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# GC-MS profiling and HPTLC analysis for Exploration of phytochemicals in the Methanolic extract of *Bauhinia tomentosa* L. Pod

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**Abstract**

*Bauhinia tomentosa* L. , an ethnomedicinal and ornamental plant , belongs to Fabaceae family. It is a small tree growing at a height of about 8 metres . Ethnomedicinally, it is utilized for the treatment of urinary tract infections, helminthiasis, liver inflammation, diarrhea, diabetes, tumors, wounds, hyperlipidemia, bleeding, animal bites, fever, and as ethnomedicines in Africa and Asia . For present study, methanolic extract was prepared using Soxhlet extraction method. For the identification of phytochemicals, prepared methanolic extract was subjected to GC-MS analysis and HPTLC analysis. The obtained spectra of GC-MS were analysed and compared with the GC-MS spectra database of online Wiley library and NIST (National Institute of Standard and Technology) to identify the phytochemical compounds. A total of 56 compounds were isolated, out of which Hexadecanoic acid, methyl ester was present in highest % as 20.12 and Pentanoic acid,2,2-dimethyl-ethyl ester in lowest % of 0.12. Major phytochemicals identified from GC-MS spectra are megastigmatrienone, caproic acid, clorius, methyl nicotinate, n-caprylic acid, caryophyllene, cedrene, caprylone, eicosane and muscalure. In HPTLC analysis the peak reports of the methanolic extract was compared with seven standards as gallic acid, quercetin, tannic acid, rutin, dl-alfa tocopherol, ascorbic acid , and carotene.

**Key words**: *Bauhinia tomentosa Linn.*, pod, GC-MS, HPTLC.

## Introduction

One of the largest genera of the plant kingdom is Bauhinia belonging to family Fabaceae and sub-family Caesalpiniaceae. It includes about 500 species of shrubs and small trees which are tropically distributed [1]. The genus Bauhinia has a treasure of alkaloids, steroids, phenolic compounds, flavonoids, and terpenes [2]. This genus also has an abundance of structurally distinct natural products [3] such as alkaloids [4], steroids[5] , cyanoglucosides [6], flavonoids [7], triterpenoids [8]. Species belonging to Genus Bauhinia genus are commonly referred as “cow’s hoof” or “cow’s paw” as the leaves resembles in shape to the cow’s paw [4,9]. Stembark of one of the species, *B. variegata* has been as ayurvedic medicine in treatment of goiter and Ashthila [10]. *Bauhinia tomentosa* is a shrub with multiple stems or

small tree with drooping branches and numerous slender twigs [2,11]. Commonly it is named as phalgu in Sanskrit. It has been observed to possess amino acids, proteins [12], lectins [13], fatty acids [14], protocatechuic acid [15], minerals [16], rutin [17], quercetin [17] and isoquercetin [18] **.** In this research work, GC-MS investigation of the methanolic extract of pod of *Bauhinia tomentosa* is carried out to detect the presence of some bioactive compounds. The detection and identification of these bioactive compounds may prove to be very beneficial for the introduction of these bioactive compounds in Fragrance industry and pharmaceutical industry. Basically ,GC-MS is a technique in which gas chromatography and mass spectroscopy are collaborated to recognize the compounds present in the sample. It has proven to be a remarkable technique to recognize the bioactive compounds present in plants, the compounds are recognized by mass spectrometer peaks procured by eluted compounds at variable times. The observed mass spectra peaks are treated as the fingermark of compounds that are recognised by matching with library. The results of gas chromatography are based on considering the concentration of compounds as a function of retention time [19].

## Materials and Methodology

### Plant material Collection and extraction

*B.tomentosa* pods were collected from JECRC University Campus and it was authenticated by Department of Herbarium, University of Rajasthan (authentication reference no. RUBL21466). The pods were thoroughly washed with running water, shade dried and coarsely powdered. Crude extract was produced after soxhlet extraction with methanol at room temperature for 72 hrs. Thereafter, the methanol extract was filtered and stored in small vial bottles for further use for GC-MS profiling.

### GC-MS profiling and HPTLC analysis of Methanolic extract of B. *tomentosa* pod

### GC-MS profiling

GC-MS profiling was carried out at Ayushraj Enterprise Pvt. Limited , Analytical Division , Jaipur. The chemicals utilized during this analysis were obtained from Loba Chemi (India) and Sigma-Aldrich (USA) [20] .

