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Antioxidant Potency of Essential Oil Extract from *Lantana Camara* Growing in the Central Region of Ghana

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ABSTRACT

Essential oils have been used in cuisine as spices or herbs as well as in fragrance, cosmetics, and medicine since ancient times. Although their primary usage in the nineteenth century was as additives for scent and flavor, their original application was in medicine. In this study, essential oil was extracted from fresh leaves of *Lantana camara* using hydro-distillation procedure. The yield of the oil obtained was 12.68g which represented a 2.54% yield. Gas Chromatography -Mass Spectrometry Analysis of the oil revealed that the essential oil from *Lantana camara* contains total of 76 compounds representing 99.40% of the oil with (E)- Caryophyllene as the predominant compound in the oil extract constituting 20.6%. The total flavonoid was determined as well as the Total Antioxidant Capacity (TAC) of the essential oil extract of the *Lantana camara* was examined using UV-Vis spectrophotometer according to the phosphomolybdenum assay. Also, the free radical scavenging activity of the essential oil extract was then determined using the DPPH assay. A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract indicated a slight difference in activity of the TAC than the TPC, meaning there are more phenolic contents present in the oil extract, with a lower antioxidant capacity. Its (*Lantana camara*) widespread usage as a medicinal plant with the curative effect against a variety of diseases and conditions is supported by the presence of antioxidant qualities in the essential oil.

1.0 Introduction

Plants have been recognized many years ago for their therapeutic properties. These plants have been a great source of medicine due to the presence of bioactive natural compounds. These bioactive natural compounds are widely used in both traditional and modern therapies for improving human health with relatively less or no side effects [1]. Globally, various medicinal plants have been well explored to discover novel drug molecules to combat the threat of ever-increasing human diseases [2].

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant [3]. Essential oils have been used for centuries in medicine, perfumery, cosmetic, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century their use as aroma and flavour ingredients increased and became their major employment[4]. The antibacterial properties of secondary metabolites were first evaluated using essential oil vapours by De la Croix in 1881[4]. Since then, essential oils or their components have been shown to not only possess broad-range antibacterial properties [5], but also anti-parasitic, insecticidal [6] and antioxidant [7] properties

Lantana camara is listed as one of the significant medicinal plants of the world [8]. The plant *Lantana camara*, generally known as wild or red sage is the most widespread species of this genus and it is a woody straggling plant with various flower colours, red, pink, white, yellow and violet. It is an ever-green strong-smelling shrub, with stout recurred prickles, leaves opposite, ovate, acute or sub-acute, crenate - serrate, scab ride on both sides. *Lantana camara* shrub belongs to the family Verbanaceae and is located in the tropical parts of earth. It is native to tropical American countries like Brazil, Colombia, and Mexico[9]. *Lantana camara* has been used in many traditional medicinal applications but there is inadequate scientific confirmation on these traditional applications. It is known to be used in the treatment of itches, cuts catarrh, tumour, headache, ulcers, eczema, tetanus[10]. In Ghana, infusions of the plant are used to treat bronchitis and the powdered root is added to milk and given to children to treat stomach ache[11]. Barreto et al., [12] published that Lantana is used as an antiseptic and antitumoral agent and that, its roots have been used for the treatment of malaria. The leave extracts of Lantana have shown analgesic and antimicrobial properties in many experiments[13].

2.0 Materials and Method

2.1 Sample Collection and Treatment

The leaves of *Lantana camara* were collected from Amamoma near the University of Cape Coast botanical gardens and sent to the Natural Product Research laboratory of the Department of Chemistry, University of Cape Coast for the essential oil extraction. The sampled leaves were weighed, washed and cut into smaller pieces to enhance its surface area for the oil extraction.

This was then packed into a still compartment of the hydro distillation setup as shown in Figure1.

Figure 1 A Hydro-Distillation Setup

2.2 Extraction of essential oil

500g of the treated leaves were packed into the still compartment (round-bottom flask) seated in the heating mantle. 300ml of distilled water was then added and the mixture distilled. The distillate was collected in a flat-bottom flask and allowed to cool to room temperature with aluminium foil seal to prevent volatilization of the essential oil.

