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Study of GC-MS Compotition and Chemical Structure of Extract Hexane of Red Betel Leaf *Piper cfcrocatum* Blume

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Abstract
Red betel extraction with n-hexane solvent was performed. The extraction results were characterized by GC-MS to find out the chemical structure of red betel extract with n-hexane solvent. Based on fragmentation on GC-MS, chemical compounds extracted from n-Hexane such as butyl etanoate with BM (Molecular weight)= 116 was $C_6H_{12}O_2$, α-pinen with BM = 136 was $C_{10}H_{16}$, limonen with BM= 136 was $C_{10}H_{16}$, cineol-1,8 with BM = 154 was $C_{10}H_{18}O$, terpinen-4-ol with BM = 154 was $C_{10}H_{18}O$.

Keywords: exctract of *Piper cfcrocatum* Blume, GC-MS, Extract n-Hexane, Leaf *Piper cfcrocatum* Blume, red betel plants

Introduction
Red betel (*Piper cf. arcuatum* Blume) was one of original plant from Indonesia which widely used by people as a traditional medicine. Red betel leaf was part of the plant that was empirically used by communities to cope with various diseases, including cancer [1-2, 9] Red betel was known to contain flavanoid, alkaloid are polyphenol compounds, tannin, and essential oil. Flavanoid act as antibacterial by forming complex compound against extracellular protein that interfere with the integrity of bacterial cell membrane. According Dwidjoseputro [3], flavanoid was phenol compound while phenol compound can be protein coagulator.
Alkaloid has the ability as an antibacterial. The alleged mechanism was interfered with the peptidoglycan component of the bacterial cell, so that the cell wall layer was not completely formed and cause the cell's death [4]. Tannin has the ability as an antibacterial, outline the estimated mechanism was toxicity of tannin can damage the cell membrane of bacteria, the astringent tannin compound can induce the formation of a bonding compound complex against microbial enzymes or subtracts and the formation of a tannin bonding complex against ametal ion which can increase the toxicity of tannin itself[5]. Tannin was thought to be able to shrink the cell wall or membrane cells that disrupt the permeability of the cell itself. Tannin also has antibacterial power by precipitating protein. Essential oil act as antibacterial by interfering with the formation of membrane or cell wall so they are not formed or formed imperfectly [7]. The essential oil that are active as antibacterial generally contain hydroxyl (-OH) and carbonyl functional group. Phenol derivates interact with bacterial cell through an adsorption process involving hydrogen bond. In this study, it will be examined the content of compound to be extracted from red betel leaves with n-Hexane solvent.

Experimental
General Experimental Procedur
Red betel plants (*Piper cf. crocatum* Blume) were harvested from Ciapus, Bogor. The leaves were washed with running tap water to remove the dirt, prior to the drying process. The leaves were cut into small pieces, dried in room temperature, and then were powdered.
All chemicals used were of analytical grade. Methanol, ethyl acetate, *n*-hexane, concentrated sulfuric acid, concentrated HCl, ferric chloride hexahydrate ($FeCl_3.6H_2O$), DMSO, acetic acid anhydride, acetic acid glacial, chloroform were purchased from Merck.

GC-MS apparatus and chromatographic conditions
Separation process was conducted by Thin Layer Chromatography, and further structure elucidation was performed by GC-MS, solvent removal was done by rotary evaporator.

Sample Extraction

Sample preparation was conducted by maceration using several organic solvents. A 100 g of powdered red betel leaves were immersed in 5 L of n-hexane for 3 days, and then filtered. Filtrate was evaporated until dry sample was obtained, and this step resulted in raw extract of n-hexane. The residue from first immersion was entirely immersed back in 5 L ethyl acetate for 3 days to obtain raw extract of ethyl acetate. The solution was then filtered and evaporated, and the residue from this step was immersed in methanol for 3 days, resulted in raw methanolic extract. The maceration process was repeated several times to obtain clear extract containing all of expected chemical species.Azmir *et al*. states that the efficiencies of extraction methods mostly depend on the understanding the nature of plant matrix and chemistry of bioactive compounds [8].

Separation by Column Chromatography

Beaker glass containing silica gel (50 g) was added by *n*-hexane, stirred homogeneously until the gel appears like porridge. This porridge was introduced into a-50 cm length, 2 cm diameter-column, which has cotton lid at the bottom of column. The tap of the column was opened thus the n-hexane solvent can drip and be collected in a flask. After the surface of remaining n-hexane was about 3 cm on silica gel surface, the tap was closed. This silica gel was acted as stationary phase.