GC-MS profiling was conducted by utilizing Shimadzu GC-MS-QP2010 Ultra system. The injector's temperature was kept at 280°C, with samples injected at a volume of 1 µl and at a ratio of 1:25 in split mode. Conveyor gas was taken as Helium , flowing consistently at 1.00 ml $min^{-1}$ through a capillary column Rtx-5MS which comprises of 5% Diphenyl & 95 % Dimethyl Polysiloxane and having dimensions of 30 m x 0.25 mm x 0.25 µm [21].

In the beginning for two minutes, the temperature of the oven was kept at 60°C , then it was raised at a rate of 10°C per minute until reaching 260°C, where it remained for 10 minutes. MS ionization potential stood at 70 eV, with the following temperature settings , ion source at 280°C, interface at 260°C, and a mass scan range of 40-550 [19]. Analysis of chromatograms involved assessing constituent compounds based on their retention time (RT) ,refractive index (RI) , and juxtaposition of mass spectra with those in NISTMS (National Institute of Standards and Technology Mass Spectral library of the GC-MS data system and co-inoculation with validated compounds for verification [21].

### HPTLC fingerprint profile of *B. tomentosa*

HPTLC analysis of methanolic extract of *B. tomentosa* pod extract was performed at Ayushraj Enterprise Pvt. Limited , Analytical Division , Jaipur.

### HPTLC conditions

CAMAG Linomat V Automatic Sample Spotter was utilized as the spotting device. 100 ml Syringe was used and the developing chamber was a CAMAG glass twin trough chamber (20 x 10 cm). Silica gel covered TLC plates, measuring 20 x10 cm with uniform thickness of 0.2 mm, supported by aluminium sheets were utilized. CAMAG TLC Scanner 3 linked to WINCATS software constitute

densitometer .Toluene: Ethyl acetate: Formic acid (5:4:1) was utilized as mobile phase and Silica Gel 60 F254 was taken as stationary phase [21,22].

**Results and Discussion**

GC-MS study of methanolic extract of B. *tomentosa* pod samples produced a chromatogram with a runtime of 22 minutes as shown in Figure -1 and a list of all the components detected is shown in Table 1. HPTLC peak reports were obtained by running seven standards as gallic acid, quercetin, tannic acid, rutin, dl-alfa tocopherol, ascorbic acid , and carotene and the results were depicted in the form of peak reports observed as shown in Figure 2 - Figure 8 and peak report of *B. tomentosa* pod methanolic extract is shown in Figure 9. HPTLC results of seven standards and *B. tomentosa* pod methanolic extract at 254 nm and 366 nm is shown in Figure 10 and Figure 11 respectively depicting the fingerprint for the standardisation of the bioactive compounds.

GC-MS chromatogram of B. *tomentosa* pod revealed 57 peaks, out of which the presence of 56 different compounds having retention time more than five minutes are depicted in table 1. Identification of the active components in the *B.tomentosa pod's* methanolic extract was performed by comparing their peak area percentage, mass area fragmentation pattern and retention indices, with database from NIST (National Institute of Standard and Technology) and Pubchem. The relative percentage amount of every chemical constituent was calculated by juxtaposing its average peak area to the total observed peak areas. Database from NIST (National Institute of Standard and Technology) was considered for performing interpretation on GC-MS of *B. tomentosa* pod extract and Table 1 shows the list of the constituent compounds observed. Muscalure has been reported in pubchem to be used as an active ingredient for making pesticides. Almost all the bioactive constituents reported in *B.tomentosa* pod extract possess the property of being used in Perfume industry and as flavoring agents. Megastigmatrienone, also called as "tabanone", a volatile compound was reported Ohloff et al.in 1985 [23] to be key ingredient responsible for flavor of tobacco [24] and latterly it has been quantified in wines also [25]. Caryophyllene has been reported in the leaves *B.rufa* and *B. dumosa* [26]. Eicosane possess anti-analgesic and anti-pyretic properties [11,27]. Caprylic acid was reported in *Bryonia dioica* and *Galium aparine* [28] .Caprylic acid helps in treatment of Crohn's disease. It is an essential ingredient in products meant for skin care [29,30].

