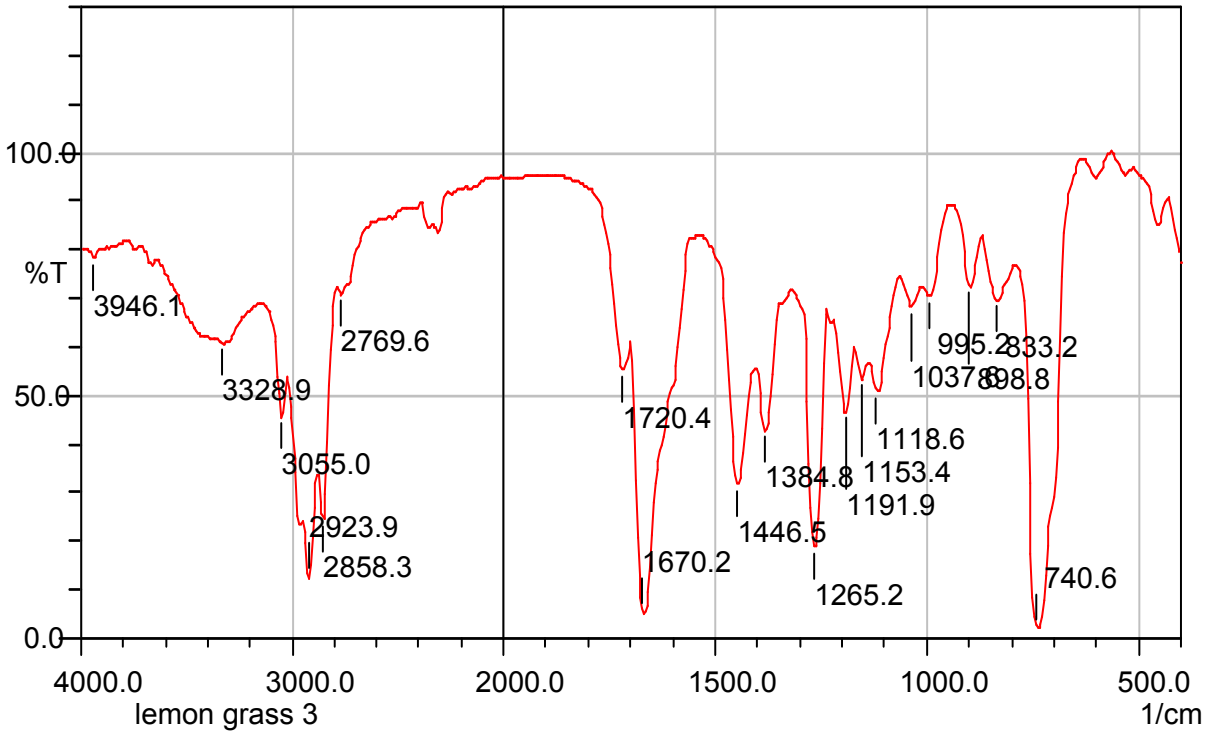

Figure 1: FT-IR spectrograph of the sample

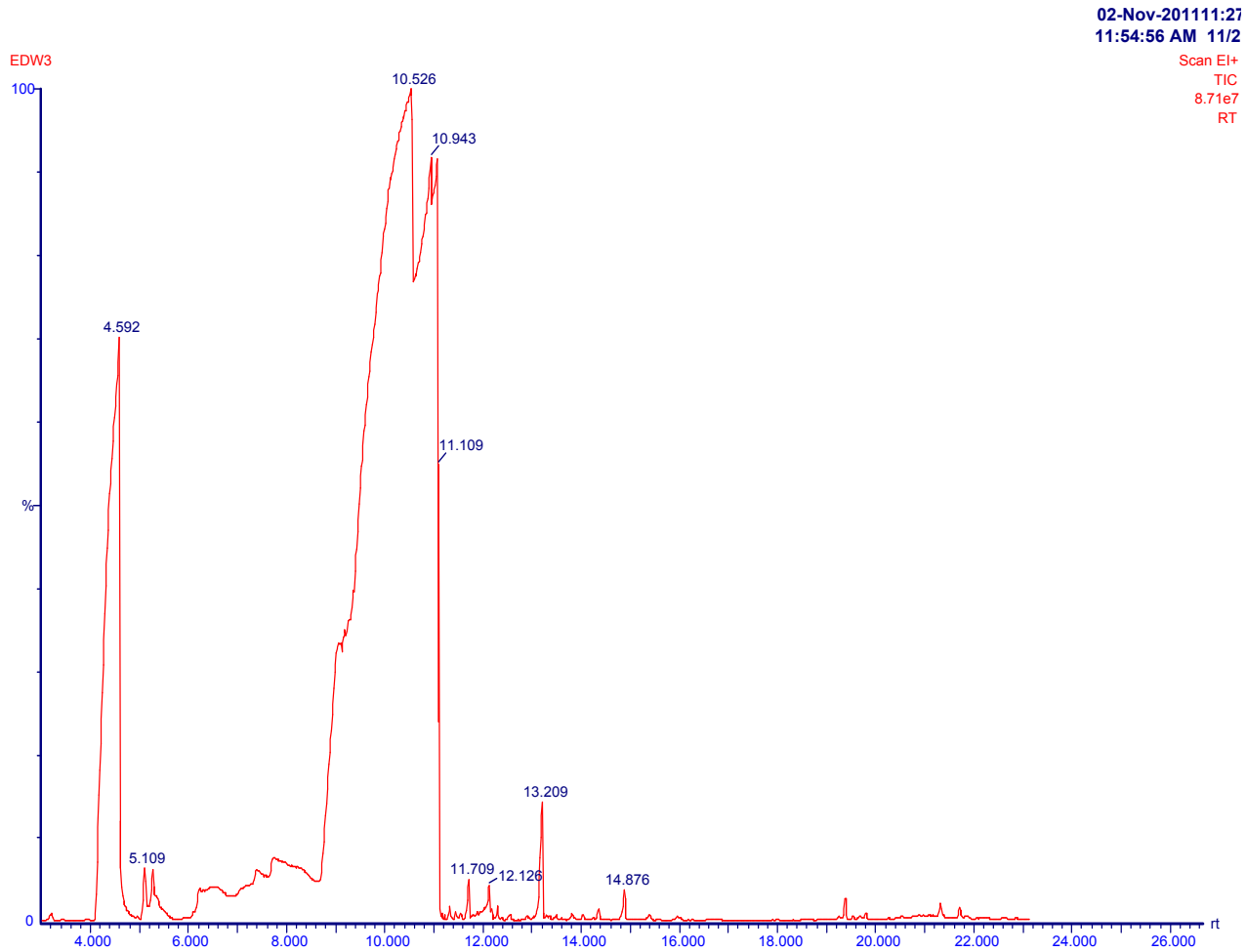

