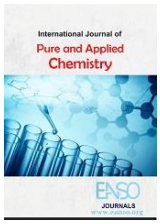

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Original Article

Attenuated Total Reflectance –Fourier Transform lnfrared (ATR-FTlR) Analysis of Ocimum kenyense Essential Oils

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Keywords: *Ocimum Kenyense, ATR-FTIR, 1, 8-Cionele, Eucalyptus Oil, Analytical Chemistry.*

ABSTRACT

ATR-FTIR analysis is a robust method that is used to analyse solids and liquid samples with minimal sample preparation. The samples are usually placed on the ATR crystals and pressure applied to obtain a clear spectrum. Essential oils from O. kenyense were extracted through hydro distillation using the Clavenger apparatus for 8 hours. The essential oils were dried by passing through anhydrous sodium sulphate after which they were placed in brown viols and refrigerated at 4°C. The sample of oils was latter analysed at the government chemist in Nairobi County in Kenya. Compounds that recorded a high hit quality of 600 and above had the highest probability of being present in the oil. Eucalyptus oil had a hit quality of 673 and 1, 8-cionele had a hit quality of 655 respectively. Therefore, the two were presumed to be present in O. kenyense essential oils.

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INTRODUCTION

Analytical chemistry is a multidisciplinary subject cutting across various fields. Analytical chemistry thus contributes to the wealth of knowledge in various fields' such as: organic chemistry, medicine, pharmacy, agriculture, natural products chemistry, and environmental chemistry (Gary, 2014). The discipline of analytical chemistry cuts along two divides, the classical wet methods chemistry and instrumental analytical methods (Irving, 1977). The aim of analytical chemistry is to determine the quantitative composition (how much) and the qualitative analysis (what is it) (Gary, 2014) . Classical methods are tedious and less accurate than the modern instrumental methods. The major categories of instrumental methods are the spectral, electroanalytical, and separatory (Robinson et al., 2014). Some of the modern instrumental methods used in organic molecules analysis include; ultra-violet spectroscopy, infrared spectroscopy, mass spectrometer, and nuclear magnetic resonance. Each of the instrument is used to measure a certain aspect of the analyte. For example, Ultra-violet spectrophotometer is used to assess the level of conjugation, Infrared spectrophotometer is used to identify the functional group, and mass spectrometer is used to identify the molecular mass and nuclear magnetic resonance (NMR) for structural elucidation (Robinson et al., 2014). In this study ATR-FTIR was used to identify functional groups and in the subsequent identification of the molecules present by comparing with the ATR-FTIR LIB-PHARMA-2-472-2SO11R libraries of Alpha-Bruker ATR-FTIR instrument.

History of Fourier Transform Instruments

IR instruments were first developed in the 1940s in the USA, the instruments are designed to use the IR radiation of electromagnetic spectrum. The IR region ranges from 700-1000 nm. The IR spectrometer measures the relative amount of energy as the function of the wavelength of IR-radiation when it passes through a sample (Khan et al., 2018)

Principle of FTIR analysis

The principle behind IR radiation is that the samples absorb specific frequencies since they are in continuous vibration. When a beam of IR radiation is passed through the sample the molecules absorb the frequencies that are resonant to their molecular vibrations (Khan et al., 2018). The absorbed or transmitted radiations are recorded as percentage transmittance or absorbance.

Types of FTIR instruments

There are two types of infrared spectrometers that is; 1. Dispersive infrared spectrometer (DS) 2. Fourier transform infrared spectrometer (FTIS)

Dispersive Infrared Spectrometer

The dispersive infrared spectrometer emerged in the 1940's. This design helped to spread the use of infrared spectroscopy as a common analytical technique for organic compound characterization in laboratories (Chan et al., 2014). Dispersive spectrometers usually generate spectra by optically dispersing incoming radiation into its frequency or spectral components. Prisms and gratings are the common dispersive elements (Khan et al., 2018). Monochromators and spectrographs are the two different forms of dispersive spectrometers. A monochromator has a single detector, small slit(s) and the revolving dispersive element that allows user to view a specific wavelength range. The dispersive IR spectrometer include a radiation source, sample, and reference cells, monochromators, detector, amplifier, and recorder (Robinson et al., 2014)

Fourier Transform Infrared Spectroscopy (FT-IR)

In the 1960s, commercial Fourier transform infrared spectrometers were developed. An FT-IR instrument uses a system called an interferometer to collect a spectrum. The interferometer consists of a source, beam splitter, two mirrors, a laser, and a detector (Khan et al., 2018). The energy goes from