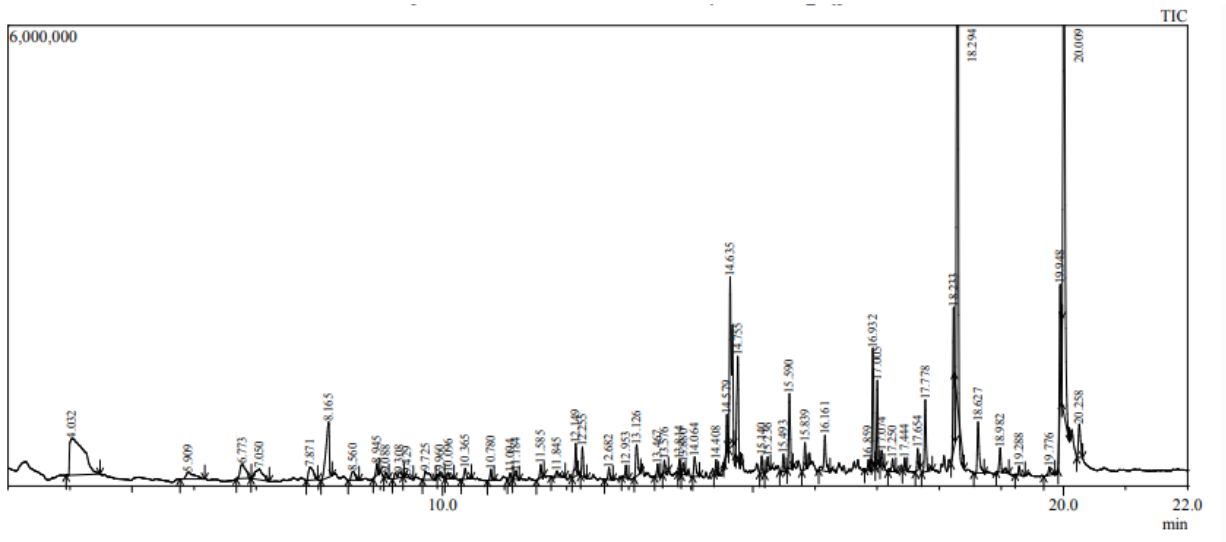
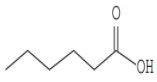
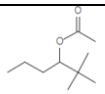
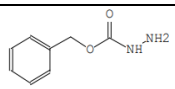
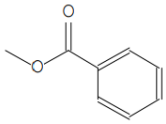
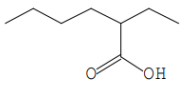
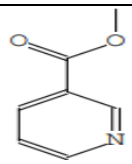
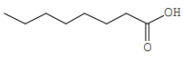
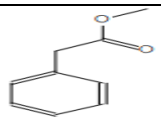
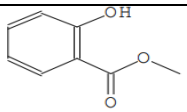
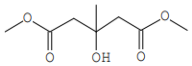
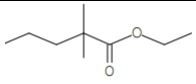
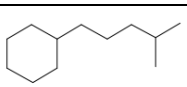
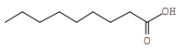
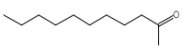
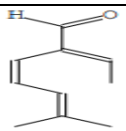
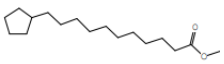
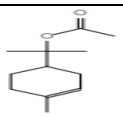
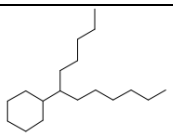
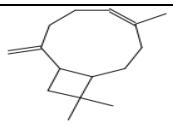
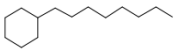
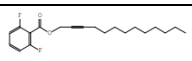
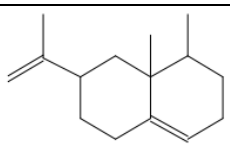
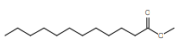
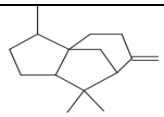
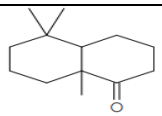
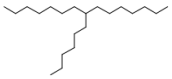
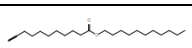
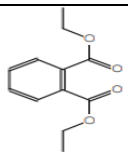
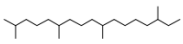
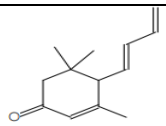
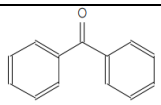
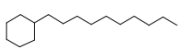
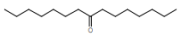
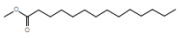
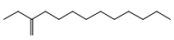
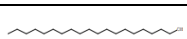
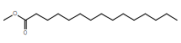
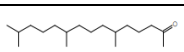
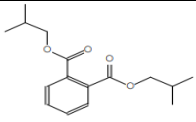


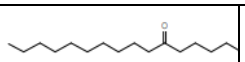
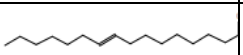
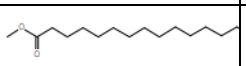
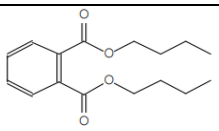
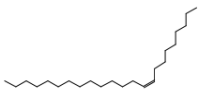
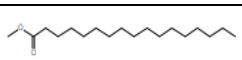
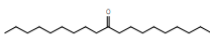
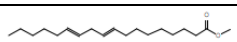
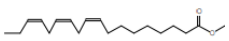
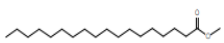