The essential oil in the distillate was extracted using separating funnel. The organic solvent, diethyl ether was used to extract the oil. For each 50 mL of the distillate in the separating funnel, 10 mL of the diethyl ether was added and shaken for several times with the release of pressure build up at time intervals. This was then mounted on a retort until there is clear separation of the organic layer and the aqueous phase. The organic phase (the essential oil) was transferred into a conical flask and covered with aluminum foil. The extraction with diethyl ether was repeated three times to ensure maximum extraction of the desired oils. The combined organic phase extracts were dried using anhydrous sodium carbonate (Na2CO3) (covered with aluminum foil) for about 15 minutes to remove any traces of moisture. This later was filtered through a glass wool into a beaker to get rid of the drying agent. The diethyl ether solvent was removed through vacuo in a fume chamber leaving behind the desired essential oil.

2.3 Gas Chromatography -Mass Spectrometry Analysis

100 mg of the sample was weighed and dissolved in dichloromethane (1 mL), vortexed for 10 seconds, sonicated for 1 hour., centrifuged at 14,000 rpm for 5 min and then dried by passing through anhydrous sodium sulphate before analysis (100 ng/µl, 1µL) by an Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer in full scan mode. The extraction and analysis were carried out in triplicates.

Identification of the oil components was based on their retention indices and mass spectra obtained from GC/MS analysis on an Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer in full scan mode. The GC analysis parameters are listed above and the MS ones were obtained (full scan mode: scan time: 0.3 s, mass range was *m/z* 35-450) in the electron ionization mode at 72.4 KPa. All the data were the average of triplicate analyses. Detection and identification of the constituents of the oils was based on comparisons of the individual retention times and mass spectra with those obtained from using NIST'11, 08, 05, Adams and chemecol mass spectral databases and literature.

2.4 Total Phenolic Capacity

The Total Phenolic Capacity of the essential oil extract was determined by UV-Vis spectrophotometer using the Folin-Ciocalteu's reagent, as described by Wolf et al. (2003). Gallic acid, as a standard, was prepared by dissolving 0.01g of the Gallic acid in a 100 mL ethanol, hence a 0.01% (%w/v) Gallic acid was obtained. Also, a 7.5 g of sodium carbonate (Na2CO3) was diluted to 100mL dilute water to obtain 7.5% (%w/v) Na2CO3. 10mL of Folin-Ciocalteu's phenol was prepared to 100mL by adding distilled water to obtain a concentration of 10% (%V/V). 500 µL of the essential oil was pipetted to 500 uL ethanol into a test tube. 2 mL of the 7.5% anhydrous sodium carbonate (Na2CO3) and 2 mL of the 10% Folin-Ciocalteu's reagent were added to the content of the test tube.

2.5 Estimation of Total Flavonoid Content

The total flavonoid was determined using the method of Ordonez et al. (2006). A volume of 0.5mL of 2% $AlCl_3$ ethanol solution was added to 0.5mL of the extract solution. The mixture was incubated for 1hr at room temperature for yellow colour appearance; the absorbance was measured at 530nm. Plant extract were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids content was calculated as quercetin(mg/g) using the equation obtained from the curve: $Y = 0.0004x$, $R^2 = 0.9532$, where x is the absorbance and Y is the quercetin equivalent.

2.6 Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) of the essential oil extract of the *Lantana camara* was examined using UV-Vis spectrophotometer according to the phosphomolybdenum assay outlined by Wolfe *et. al*. (2003). A 1.20 g Na_2SO_4 was dissolved in a 250 mL volumetric flask of H_2SO_4 at initial concentration of 12.48 M. Also, a 1.25 g of ammonium molybdate was dissolved into the 250 mL H_2SO_4 volumetric flask. The mixture was swirled to ensure homogeneity. Using ascorbic acid as the standard, a mass of 0.01 g of $C_6H_8O_6$ was dissolved with distilled water to 100mL to obtain a concentration of 0.01% (% w/v). 500 µL of the essential was pipetted into three different test tubes; C1, C2 and C3. 3mL of the H_2SO_4 together with sodium phosphate and ammonium molybdate (phosphomolybdenum) was added to each of the three test tubes. The test tubes were incubated for 60 minutes in a water-bath at 95 ^{O}C and allowed to cool to ambient temperature. Serial dilutions of the standard ascorbic acid were also prepared in ten different test tubes, labelled S_1 to S_{10}, in varying concentrations of 10 µg/mL to 100 µg/mL respectively, with the addition of 3 mL phosphomolybdenum solution. These test tubes were also incubated for 60 minutes at 95 ^{O}C. A blank solution was also prepared. Using the T70 UV-Vis spectrophotometer, the various abundances were obtained for the standard, essential oil and the blank. A standard calibration curve of absorbance against concentration was constructed from the abundance values of the ascorbic acid.

