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# Effect of extraction solvents/techniques on polyphenolic contents and antioxidant potential of the aerial parts of *Nepeta leucophylla* and the analysis of their phytoconstituents using RP-HPLC-DAD and GC-MS

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**S1:** Linear equations and $R^2$ for different standards used to calculate the results of different assays

| Sr. No. | Name of Assay | Name of Standard and Concentration | Linear Equation | $R^2$ |
|---|---|---|---|---|
| 1 | Total Polyphenolic Content (TPC) | Gallic Acid (0-150mg/L) | Y = 9.788x + 0.0137 | 0.9993 |
| 2 | Total Flavonoids Content (TPC) | Rutin Trihydrate (0-300mg/L) | Y = 1.1643x + 0.0018 | 0.9984 |
| 3 | Ferric Reducing Antioxidant Power (FRAP) | Ferrous Sulphate (0-300mg/L) | Y = 4.5682x – 0.0347 | 0.9993 |
| 4 | Total Antioxidant Capacity (TAC) | Vitamin C (0-300mg/L) | Y = 3.5665x – 0.0503 | 0.9992 |

**S2:** Linear equations, $R^2$, LOD and LOQ values of différent standards used to calculate the results of different polyphenolic compound by RP-HPLC analysis:

| Sr. No. | Name of Standard (Conc. -0.2 – 1 mg/mL) | Linear Equation | $R^2$ | LOQ* (µg/ml) | LOD* (µg/ml) |
|---|---|---|---|---|---|
| 1 | Myricetin | Y = 184.8x – 6.9079 | 0.9954 | 2.1 | 0.69 |
| 2 | Catechin Hydrate | Y = 114.42x – 1.9269 | 0.9999 | 1.67 | 0.55 |
| 3 | Chlorogenic acid | Y = 290.08x – 17.903 | 0.9987 | 1.58 | 0.52 |
| 4 | Rutin trihydrate | Y = 110.36x – 5.5993 | 0.9916 | 1.66 | 0.5 |
| 5 | Caffeic Acid | Y = 581.4x – 26.194 | 0.9952 | 1.01 | 0.34 |
| 6 | Syringic Acid | Y = 279.83x – 2.409 | 0.998 | 1.05 | 0.35 |
| 7 | Vanillic acid | Y = 666.11x – 7.0634 | 0.9994 | 0.46 | 0.15 |

LOQ and LOD was calculated on the basis of S/N, (for LOQ S/N ≥ 10, for LOD ≥ 3.3)

**S3:** Method used for the quantification of different phenolics using RP-HPLC-DAD:

In order to construct the standard curve for quantification of different polyphenolics in plant extracts, the standards and samples were dissolved in methanol (HPLC grade) and then filtered by syringe filter (0.22 µm, Millipore Millex GV, hydrophilic PVDF). A stock solution of different standards **(1 mg/mL)** and samples (**4 mg/mL**) were prepared and stored in a deep freezer (-40 °C) for further use. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (**0.1 mg/mL to 1mg/ mL i.e. 0.2, 0.4, 0.6, 0.8, 1 mg/mL)** of different standard compounds. The different dilutions were done using methanol. LOD and LOQ were also calculated for every standard separately. The peak area for different concentration was calculated from the corresponding chromatogram of different concentration. The linear equation was constructed for each standard by plotting the graph between peak area and respective concentration in excel software.

The results of RP-HPLC analysis was calculated from the linear equation obtained from standard curve as follow:

$$Y = AX \pm B$$

Where, Y refers to the absorbance of the sample, A and B was given in a linear equation (The linear equation was obtained from the curve drawn from area under the peak in different concentrations of the standards). From this the value of X was calculated which was then introduced in another equation:

$$C = X * V/N$$

Where, C was the final result e.g. in case of TPC, C was equal to mg of gallic acid equvelant / g of dry plant extract. X was concentration calculated from calibration curve; V was the volume of extract used and N was the weight of plant extract taken in g.