**Figure 1 Chromatogram of methanolic extract of pod of *B. tomentosa***

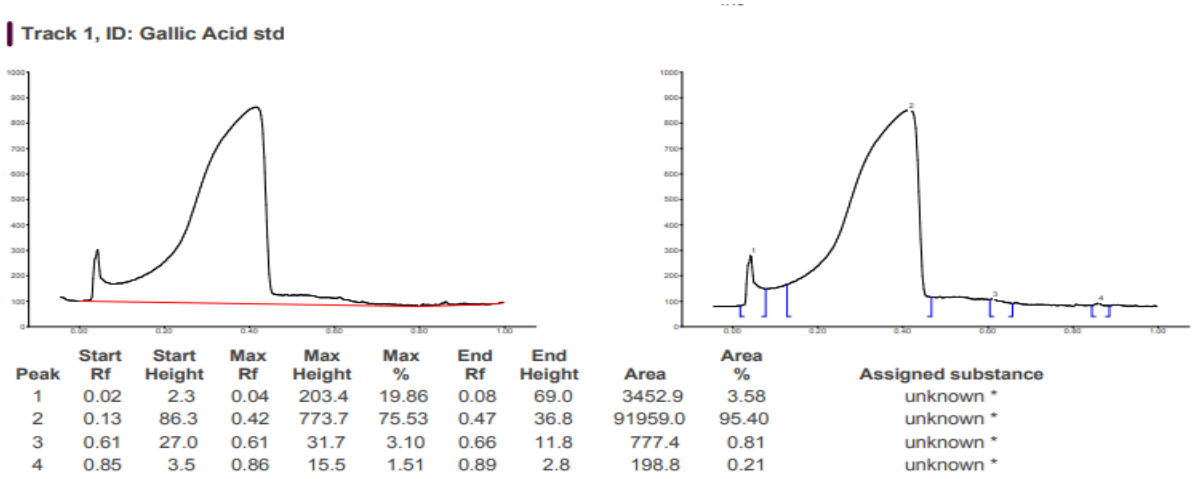
**Table 1 Compounds detected in GC-MS of Methanolic extract of *B.tomentosa* pod**

| S.No | Compound Name | Molecular structure | Retention time | Molecular weight | Molecular formula | Area % | CAS No. |
|---|---|---|---|---|---|---|---|
| 1. | Caproic acid |  | 5.909 | 116 | $C_6H_{12}O_2$ | 0.94 | 142-62-1 |
| 2. | 2,2-Dimethyl-3-hexanol acetate |  | 6.773 | 172 | $C_{10}H_{20}O_2$ | 1.31 | 4166-44-3 |
| 3. | Hydrazinecarboxylic acid , phenylmethyl ester |  | 7.050 | 166 | $C_8H_{10}N_2O_2$ | 1.35 | 5331-43-1 |
| 4. | Clorius |  | 7.871 | 136 | $C_8H_8O_2$ | 1.05 | 93-58-3 |
| 5. | Hexanoic acid, 2-ethyl- |  | 8.165 | 144 | $C_8H_{16}O_2$ | 3.13 | 149-57-5 |
| 6. | Methyl nicotinate |  | 8.560 | 137 | $C_7H_7NO_2$ | 0.53 | 93-60-7 |
| 7. | n-Caprylic acid |  | 8.945 | 144 | $C_8H_{16}O_2$ | 0.32 | 124-07-2 |
| 8. | Benzeneacetic acid, methyl ester |  | 9.088 | 150 | $C_9H_{10}O_2$ | 0.21 | 101-41-7 |
| 9. | Hexadecen-1-ol |  | 9.308 | 240 | $C_{16}H_{32}O$ | 0.49 | 64437-47-4 |
| 10. | Methyl salicylate |  | 9.429 | 152 | $C_8H_8O_3$ | 0.15 | 119-36-8 |
| 11. | Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate |  | 9.725 | 190 | $C_7H_{12}O_5$ | 0.74 | 19020-62-3 |
| 12. | Pentanoic acid,2,2-dimethyl-ethyl ester |  | 9.960 | 158 | $C_9H_{18}O_2$ | 0.12 | 5837-93-4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13. | Cyclohexane,(4-methylpentyl)- |  | 10.096 | 168 | $C_{12}H_{24}$ | 0.24 | 61142-20-9 |
| 14. | Nonanoic acid |  | 10.365 | 158 | $C_9H_{18}O_2$ | 0.40 | 112-05-0 |
| 15. | 2-Undecanone |  | 10.780 | 170 | $C_{11}H_{22}O$ | 0.31 | 112-12-9 |
| 16. | 3,5-Heptadienal,2-ethylidene-6-methyl- |  | 11.094 | 150 | $C_{10}H_{14}O$ | 0.21 | 99172-18-6 |
| 17. | Methyl dihydrohydnocarpate |  | 11.184 | 268 | $C_{17}H_{32}O_2$ | 0.39 | 25779-85-5 |
| 18. | Alpha-Terpinyl acetate |  | 11.585 | 196 | $C_{12}H_{20}O_2$ | 0.28 | 80-26-2 |
| 19. | Dodecane, 6- cyclohexyl- |  | 11.845 | 252 | $C_{18}H_{36}$ | 0.25 | 13151-86-5 |
| 20. | 1-Tridecene |  | 12.149 | 182 | $C_{13}H_{26}$ | 0.95 | 2437-56-1 |
| 21. | Tetradecane |  | 12.255 | 198 | $C_{14}H_{30}$ | 0.71 | 629-59-4 |
| 22. | Caryophyllene |  | 12.682 | 204 | $C_{15}H_{24}$ | 0.34 | 87-44-5 |
| 23. | Cyclohexane, Octyl- |  | 12.953 | 196 | $C_{14}H_{28}$ | 0.31 | 1795-15-9 |
| 24. | 7-Heptadecene,1-chloro- |  | 13.126 | 272 | $C_{17}H_{33}Cl$ | 1.14 | 556554-78-0 |
| 25. | 2,6-Difluorobenzoic acid, tridec-2-ynyl ester |  | 13.467 | 336 | $C_{20}H_{26}F_2O_2$ | 0.37 | - |
| 26. | Eremophila-1(10),11-diene |  | 13.576 | 204 | $C_{15}H_{24}$ | 0.19 | 10219-75-7 |
| 27. | Lauric acid , methyl ester |  | 13.814 | 214 | $C_{13}H_{26}O_2$ | 0.31 | 111-82-0 |
| 28. | Cedrene |  | 13.880 | 204 | $C_{15}H_{24}$ | 0.34 | 11028-42-5 |