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the source to the beam splitter which splits the beam into two parts. One part is transmitted to a moving mirror and another part is reflected to a fixed mirror. The moving mirror moves back and forth at a constant velocity. This velocity is timed according to the very precise laser wavelength in the system which also acts as an internal wavelength calibration. The two beams are reflected from the mirrors and recombined at the beam splitter. The beam from the moving mirror travels a different distance than the beam from the fixed mirror (Vij, 2015). When the beams are combined an interference pattern is created, since some of the wavelengths recombine constructively and some destructively. This interference pattern is called an interferogram. This interferogram then goes from the beam splitter to the sample, where some energy is absorbed and some is transmitted. The transmitted portion reaches the detector (Chan et al., 2014). The detector reads information about every wavelength in the infrared range simultaneously. To obtain the infrared spectrum, the detector signal is sent to the computer, and an algorithm called a Fourier transform is performed on the interferogram to convert it into a single beam spectrum (Chan et al., 2014).

Attenuated ***Total Reflectance-****Fourier* ***Transforms Infrared Spectroscopy (ATR-FTIR)***

Attenuated total reflection is a sampling technique that is used in conjunction with IR spectroscopy, which enables samples to be examined directly in the solid or liquid state without further preparation (Kassis, 2011). Instruments uses the property of total reflection resulting into an evanescent wave. The sample is usually in contact with ATR crystal. The radiation interacts with the sample on the surface in contact with ATR crystals. The evanescent wave extends to the sample where the specific parts of the evanescent wave are absorbed based on the sample composition. The resulting wave lack some parts and hence attenuated (Kassis, 2011). The attenuated beam returns to the crystal then exits the opposite end of the crystal and is directed to the detector in the IR-spectrometer. The IR spectrometer detector records the attenuated IR beam as an interferogram signal can be used to generate an IR spectrum. ATR-FTIR has the following advantages over the FTIR instruments by minimal sample preparation, fast and easy clean up, analysis of samples in their natural state excellence in thick and strongly absorbing samples analysis.

METHODOLOGY

Material required

Leaves of *Ocimum kenyense* were collected from Ngon'g Forest Langata Road in Nairobi County Kenya. Distilled steriled were sourced from industrial chemistry laboratories at the Mount Kenya University. Kitchen blender, Clavenger apparatus and amber vials used were from the pharmacognosy laboratories at the Mount Kenya University. The analysis was done using a Bruker alpha 11 ATR-FTIR instrument at the government chemist in Nairobi County Kenya.

Sample collection

A sample of fresh *Ocimum kenyense* (Ayabongira) leaves were collected from Ngong forest Lang'ata road in Nairobi County. A taxonomist identified the plants from which the leaves were picked and a voucher specimen deposited in the University of Nairobi herbarium with voucher specimen as JNM/003/2021. The leaves were randomly sampled from different plants in the same pedological zone and within the same period because soil conditions and growing seasons affect the composition of natural products in plants (Fall et al., 2017). The leaves were harvested at the vegetative stage using gloves and a pair of secateurs.

The harvested leaves were washed with normal tap water followed by sterile distilled water to remove physical and chemical impurities. The samples were bagged in five-kilogram polyethene sacks from the field and be labelled as Ok and transported to Mount Kenya University pharmacognosy laboratory within three hours.

Preparation of the Sample

The washed leaves samples were air-dried at room temperature for seven days under a shade to a constant mass of 10% of the original weight in the pharmacognosy Laboratory at the Mount Kenya University. The dried leaves were pulverized into a powder using kitchen blender (Azwanida, 2015). The powder was reweighed and then stored in

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airtight polyethene bags at 4º C for later extraction and analysis

The Extraction of Essential Oils

Essential oils (EOs) from *Ocimum kenyense*, were extracted through hydro distillation using the Clevenger apparatus. Two hundred grams (200.000 g) of the dry powdered leaf samples of Sm were weighed using an analytical balance (0.000 g), then placed in 1000.00 millilitres round-bottomed flask and 500.00 millilitres of distilled water added. The sample was hydro distilled for 8 hours at 100° C and ambient atmospheric pressure (Kugara, 2019). Essential oil extracts were dehydrated using anhydrous sodium sulphate before being stored in amber vials and refrigerated at 4° C (Wei, 2018) for latter FTIR analysis.