2.7 2,2-Diphenyl-1-Picrylhydrazy (Dpph) Free Radical Scavenging Activity

The free radical scavenging activity of the essential oil extract was determined as described by Prior et. al. [14], using the DPPH assay. About 0.001 g of the powdery DPPH was weighed and dissolved in a 100mL volumetric flask using methanol. The flask was covered with aluminium foil as the DPPH is volatile. Ascorbic acid was used as the standard test. 0.01 g of C6H8O6 was dissolved in 100mL distilled water in a volumetric flask. 1mL of the 0.001% DPPH was added to C1

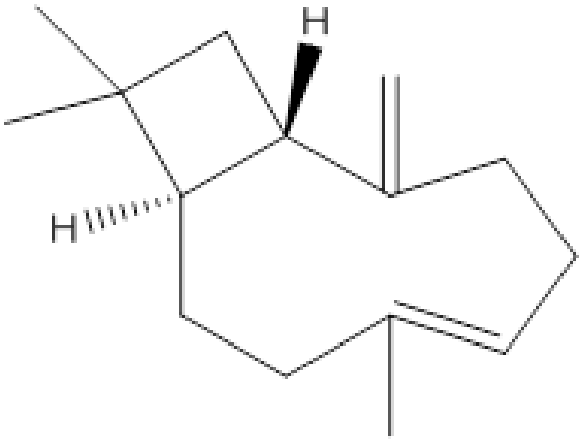
(200 µL L. camara + 800 µL methanol + 1 mL C6H8O6), C2 (400 µL L. camara + 600 µL methanol + 1ml C6H8O6), C3 (600 µL L. camara + 400 µL methanol + 1 mL C6H8O6) and C4 (800 µL L. camara + 200 µL methanol + 1 mL C6H8O6). These were allowed to stand for 30 minutes. A color change from purple to pale yellow indicates the reduction of the oil and their abundance values were determined using the T70 UV-Vis spectrophotometer at a wavelength of 517 nm.

3.0 Results And Discussions

3.1 Extraction And Percentage Yield Of The Oil Extract

Hydro-distillation is a traditional method for extracting essential oils from plant tissues in which a suitable amount of water is poured into the compartment containing the materials, which is then brought to a boil [15]. This method was used to extract the essential oil from 314.0 g of fresh *lantana camara* leaves, with diethyl ether as the extracting solvent of choice due to its low water solubility [16]. The oil extract produced had a mass of 42.0 g and a volume of 20.00 mL. This resulted in a percentage yield of 13.38 %, which is significantly greater than the 12.7% reported by Ali-Emmanuel et al. [17].

The essential oils extracted by Conventional hydro-distillation method was evaluated based on chemical composition using gas chromatography-mass spectroscopy (GC-MS). A total of 76 compounds were identified representing 99.40% of the oil with (E)- Caryophyllene (I) as the predominant compound in the oil extract constituting 20.6%.