**S4:** Main constituents (>2.50%) detected by GC-MS analysis of chloroform and hexane extracts obtained by different extraction methods.

Table S4-1. Main constituents (>2.50%) detected by GC-MS analysis of chloroform extracts obtained by different extraction methods

| Name of compound | RT | | | Peak Area (%) | | |
|---|---|---|---|---|---|---|
| | SEM | UAEM | MM | SEM | UAEM | MM |
| Linolenic acid | 30.890 | 30.927 | 30.880 | 2.82 | 3.98 | Tr |
| Abieta-9(11),8(14),12-trien-12-ol | 35.064 | 35.132 | 35.131 | Tr | 3.02 | 3.22 |
| UI | 46.533 | 46.629 | 46.629 | 3.13 | 10.03 | 6.08 |
| Squalene | 46.809 | 46.863 | - | 4.48 | 4.37 | - |
| Long chain hydrocarbon | 48.855 | 48.903 | 48.898 | Tr | 3.96 | 3.77 |
| Long chain hydrocarbon | 50.919 | 51.021 | 51.015 | 3.09 | 6.75 | 5.45 |
| UI | 51.489 | 51.593 | 51.575 | 5.57 | 8.86 | 12.2 |
| Long chain hydrocarbon | 53.416 | 53.462 | 53.465 | 13.20 | 11.57 | 15.10 |
| Vitamin E | 53.859 | 53.939 | - | 3.15 | Tr | - |
| Long chain hydrocarbon | 55.475 | 55.539 | 55.527 | 6.20 | 3.30 | 4.56 |
| Stigmast-5-en-3-ol, (3.beta.,24S) | 59.389 | 59.482 | - | 3.10 | Tr | - |
| Long chain hydrocarbon | 59.813 | 59.880 | 59.871 | 27.52 | 15.62 | 26.77 |
| Long chain hydrocarbon | 62.826 | 62.905 | 62.910 | 6.01 | 3.18 | 3.49 |
| Long chain hydrocarbon | 64.312 | 64.391 | 64.372 | 11.13 | 4.19 | 5.47 |

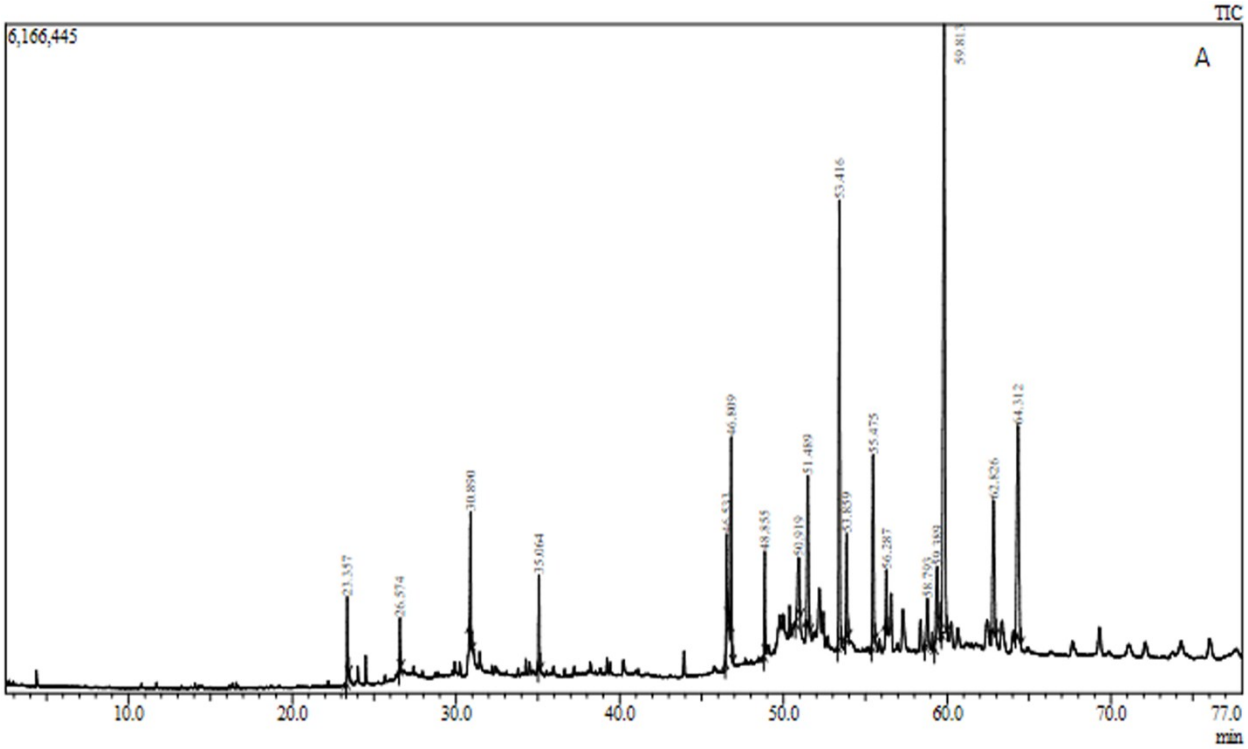
RT- retention time, UI- unidentified, - = not detected, Tr- amount present <2.5%