ATR-FTIR Analysis

The samples were analysed using an ATR-FTIR spectrophotometer at the Government Chemist Laboratories in Nairobi County Kenya. The sample was placed at the ATR crystal and pressure applied to obtain the spectrum. The peaks in the spectrum were analysed and corresponding functional groups assigned and the probable molecules identified through comparison with the computer library database (Krishnaveni & Kumar, 2016).

RESULTS AND DISCUSSION OF ATR- FTIR ANALYSIS

The IR spectra were reported in % transmittance and the finger print region used to match the compounds. The wave number region for the analysis was 4000-500 cm^{-1}(in the mid-infrared range). Each molecule produced a unique spectrum that was used as fingerprint for molecular identifications. Based on the ATR-FTIR profiles of the essential oil samples, the natural products present were identified by ATR- FTIR and subsequent comparison with the ATR-LIB-PHARMA-2-472-2S01 libraries. The compounds with a hit quality of 600 and above as analysed by ATR-FTIR show a high probability of being present in the essential oils. Hit quality was marked as the similarity between the essential oils spectra and the library correlation charts for standard compounds (Njuguna, 2021).

From the ATR-FTIR profiles of essential oils of *O. kenyense*, the following compounds were identified; Eucalyptus oil and 1, 8-Cionele which confirms findings by Obeng-Ofori (1997) that 1,8- Cionele is one of the major components of essential oils from Ocimum *kenyense* which is classified as a monoterpene (Obeng-Ofor, 1997). According to Bekele (1994) terpenes are the most conspicuous components of the essential oils. Eucalyptus oil on the other hand is a mixture of several terpenenes such as; α-pinene, p-cymene, aphelland-rene, 1,8-Cineole, c-terpinene, and limonene (Almas et al., 2021). Substances with hit quality above 600 had a higher probability of being present in the essential oils (Njuguna, 2021). Below shows chemical constituents as identified by comparison with ATR-LIB-PHARMA-2-472-2S01 libraries.

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Table 1: Chemical constituents of the essential oil derived from *O. kenyense* ATR-FTIR analysis

Compound name	Hit quality	Major peaks Wave numbers	Molecular formula	Classification
Eucalyptus oil	673	2800 cm^{-1}	N/A	α-pinene, p-cymene, aphelland-rene, 1,8cineole, c-terpinene, and limonene
1,8-Cionele	655	2800 cm^{-1}	$C_{10}H_{18}O$	Monotepene
Squalane	222	2800 cm^{-1}	$C_{30}H_{62}$	Hydrocarbon
Prenol	225	2800 cm^{-1} and 3300 cm^{-1}	$C_5H_{10}O$	Terpenoid
Anethole	368	3000 cm-1	C10H12O	Phenylpropanoid
Cajeput oil	575	3500 cm-1 2900 cm-1	N/A	Mixture of several terpenes and terpeenoids (eucalyptol, γ-terpinene, terpinolene, β-eudesmene, α- selinene, α-terpineol, 1R-α-pinene (2.158%), caryophyllene, and α-caryophyllene
Anise oil	218	2800 cm-1	N/A	limonene, carvone, myristicin
Spicae oil	263	3300 cm-1 2800 cm-1	N/A	eugenol (85.5%), β-caryophyllene (10.54%) and α-humulene (3.12%) while β-pinene (12.75%), α-pinene

Other components of *O. kenyense* oils had a hit quality below 600, this means they had very low probability of being present in the essential oils (Njuguna, 2021). The compounds that had a hit quality of less than 600 included; cajeput oil, anise oil, spicae oil, anethole, prenol and squalane. After obtaining the spectrum, the process of interpretation follows that involves the correlation of absorption bands of the unknown compound with the known frequencies from the IR correlation charts. Majority of the spectra obtained had a strong peak at 2800 cm^{-1} that corresponds to –C-H stretching in alkanes while the peaks at 3300 cm^{-1} corresponds to –OH stretching.

CONCLUSION

The study confirms the results of a study by Bekele (1994) that the major component of essential oils from *O. kenyense* is 1, 8- Cionele (Eucalyptol, however the analysis may require a more sensitive and robust chemical analysis method to fully analyse the components of essential oils and elucidate their structures.