Raw extract of n-hexane (0,2 g) was dissolved in small amount of n-hexane, added by silica gel, and homogenized. The *n*-hexane was evaporated using waterbath at temperature of 40ºC until residue was obtained, and then introduced to the column. Eluen which acts a mobile phase carefully poured into the column. The ratio of *n*-hexane to ethyl acetate was listed in the Table 1.

Table 1: The ratio of *n*-hexane to ethyl acetate as eluen mixture.

n-Hexane (mL)	Ethyl acetate (mL)
100	0
90	10
80	20
70	30
30	70
0	100

The solution obtained from purification was collected in a 10 mL- vial, and analyzed by TLC. Identification of this solution was aided by UV lamp, at λ = 254 nm and 366 nm. The solution which showed spots in TLC sheet with the same Rf was collected in the same vial. This step was also done for raw extract of ethyl acetate (0.5 g) and that of methanol (0.5 g).

TLC and Column Chromatography analysis

TLC analysis showed the difference of color andRf value between each spots in TLC layer, with or without the aid of UV lamp. The chemical separation in the n-hexane extract was done using silica gel as stationary phase and the mixture of n-hexane and ethyl acetate as mobile phase, which has gradient composition (Table 1). When the ratio of eluen mixture was 7 : 3 (n-hexane : ethyl acetate), the separation yielded seven isolates, in which isolate 1 to 6 can be combined and called as fraction A, due to the same Rf value. This fraction has weight of 0.027 g.

The separation of ethyl acetate extract was done using the eluen mixture of n-hexane: ethyl acetate which has gradient composition (Table 1).

Structural elucidation of each fraction by GC-MS

White crystal of each fraction from raw *n*-hexane, ethyl acetate, and methanol extracts were further analyzed by GC-MS to determine the species contained in the samples.

Result and Discussion

Result of Extraction Red Betel Leaf (*Piper cf.crocatum*Blume)

The extraction was done with n-Heksana solvent. The result of extraction shows Figure 1.

Fig 1: The extract of rough red betel leaf with *n*-hexane

Figure 1 shows that extract randemen from 100 g red betel leaf (*Piper cf. Crocatum* Blume) was the result rude extract n-Heksana as much as 1,70 g or 1,67 % in the form of turquoise oil.

Result of Analysis with TLC

Analysis with TLC shows the difference of color and value of Rf from spot TLC, either directly or with the aid of UV light at wavelength, λ = 254 nm dan λ=366 nm. This means that the polarity of the solvent is an important role to the secondary metabolite species contained in the plant.

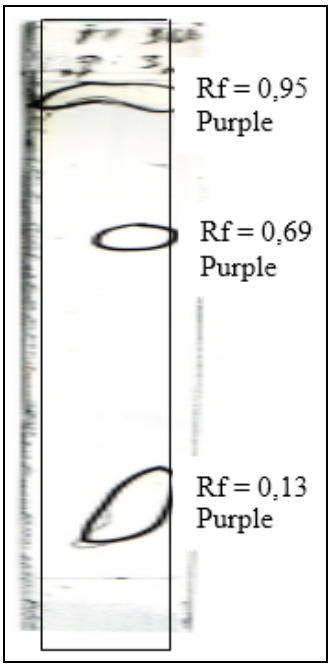


Fig 2: TLC Chromatogram of Crude extract of n-Hexane with eluent n-Hexane: Ethyl acetate (7:3).

Figure 2 shows that the minimum n-Hexane crude extract can be detected by three spots with different Rf values as shown Table 2.

Table 2: Rf Value of Crude Extract n-Hexane Color of Observation with the help of UV Light.

Spot Number	Value of Rf	Color of observation with the help of UV light	UV Light
1	0,95	Purple	366 nm
2	0,69	Purple	366 nm
3	0,13	Purple	366 nm

Result of Separation with Column Chromatography from n-Hexane Extract

Separation by column chromatography yielded seven isolates. The TLC results using eluent n-Hexane (7) : ethyl acetate (3) showed that isolates 1 to 6 can be combined because they have nearly equal Rf values. The results of combining this isolate was called fraction A in the form of solids weighing 0.027 g.

Result of Compound Analysis of Fraction A with GC-MS

The result of fraction A analysis with gas chromatography can be obtained by chromatogram as in Figure 3.