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3.2 Gas Chromatography-Mass Spectrometry (Gc-Ms) Analysis of Oil Constituent

The GC-MS results of the oil extract shows that oil extract contains mixtures of various groups of compounds. A split ration of fifty (50) was used so as to obtain much clearer. peaks. The following are table of the GC-MS results and chromatogram of the oil extract

The chromatograms from the GC-MS analysis as shown in figure 2 represent the chemical composition of the essential oil extract from the fresh leaves of *Lantana camara* oil. The respective retention indices and relative peak area, along with their percentage abundance (%) are indicated on the chromatograms. The percentage peak areas were taken to represent the proportion of each compound relative to the total. The analysis revealed the identification of seventy-six (76) unique constituents in the oil extract. The oil is consisted of a complex mixture of numerous compounds, many of which are present in low concentration quantities. The identified compounds comprise of 99.4% of the total extracted oil. The results could be compared to those reported by Limberger et al. [18], obtained through hydro-distillation method of oil extraction and GC-MS technique of L. camara. Limberger et al., [18] also reported concentration of constituents of Lantana similar to that recorded for this analysis.

The 76 chemical components analyzed by GC-MS can be found in table 1. The predominant constituent compounds of the oil extract are (E)-Caryophyllene and α-Humulene (II) with percentage abundance of 20.6% and 13.5% respectively. Other major chemical components analyzed are 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl) (2.7%), 3Z-Hexenol (5.4 %), Caryophyllene oxide (5 %), (-)-Aristolene (2.3%), 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-

tetramethyl (3.7%), 1,6-Cyclodecadiene, cubedol (3.7%), Linalool (2.6%) and 2-ethoxy-2-methylpropane (2.8%).

The chemical composition of the essential oils extracted from the L. camara leaves was consistent with previously works reported by [19] with some differences within the relative quantities of the volatile compounds. The observed differences in composition of the extracted oil and other studies could be due to climate differences in the harvesting of the Lantana camara leaves topographical location difference as well as the season. Most of the identified compounds, namely a 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl, 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl, and α-Muurolene are known for pharmaceutical applications as was reported by [20]. Thus, α-Muurolene (III), has been reported as having pain-relieving and anti-inflammatory properties, and it exhibits antifungal activity against dermatophytes. Oxabicyclododeca oil, which is rich in dodecene (> 73%), has been reported to show antioxidant, antibacterial, and insecticidal activities [21]. Apart from the medicinal value of L. camara oil, the sustained demand for synthetic flavorings and fragrances to be used within the pharmaceutical, food, and cosmetic industries makes this essential oil valuable for exploitation in these industries additionally. Again, the method of extraction of these essential oils can also bring about differences in the chemical composition of the recovered oil. Some researchers had used methods such as Conventional hydro-distillation, Clevenger-assisted hydro-distillation, Steam distillation, Microwave-assisted distillation etc. These had led to differences in the constituent of the oil extract in their concentrations and some cases differences in the chemical constituents of the oil.

3.2 Determination of Total Phenolic Content (TPC)

The Standard calibration curve of Gallic acid as well as the Absorbance and Concentration values of TPC of the oil extracts are represented in fig. 3 and table 2 below respectively. The total phenolic content was calculated using the graph and the standard curve equation is $y = 0.0402x + 0.1733$, with $R2 = 0.988$, implying a 99 % correlation between the data sets. The concentrations of essential oil extract were calculated using this equation, and the average is 14.966 ± 4.43 µg/mL. Thus, the oil extract as calculated shows a total phenolic content (Gallic acid equivalent, mg/g) of 14.966 ± 4.43 mg GAE/g.

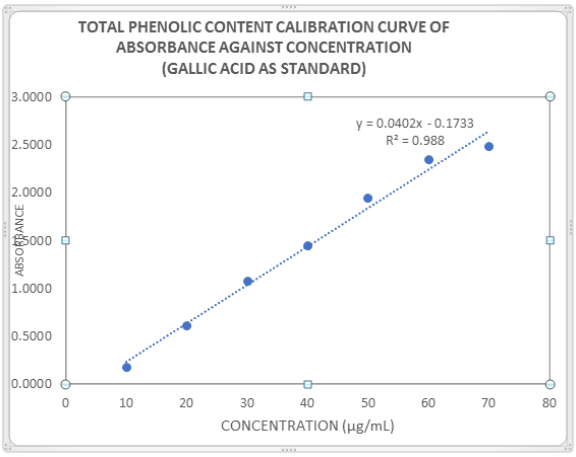

Figure 3 Standard Calibration Curve Of Gallic Acid

Table 2 Absorbance and Concentration values of TPC

Sample	Absorbance	Concentration (µG/ML)
C1	0.633	20.057
C2	0.343	12.843
C3	0.309	11.998
AVERAGE		**14.966 ± 4.43**