Figure 2: GC-MS chromatogram obtained for a sample of lemon grass essential oil

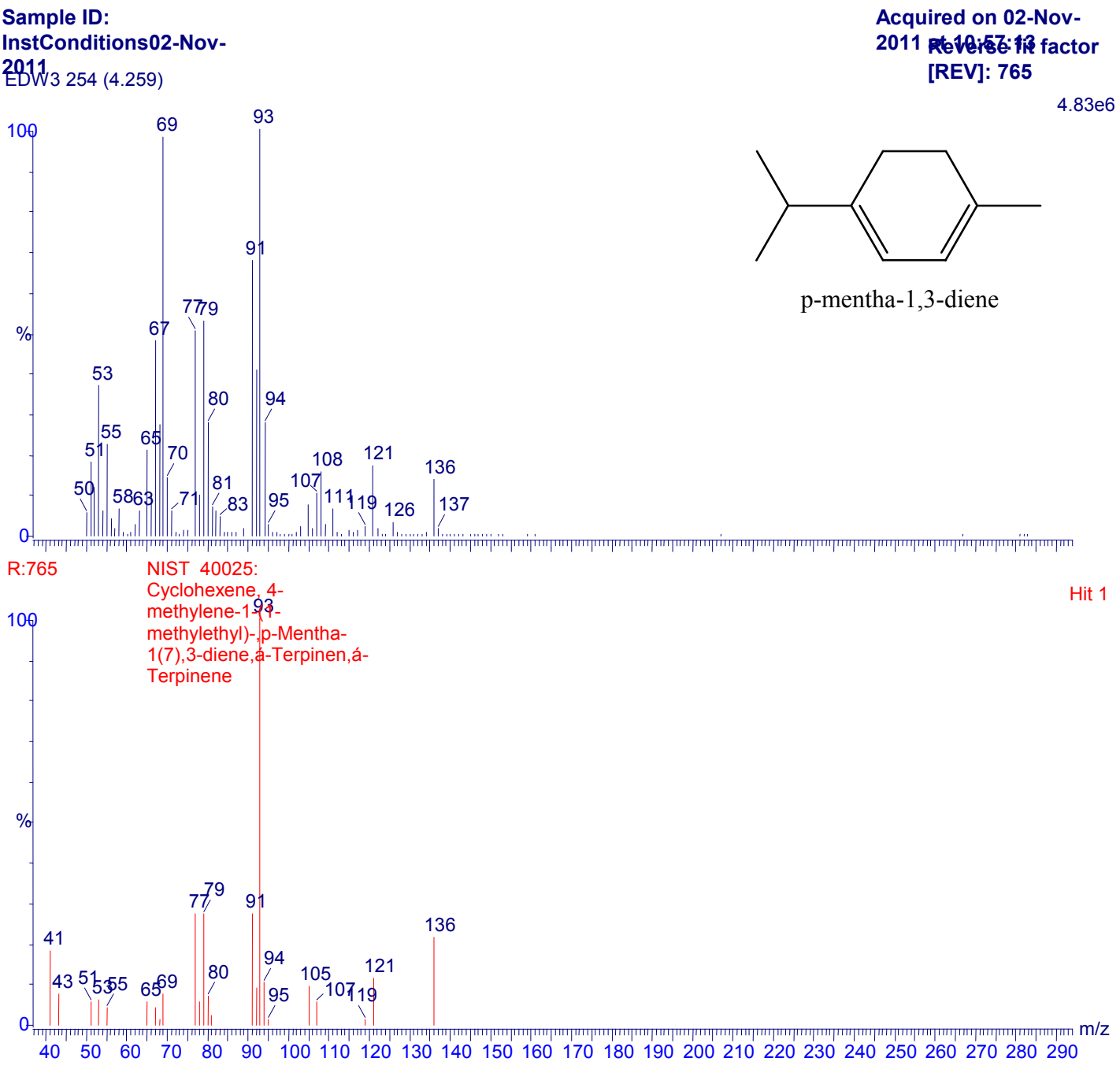