Table S4-2. Main constituents (>2.50%) detected by GC-MS analysis of hexane extracts obtained by different extraction methods

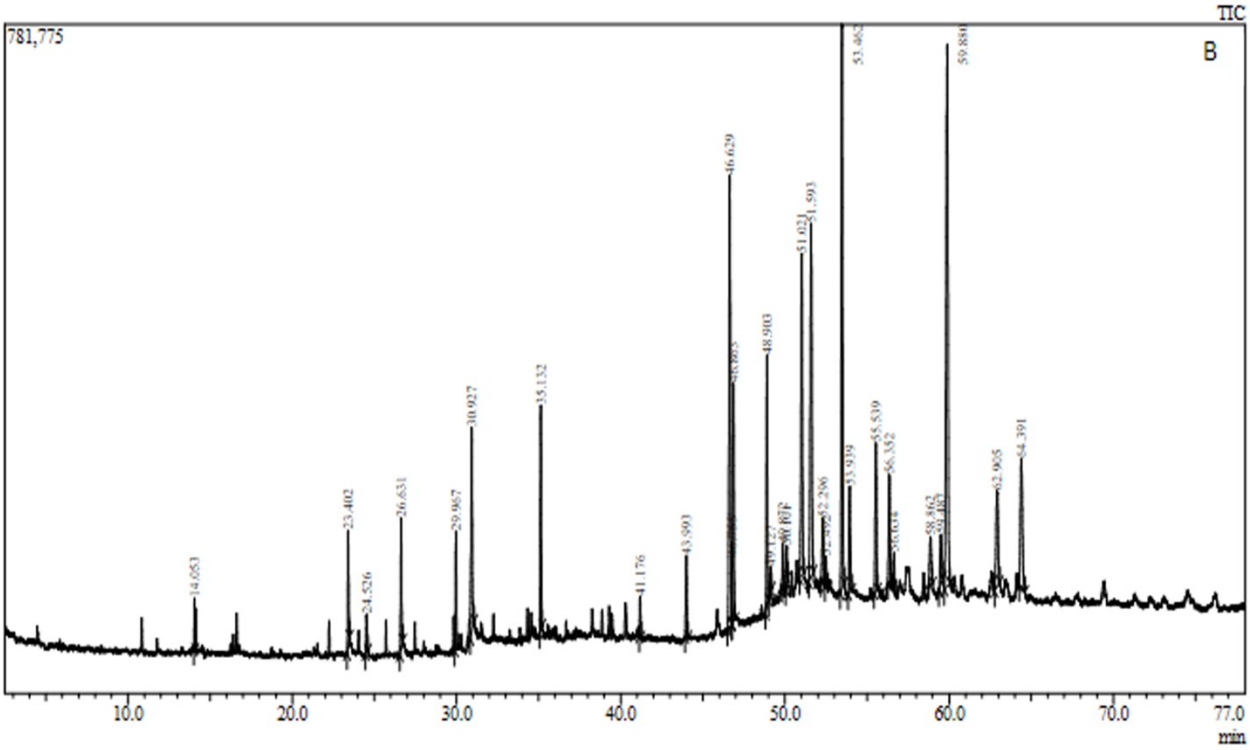
| Name of compound | RT | | | Peak Area (%) | | |
|---|---|---|---|---|---|---|
| | SEM | UAEM | MM | SEM | UAEM | MM |
| Hexadecanoic acid, methyl ester | - | - | 25.736 | - | - | 3.65 |
| Hexadecanoic acid | - | 26.674 | 26.740 | - | 3.48 | Tr |
| Linolenic acid | 31.031 | 30.986 | 31.018 | 4.95 | 5.47 | Tr |
| Abieta-9(11),8(14),12-trien-12-ol | 35.086 | 35.145 | 35.158 | 2.61 | 5.26 | Tr |
| UI | - | 46.638 | - | - | 2.76 | - |
| Squalene | 46.811 | 46.875 | 46.874 | Tr | 4.45 | Tr |
| Long chain hydrocarbon | 48.863 | 48.910 | 48.923 | 2.50 | 2.73 | 3.21 |
| Long chain hydrocarbon | 53.491 | 53.485 | 53.778 | 12.63 | 13.76 | 15.35 |
| Long chain hydrocarbon | 55.512 | 55.552 | 55.595 | 5.57 | 5.01 | 3.65 |
| Long chain hydrocarbon | 56.326 | 56.362 | 56.407 | 3.11 | 2.57 | 4.19 |
| Stigmast-5-en-3-ol, (3.beta.,24S) | 59.407 | - | - | 3.84 | - | - |
| Long chain hydrocarbon | 60.020 | 59.940 | 60.146 | 28.97 | 28.65 | 32.82 |
| Long chain hydrocarbon | 62.907 | 62.955 | 63.017 | 7.49 | 3.96 | 4.64 |
| Long chain hydrocarbon | 64.432 | 64.435 | 64.527 | 10.85 | 7.57 | 7.23 |