A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract as shown in fig. 4 below, indicate a slight difference in activity of the TAC than the TPC. The R2= 0.9033 (90%) indicates a stronger correlation between the two data set. The P-value = 0.0036, therefore P<0.05, meaning there is a statistical significance difference their mean value. This means there are more phenolic contents present in the oil extract, with a lower antioxidant capacity.

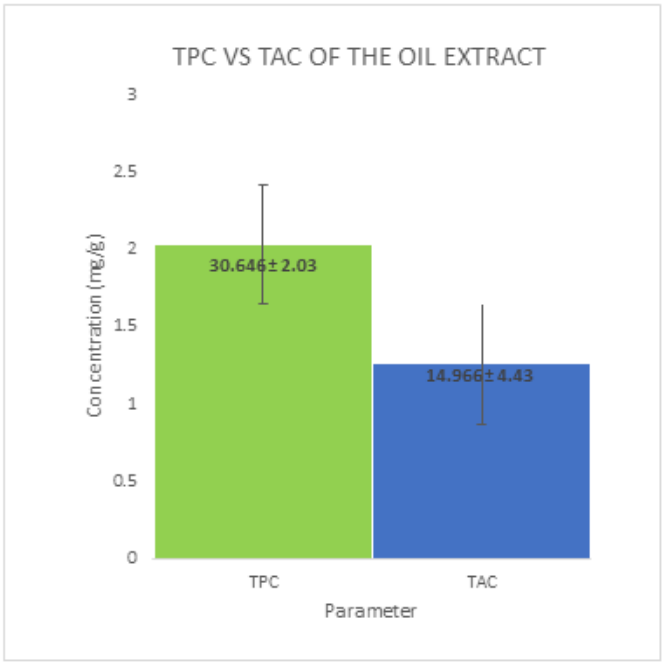

Figure 4 Comparison of TAC and TPC analysis of the oil extract .

A phenol loses an H+ ion to generate a phenolate ion, which decreases the Folic-Ciocalteu reagent in the basic reaction conditions [22]. The change is measured using spectrophotometry. Because phenolics (including many flavonoids) include polar phenolic hydroxyl group(s), Gallic acid's greater TPC is understandable. Similarly, the oil extract's lower TAC could be explained in the same way.

3.3 Determination of DPPH radical scavenging activity

The DPPH assay relies on the aptitude of an antioxidant to donate a hydrogen radical or an electron to DPPH radical, which is stable radical with deep violet color. When an odd electron becomes paired within the presence of atom scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH -H form [14] and therefore the solution gets decolorized from its initial deep violet to light yellow colour. The degree of fall within the absorbance measured is proportional to the concentration of the antioxidant. The measured absorbance at various concentrations is used to calculate the percentage radical scavenging activities of both the oil extract and standard ascorbic acid, obtaining a regression line of y = 0.1928x + 15.107 and coefficient of correlation, R2 = 0.8607, which showed a comparatively good correlation (86%) between the % RSAs with a mean of 24.75± 5.37. This could be observed from fig. 5. Also, the % RSA of the standard ascorbic acid from fig. 5 below gave a linear equation of y = 0.0947x + 52.492 and coefficient of correlation, R2 = 0.9296, which showed much higher correlation (93%) than that of the oil extract, averaging 57.22 ± 2.54. These %RSA averages of the samples indicated that the radical scavenging activity of the standard ascorbic acid had a higher capability against the free DPPH compared to the Lantana camara oil extract.