Figure 3: p-mentha-1,3-diene identified by the mass spectrometer detector

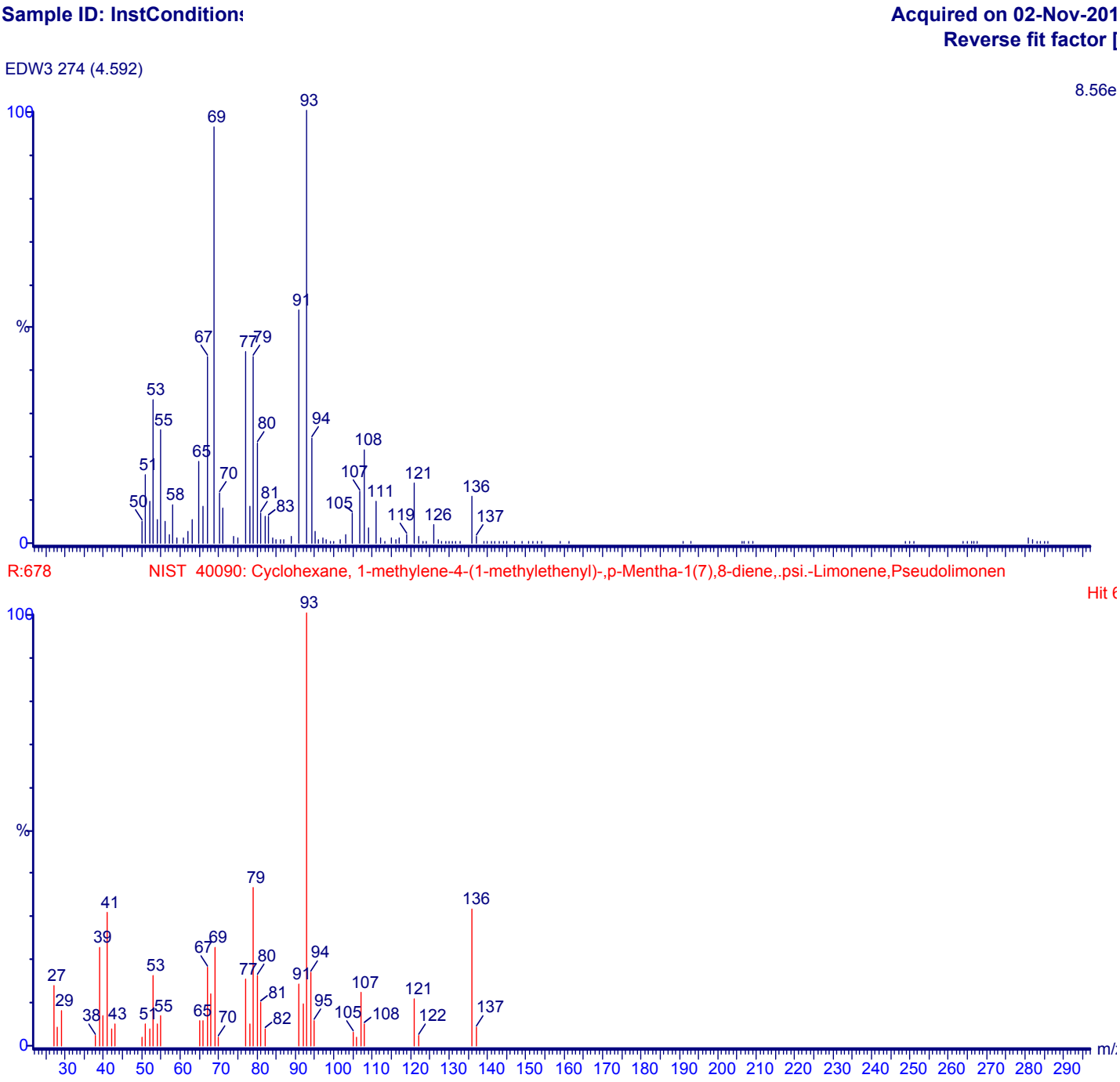

Figure 4: p-Mentha- 1(7), 