RT- retention time, UI- unidentified, - = not detected, Tr- amount present <2.5%

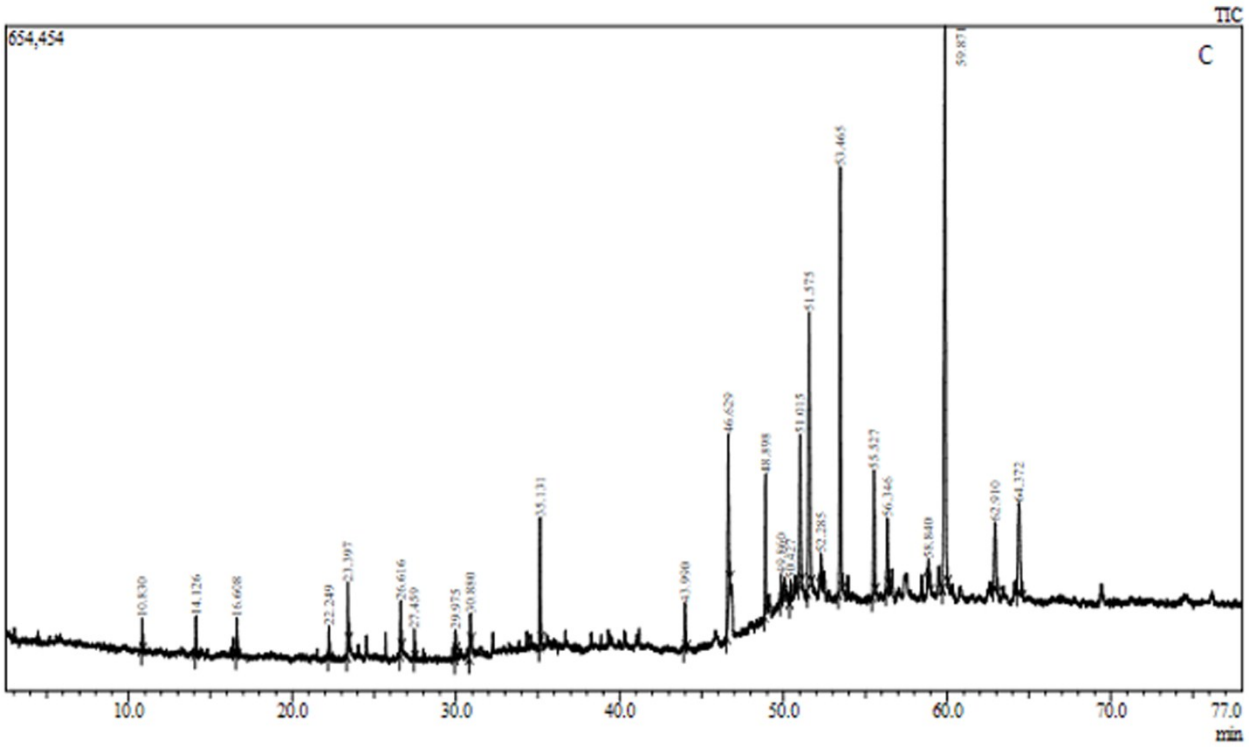
**S5:** GC-MS chromatograms of chloroform and hexane extracts obtained by different extraction methods (A) Chloroform extract obtained by SEM (B) Chloroform extract obtained by UAEM (C) Chloroform extract obtained by MM (D) Hexane extract obtained by SEM (E) Hexane extract obtained by UAEM (F) Hexane extract obtained by MM