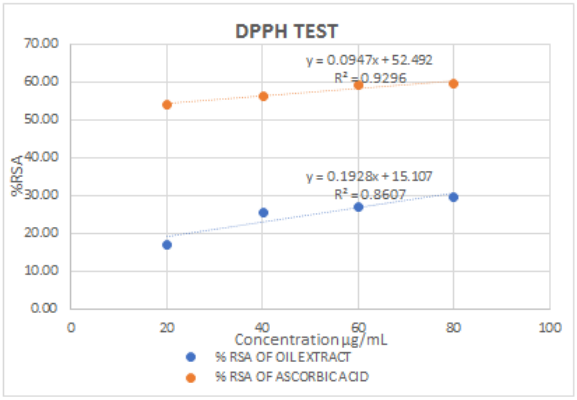

Figure5 %RSA of both Lantana camara oil extract and standard ascorbic acid.

The DPPH Radical Scavenging Activity of oil extract and standard ascorbic acid at modified concentrations is presented in table 4 below.

Table 4. DPPH Radical Scavenging Activity of Oil Extract and Standard Ascorbic Acid at Modified Concentrations.

Concentration (µg/mL)	% RSA Oil Extract	% RSA Ascorbic Acid
20	17.10	54.15
40	25.58	56.15
60	26.74	59.14
80	29.57	59.47
AVERAGE	**24.748 ± 5.37**	**57.223 ± 2.54**

The free radical scavenging property of essential oil extracts from the Conventional hydro- distillation was dose dependent. The data obtained for the radical scavenging activity of the oil extract was compared with standard anti-oxidant drugs ascorbic acid and gallic acid with the different concentrations ranging from 20, 40, 60 and 80 µg/ml. In table 5, the DPPH free radical scavenging activity of the oil extract from the fresh leaves of Lantana camara is indicated whereby ascorbic acid was employed as a comparative standard antioxidant drug in the analysis. The percentage of inhibition of DPPH free radical for the oil extract showed dose dependent trend with these observed values 17.10, 25.58, 26.74, 29.57 (mean 24.75± 5.37) for oil obtained from the Conventional hydro-distillation compared to that of the standard ascorbic acid with values 54.15, 56.15, 59.14, 59.47 (mean 57.22±2.54) with p-value 0.200. This is an indication of the superior antioxidant property of the standard drug ascorbic acid due to the higher DPPH free radical percentage inhibition compared to the value found for the oil extract. The DPPH free radical scavenging activity of the oil extract could be attributed to the constituents and the concentrations of the individual components of the oil extract as shown by the GC-MS data in table 1.

4.0 Conclusions

Based on the findings of this study, it can be concluded that the leaves of Lantana camara contain essential oils with total of 76 compounds identified representing 99.40% of the oil with (E)- Caryophyllene as the predominant compound in the oil extract constituting 20.6%. A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract indicated a slight difference in activity of the TAC than the TPC, meaning there are more phenolic contents present in the oil extract, with a lower antioxidant capacity. It can also be concluded that, the presence of antioxidant properties in the essential oil provides the basis for its (Lantana camara) wide use as a medicinal plant with the curative effect against numerous diseases and conditions.

Table 1. Gc-Ms Result Of The Oil Extract

RT	Library/ID	Peak Area_1	Peak Area_2	Peak Area_3	Mean Area	Abundance(%)
3.48	2-Pentanone	368884	405772	331995	368884	0.3
4.29	2,3-dimethyl-2-butanol	638409	702249	574568	638409	0.5
4.38	2-ethoxy-2-methylpropane	3532653	3885918	3179387	3532653	2.8
10.63	β-pinene	1310637	1441700	1179573	1310637	1.0
10.69	Limonene	650071	715078	585063	650071	0.5
10.78	1-Octen-3-ol	2466746	2713420	2220071	2466746	2.0
17.34	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	3390201	3729221	3051180	3390201	2.7
17.76	(*E*)-Caryophyllene	25704219	28274640	23133797	25704219	20.6
17.87	β-Copaene	2193445	2412789	1974100	2193445	1.8
17.96	α-Guaiene	305053	335558	274547	305053	0.2
18.13	(*Z*)-β-Farnesene	1298872	1428759	1168984	1298872	1.0
18.20	α-Humulene	16887538	18576291	15198784	16887538	13.5
18.30	α-Muurolene	876525	964177	788872	876525	0.7
18.49	Benzene, 1-(1,5-dimethyl-4-hexenyl)- 4-methyl-	669011	735912	602109	669011	0.5
18.62	β-Selinene	1057188	1162906	951469	1057188	0.8
18.70	4-epi-cubedol	1862459	2048704	1676213	1862459	1.5
18.73	(-)-Aristolene	2856078	3141685	2570470	2856078	2.3
18.81	Phenol, 2,4-bis(1,1-dimethylethyl)-	2241693	2465862	2017523	2241693	1.8
18.96	Cubedol	4578873	5036760	4120985	4578873	3.7
19.02	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	1802365	1982601	1622128	1802365	1.4
19.74	Spathulenol	1486019	1634620	1337417	1486019	1.2
19.82	Caryophyllene oxide	6194854	6814339	5575368	6194854	5.0
20.12	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	4651637	5116800	4186473	4651637	3.7
20.30	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1030000	1133000	927000	1030000	0.8
20.43	Epizonarene	2052237	2257460	1847013	2052237	1.6
20.50	α-Cubebene	1943027	2137329	1748724	1943027	1.6