8-diene identified by the mass spectrometer detector

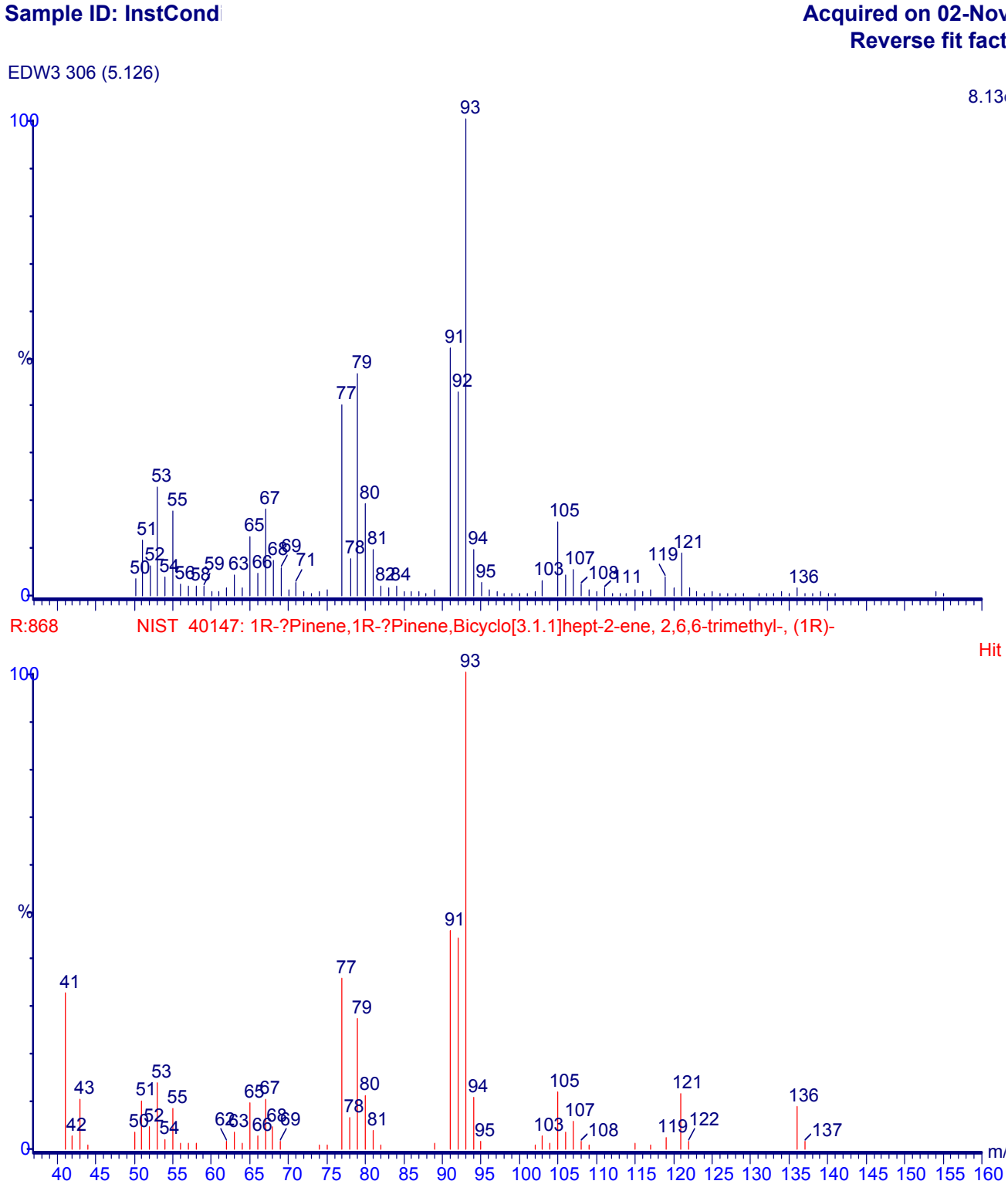

Figure 5: 1R-a-pinene identified by the