ACKNOWLEDGEMENTS

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DECLARATION OF COMPETING INTEREST

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDIX

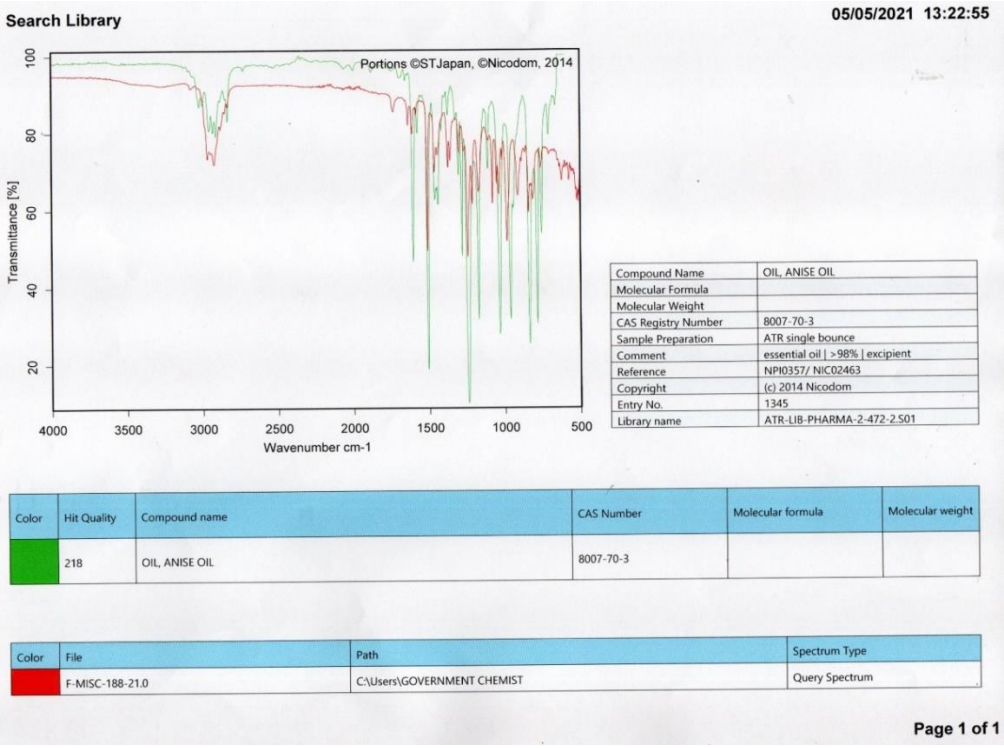