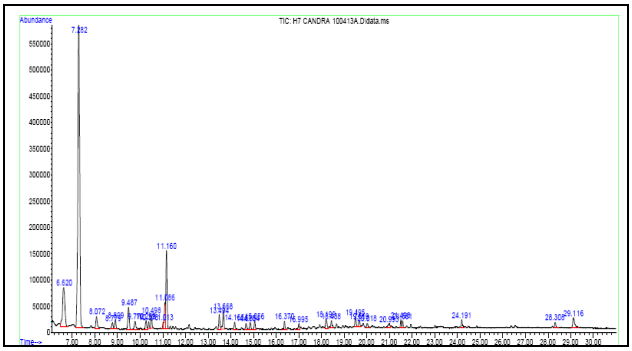


Fig 3: GC Chromatogram Fraction A

The selected chromatogram peak with a retention time of 7.282 minutes; 9.487 minutes; 11.086 minutes; 11.160 minutes; and 13.485 minutes. The mass spectra can be seen in Figure 4.5, 4.6, 4.7, 4.8, 4.9.

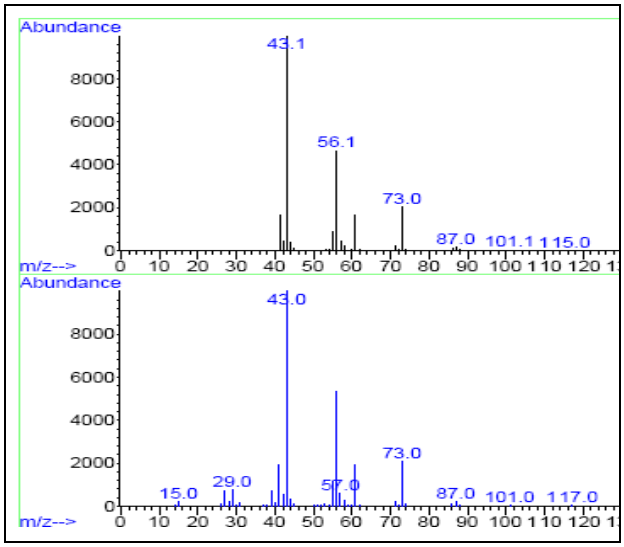
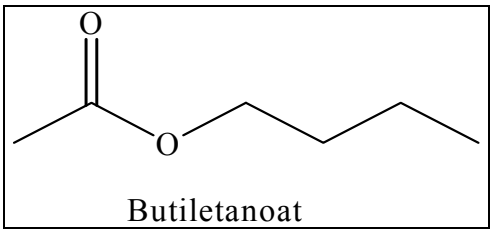
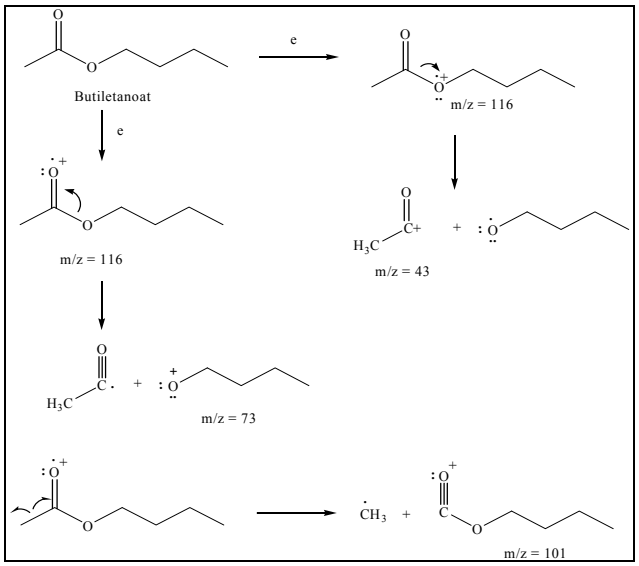


Fig 4: The mass spectra of fraction A from the top with Rt 7,282 minutes

Figure 4 shows the parent peak at m/z=116. The base peak with m/z=43 was formed when the ion parent fragmented to form ethyl ketone, while the ions with m/z =73 are the butoxide ions formed fragmentation of the ion parent. Based on fragmentation, then the possibility of molecular formula with BM = 116 was $C_6H_{12}O_2$. From the existing data base (library search report NIST05) a high similarity level (96%), the compound was determined as butyl ethanoate.