mass spectrometer detector

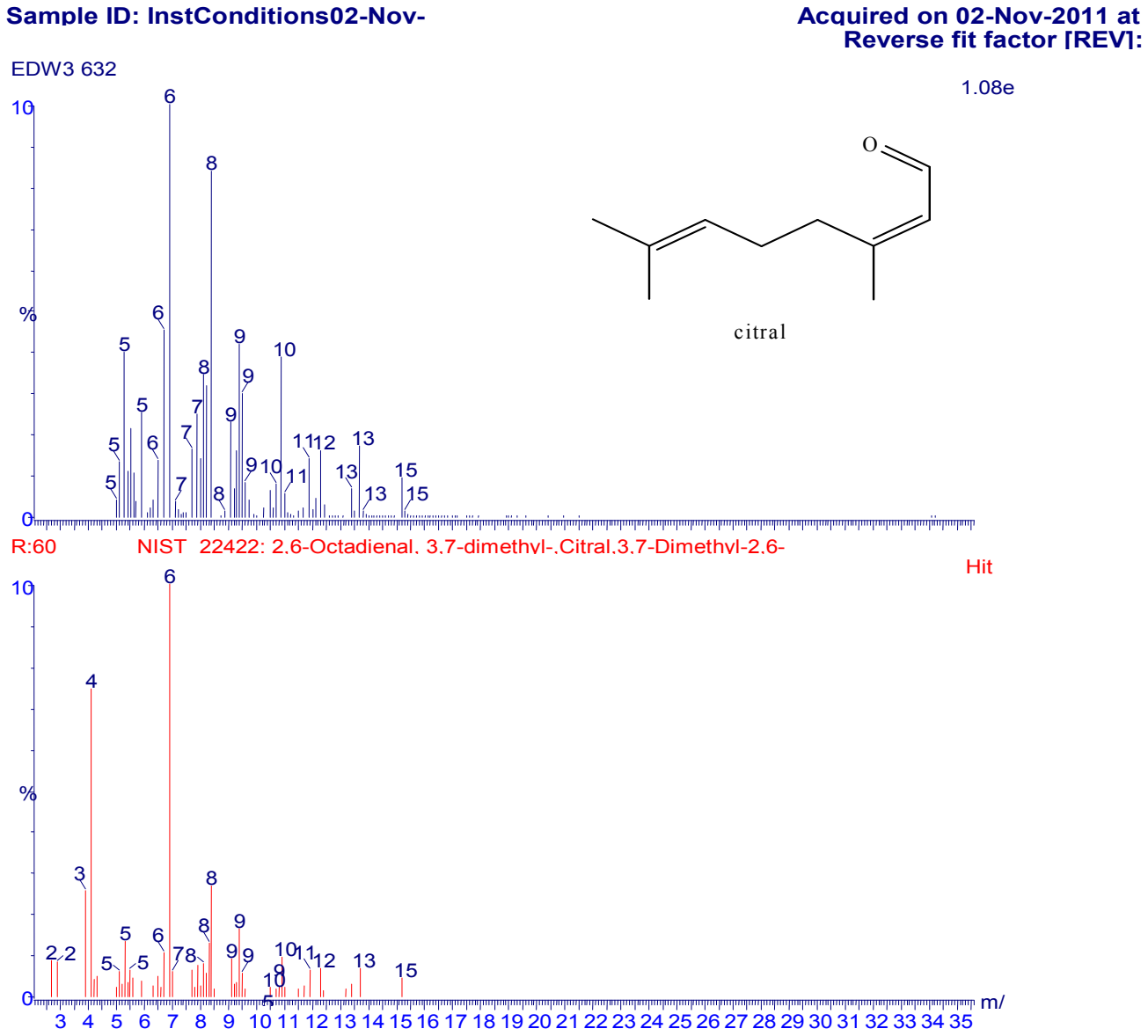

Figure 6: Citral identified by the mass spectrometer detector