Figure 1: Anise oil

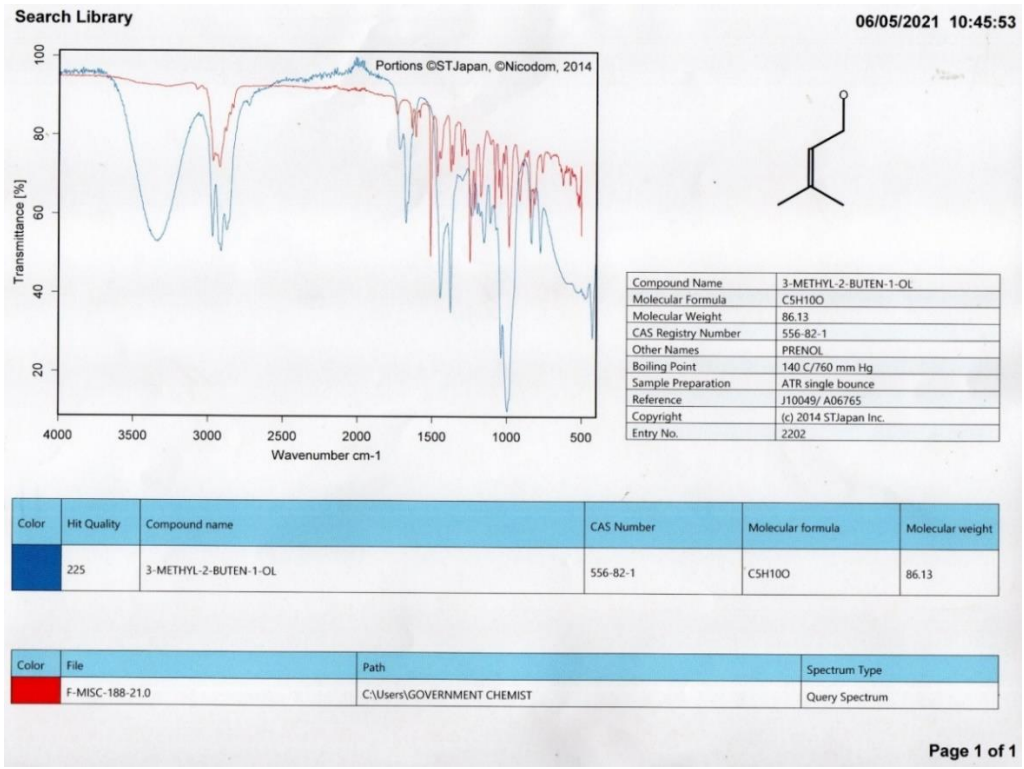

Figure 2 : Prenol

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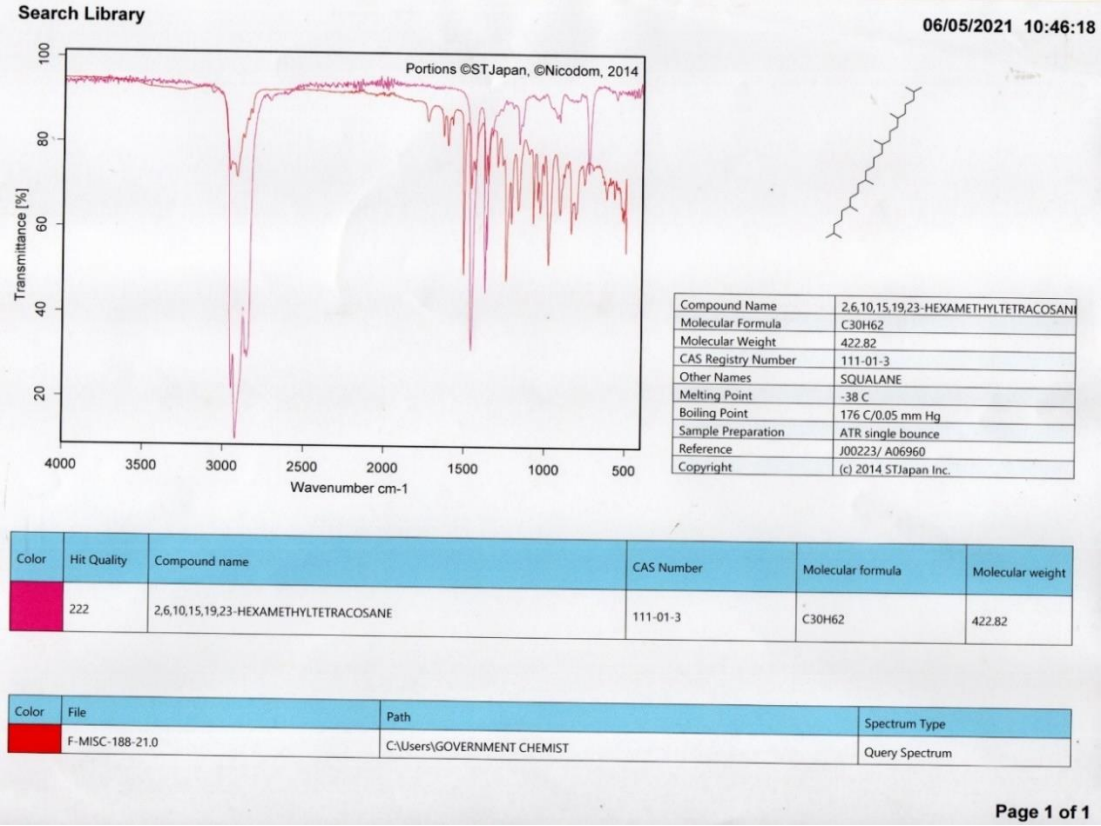