Fragmentation can be written as follow:



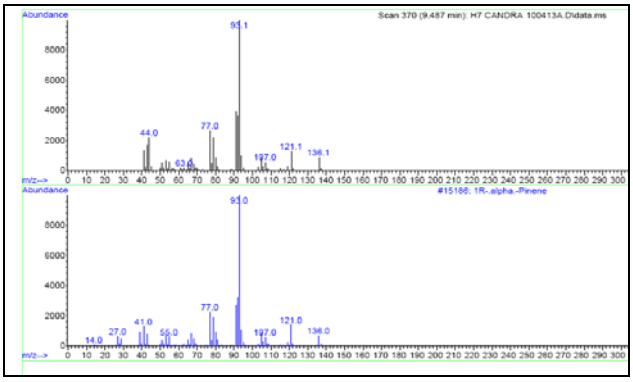
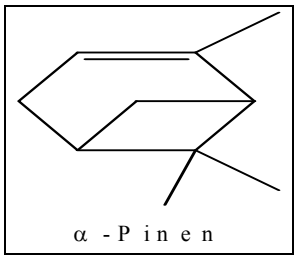


Fig 5: The mass spectra of fraction A from the top with Rt 9,487 minutes.

Figure 5 shows that the parent peak at m/z= 136. Ions with m/z= 121 were formed when the ion parent further fragmentes by releasing CH_3, while the base peak at m/z = 121 which releases two groups: CH2. Based on the fragmentation, then the possibility of molecular formula with BM = 136 was $C_{10}H_{16}$. From the existing data base (library report NIST05) high similarity level (96%), the compound was determined as α-pinen.



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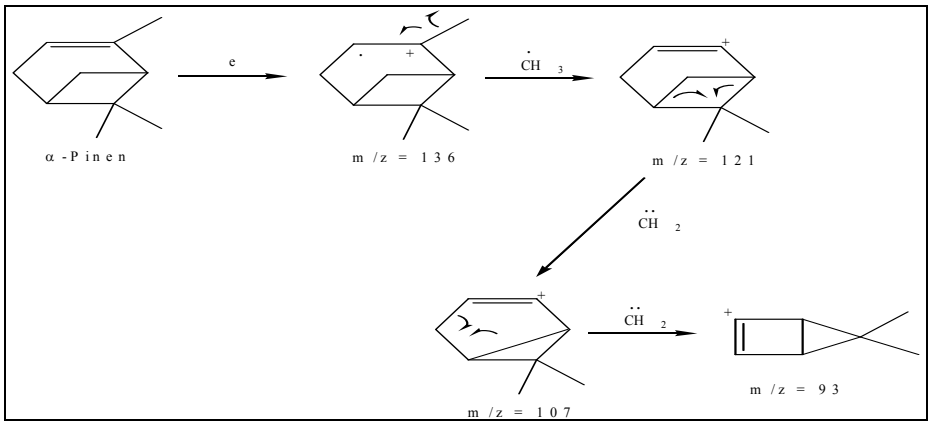


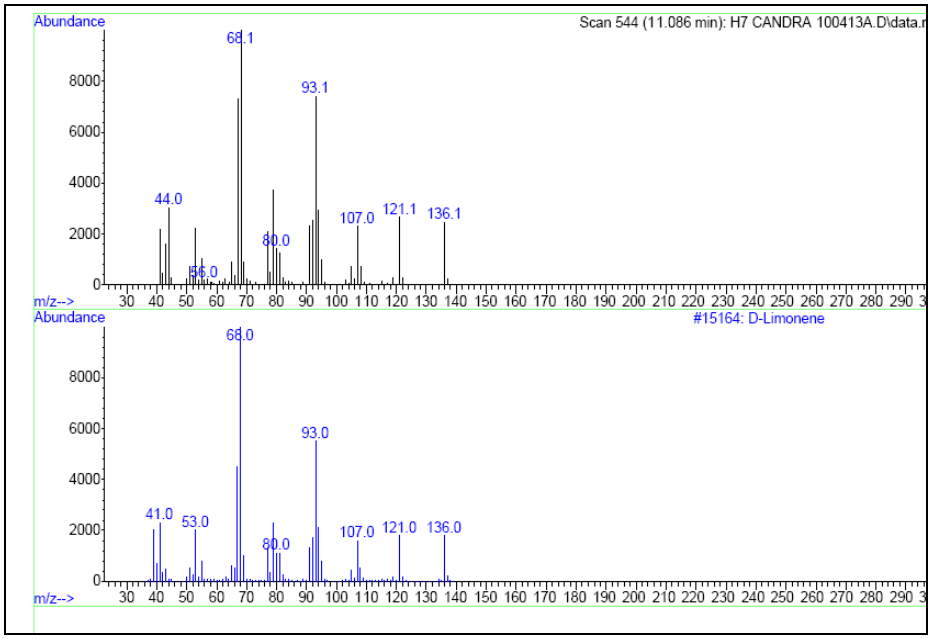
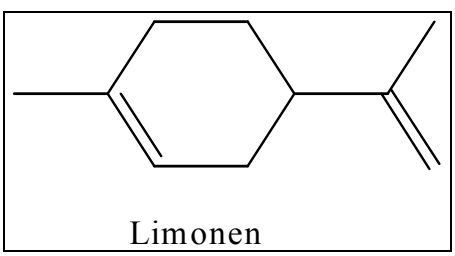