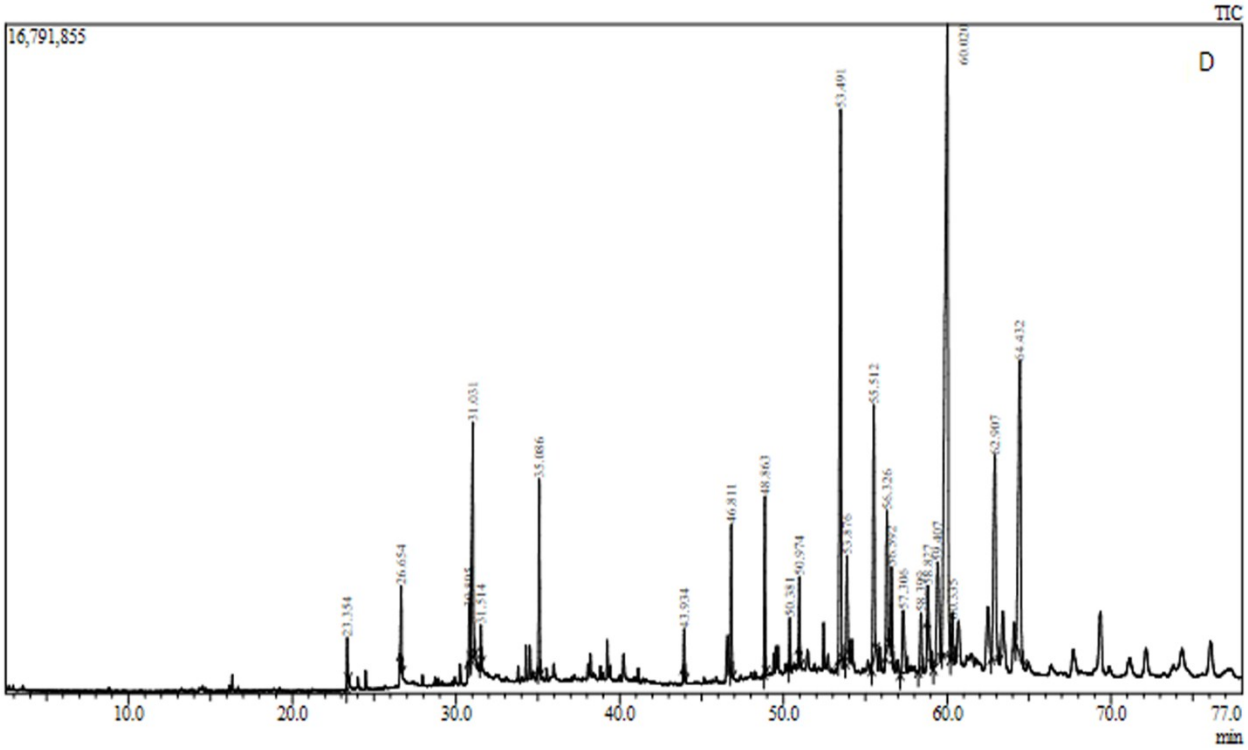

(A) Chloroform extract obtained by SEM



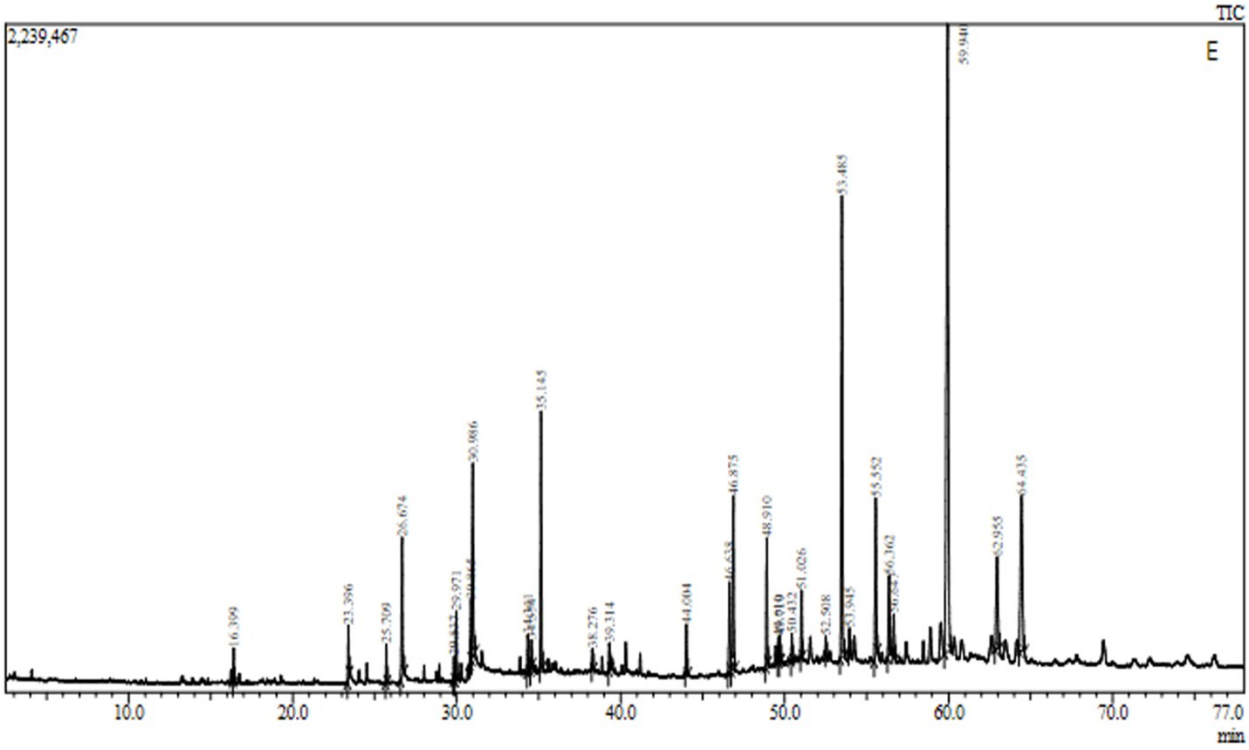