Figure 3: squalane

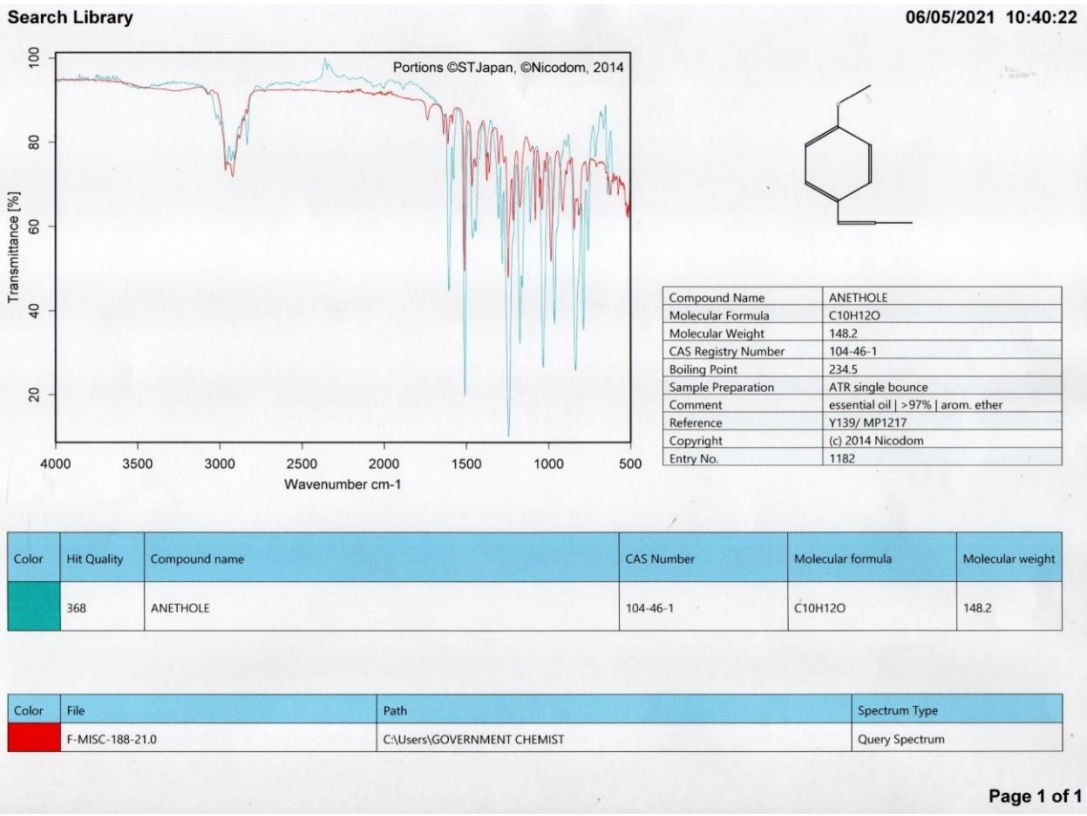

Figure 4: Anethole

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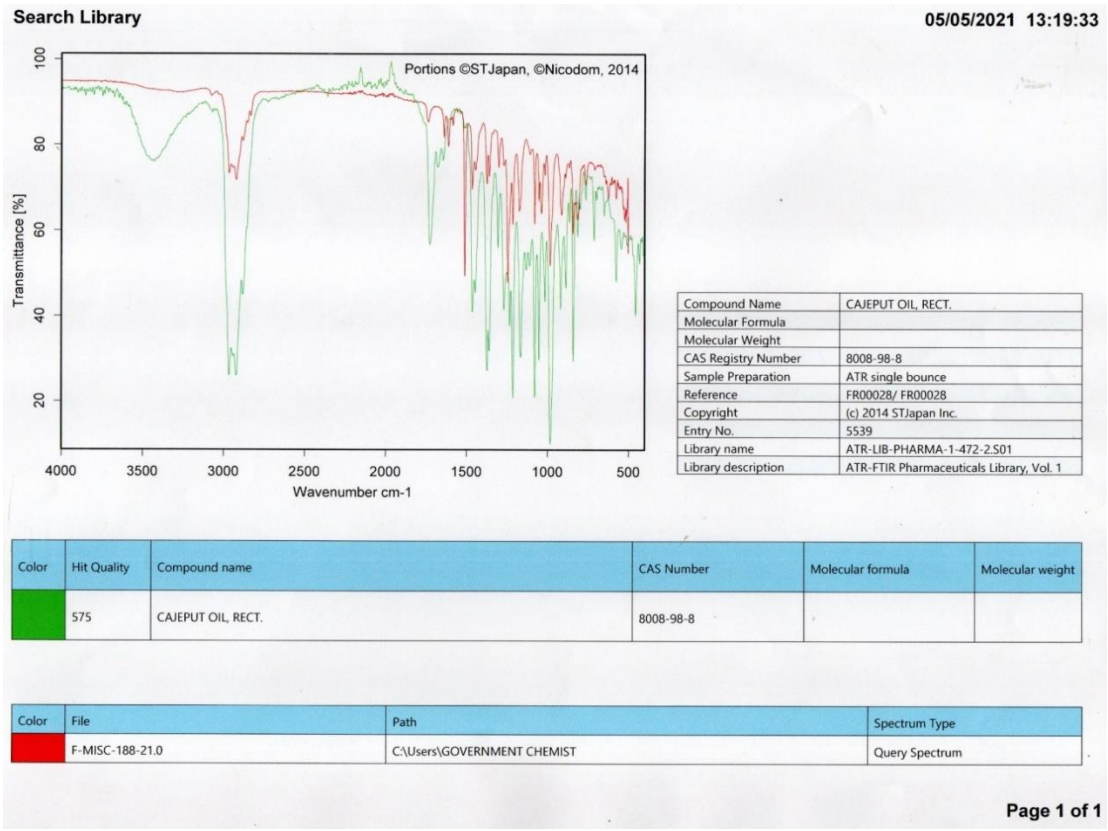