Fig 6: The mass spectra of fraction A from the top with Rt 11,086 minutes

Figure 6 shows that the peak parent at m/z=136. Ions with m/z= 121 were formed when the ion parent further fragmentes by releasing CH_3. The ion with the highest intensity at m/z= 68 was the peak base generated Retro-Diels Alder from ion parent. Ions with m/z =93 were the result of further fragmentation with m/z= 121 releasing two groups : CH_2. Based on the fragmentation, then the possibility of molecular formula with BM =136 was $C_{10}H_{16}$. From the existing data base (*library search report*NIST05) high similarity level (97%), the compound can be determined as limonene.



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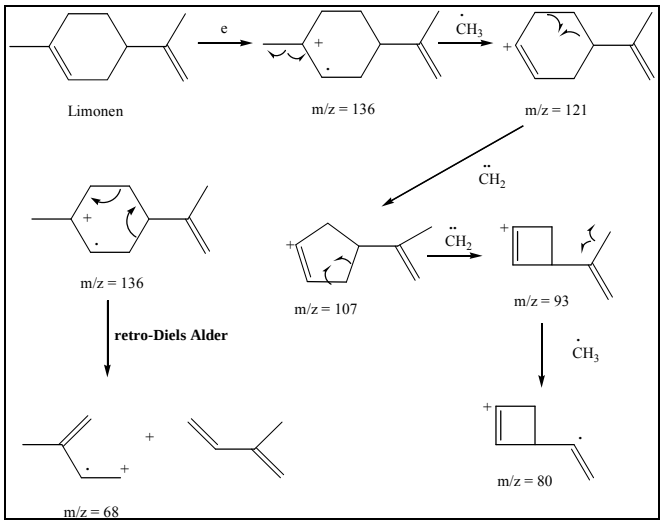


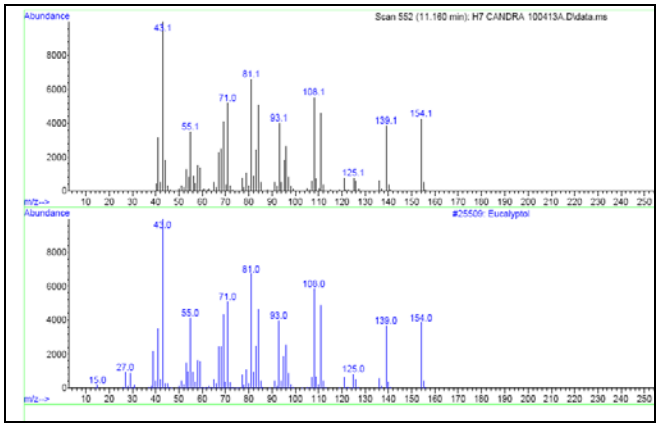
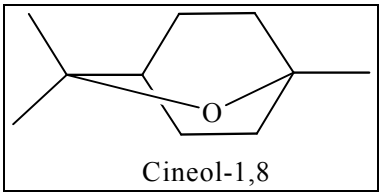


Fig 7: The mass spectrum of fraction A from the top with RT 11,106 minutes.

Figure 7 shows that the peak parent at m/z= 154. The ion with m/z= 139 was formed when the ion parent further fragmentes by releasing CH_3, while the base peak m/z=43 was the methyl ketone ion, resulting from ion parent fragmentation. Ions with m/z=125 were the result of further fragmentation with m/z =139 which releases the group $:CH_2$. Based on the fragmentation, then the possibility of molecular formula with BM=154 was $C_{10}H_{18}O$. From the existing data base (*library search report* NIST05) high resemblance level (99%), the compound was determined as ineol-1,8.