(B) Chloroform extract obtained by UAEM



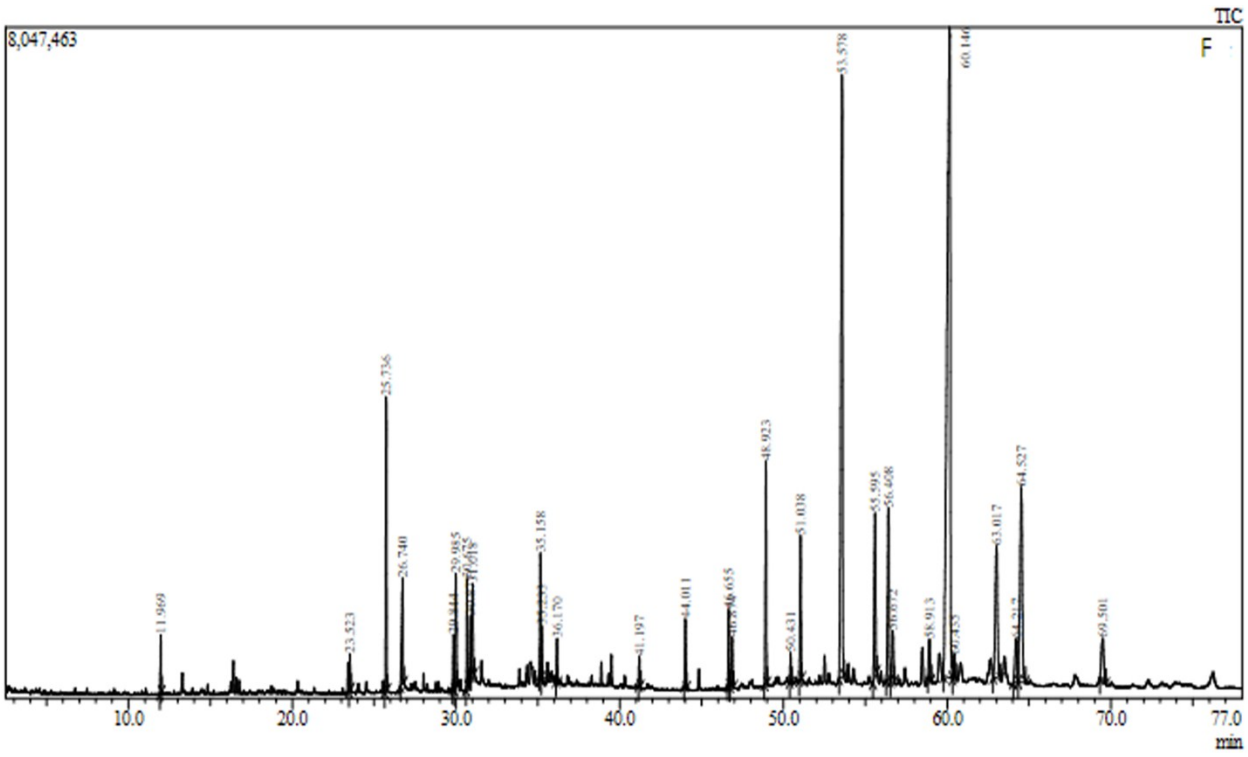

(C) Chloroform extract obtained by MM




(D) Hexane extract obtained by SEM




(E) Hexane extract obtained by UAEM




(F) Hexane extract obtained by MM