Figure 5: Cajeput oil

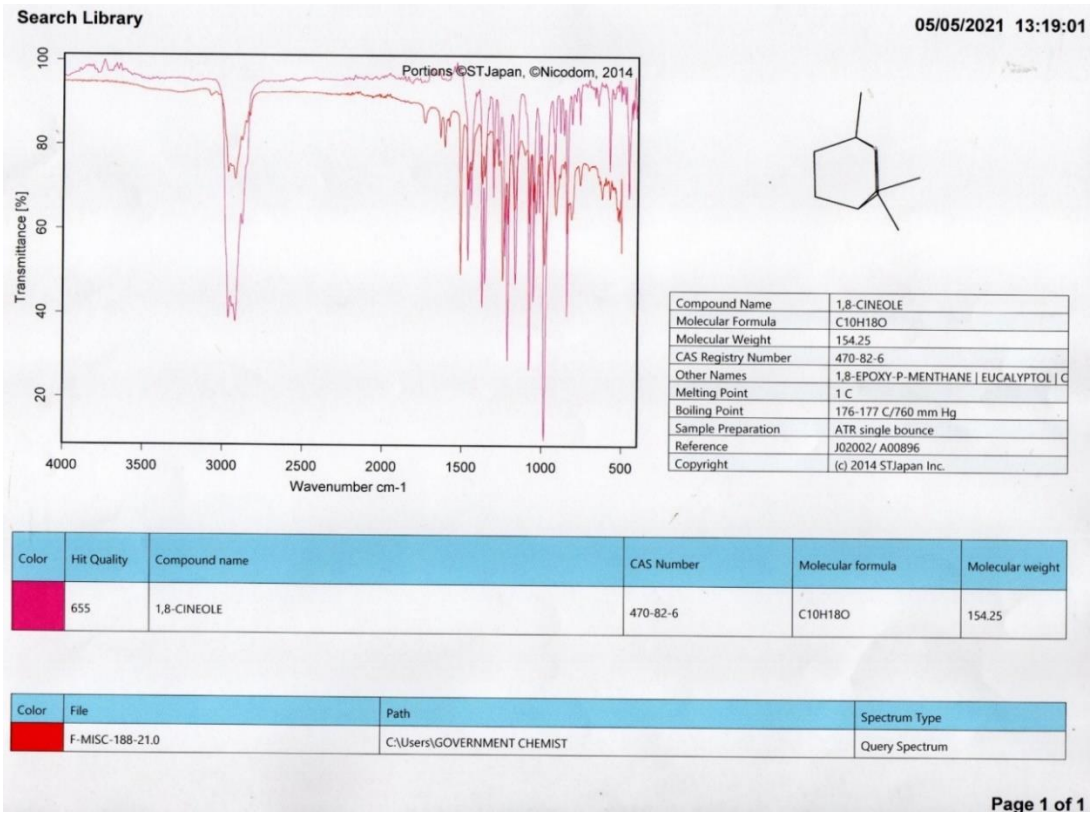

Figure 6: 1,8-Cionele

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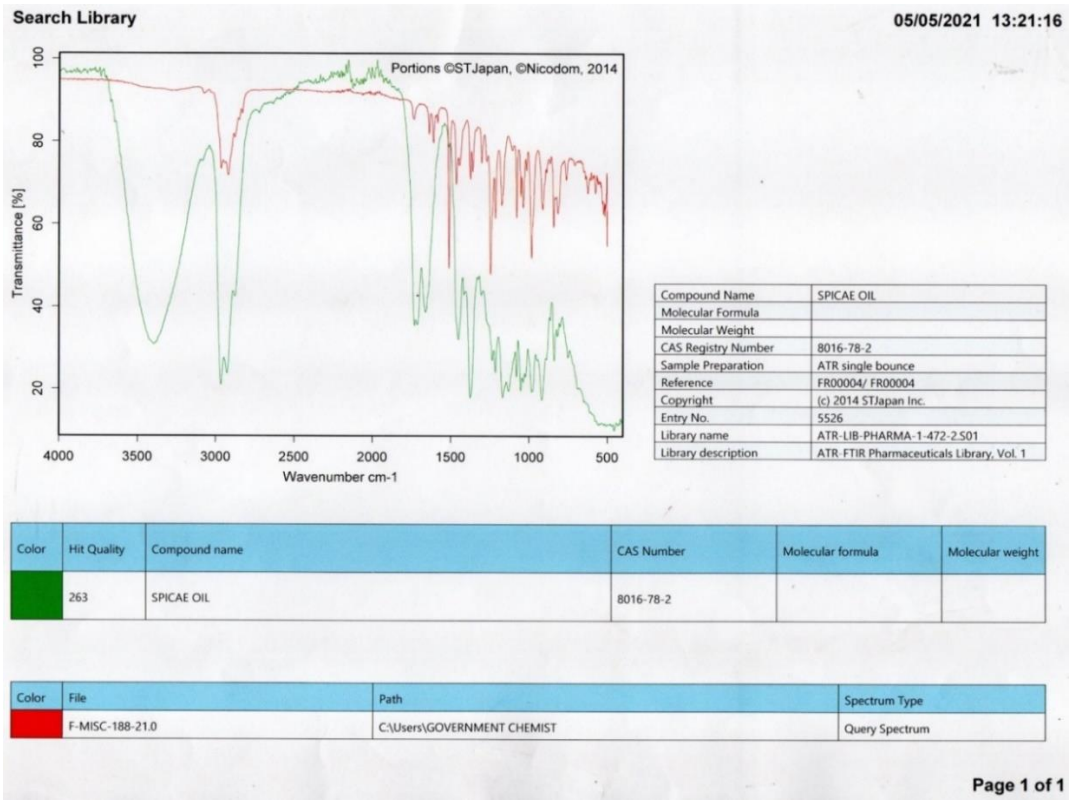