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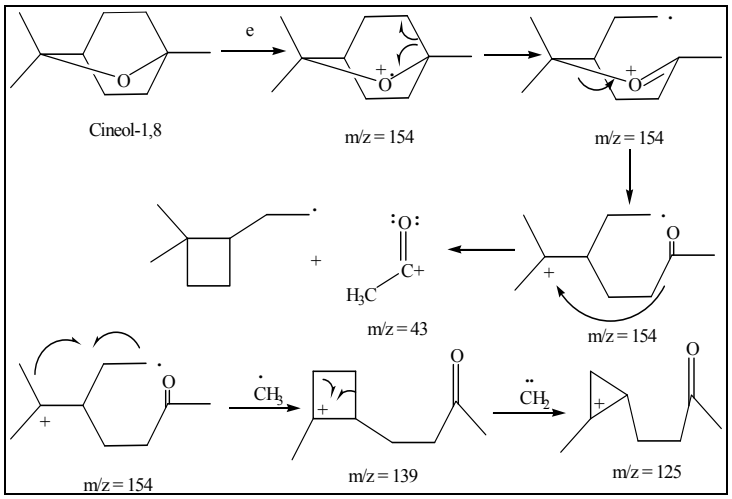


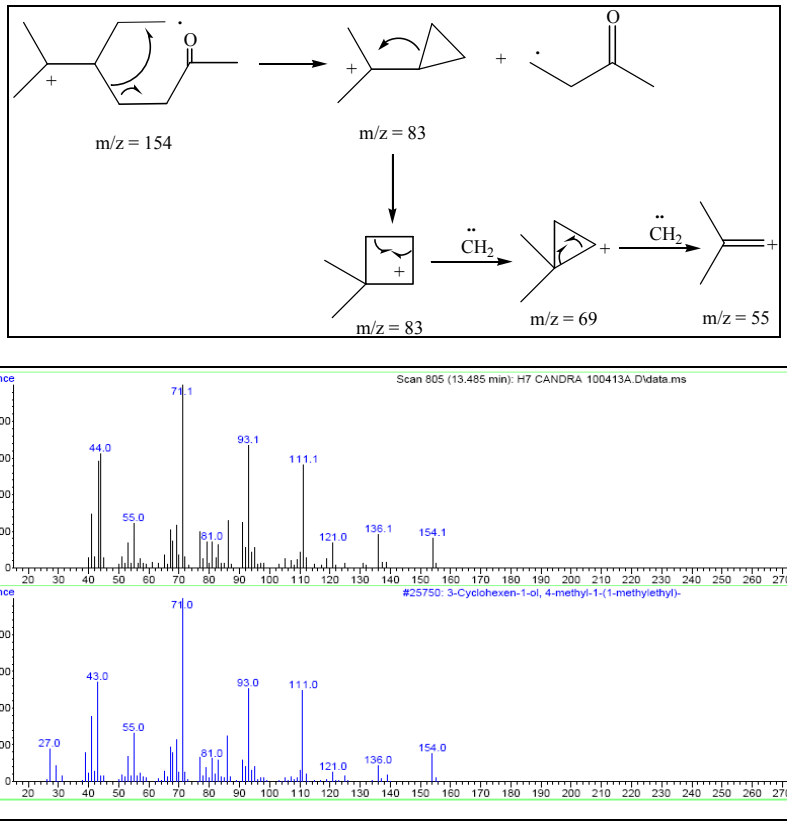
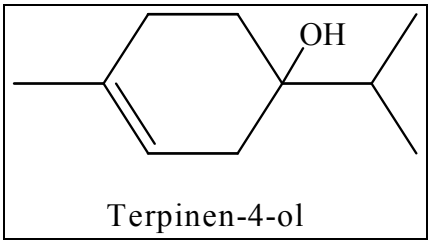
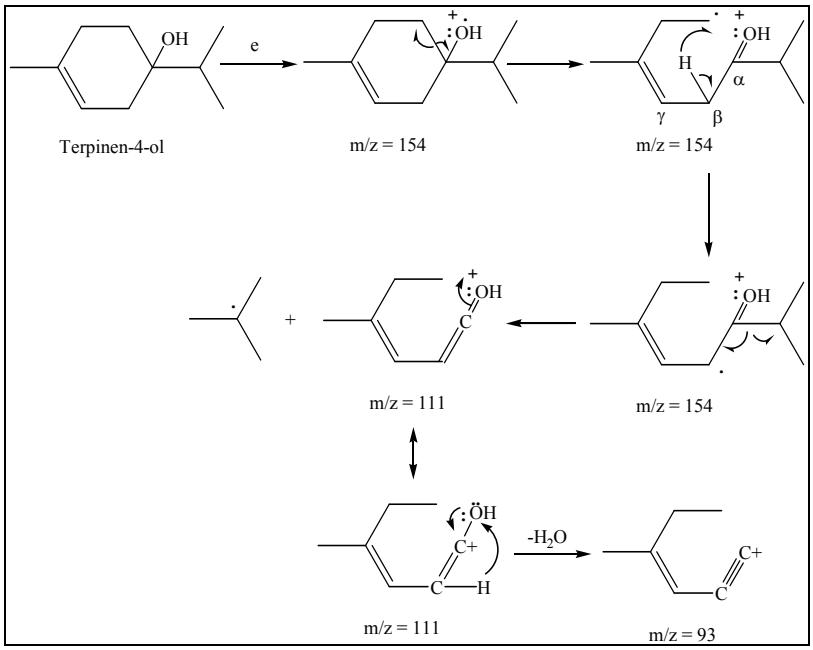