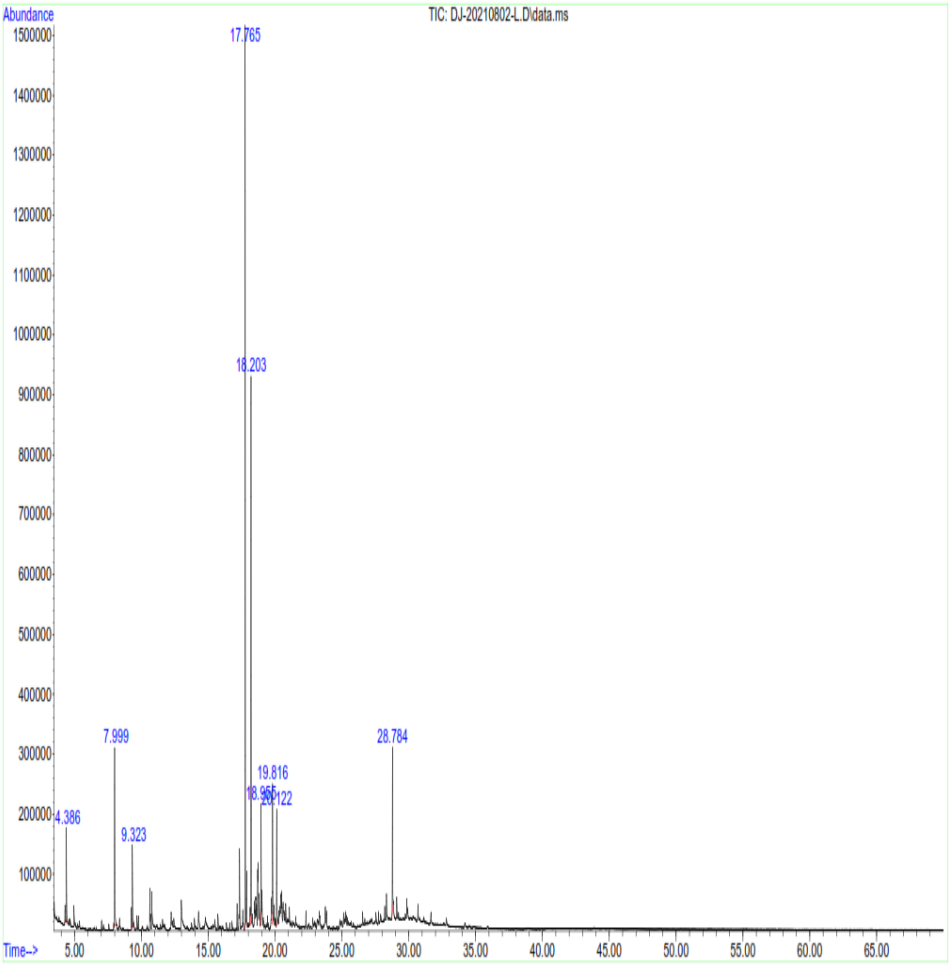

Figure 2 Non Expanded Total Ion Chromatogram With Retention Time Annotation

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