Figure 7: Spicae oil

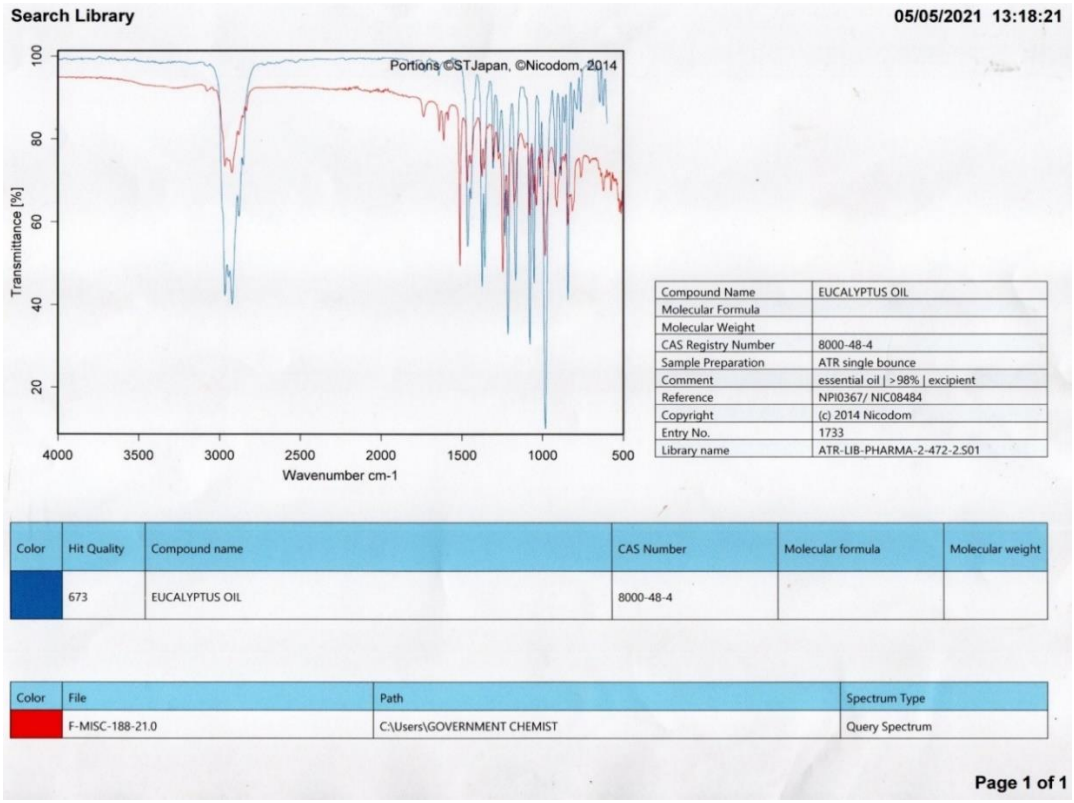

Figure 8: Eucalyptus oil

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