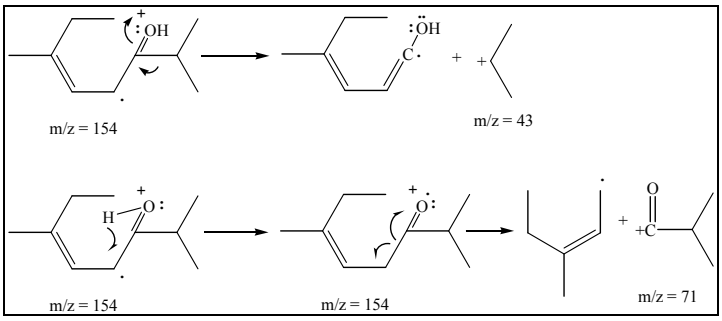
Fig 8: The mass spectra of fraction A of the peak with Rt 11,485 minutes.

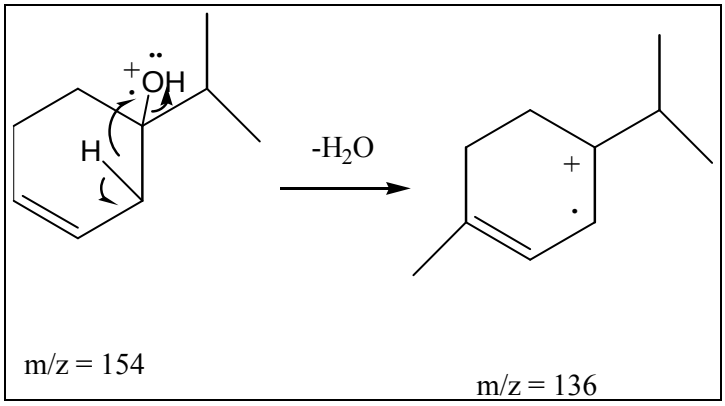
Figure 8 shows that the peak parent at m/z = 154. The ion with m/z=136 was formed when the ion parent further fragmentes by releasing H_2O, while the base peak atm/z = 71 was the isopropylceton ion produced from ion parent fragmentation. The ion with m/z=111 was the result of further fragmentation by releasing the isopropyl radical. Ions with m/z= 93 were the result from further fragmentation of ions m/z= 111 releasing H_2O. Based on the fragmentation, then the possibility of molecular formula with BM =154 was $C_{10}H_{18}O$. From the existing data base (*library search report* NIST05) high resemblance rate (96%), the compound was determined as terpinen-4-ol.



The fragmentation can be written as follow:







Conclusion

Based on fragmentation on MS, chemical compounds extracted from n-Hexane such as butyl etanoate with BM (Molecular weight) = 116 was $C_6H_{12}O_2$, α-pinen with BM = 136 was $C_{10}H_{16,}$ limonen with BM= 136 was $C_{10}H_{16}$, cineol-1,8 with BM = 154 was $C_{10}H_{18}O$, terpinen-4-ol with BM = 154 was $C_{10}H_{18}O$.

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