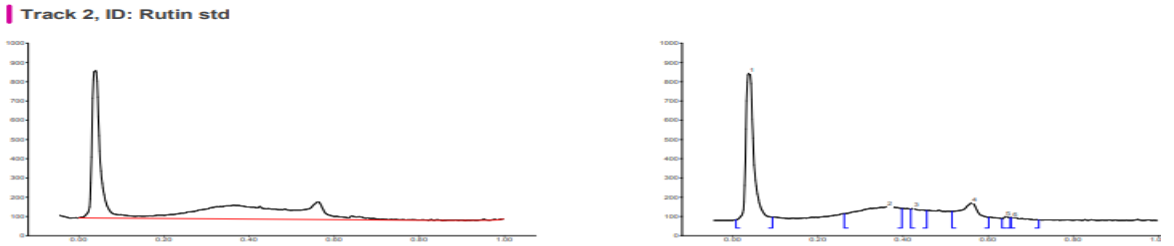
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 29. | 5,5.8a-Trimethyldecalin-1-one |  | 14.064 | 194 | $C_{13}H_{22}O$ | 0.69 | - |
| 30. | Pentadecane,8- hexyl |  | 14.408 | 296 | $C_{21}H_{44}$ | 0.30 | 13475-75-7 |
| 31. | Undec-10-ynoic acid, undecyl ester |  | 14.579 | 336 | $C_{22}H_{40}O_2$ | 0.55 | - |
| 32. | Diethyl Phthalate |  | 14.635 | 222 | $C_{12}H_{14}O_4$ | 6.66 | 84-66-2 |
| 33. | Heptadecane, 2,6,10,15-tetramethyl- |  | 14.755 | 296 | $C_{21}H_{44}$ | 2.66 | 54833-48-6 |
| 34. | Megastigmatrienone |  | 15.140 | 190 | $C_{13}H_{18}O$ | 0.45 | 38818-55-2 |
| 35. | Benzophenone |  | 15.236 | 182 | $C_{13}H_{10}O$ | 0.47 | 119-61-9 |
| 36. | Cyclohexane, decyl- |  | 15.493 | 224 | $C_{16}H_{32}$ | 0.37 | 1795-16-0 |
| 37. | Caprylone |  | 15.590 | 226 | $C_{15}H_{30}O$ | 1.86 | 818-23-5 |
| 38. | 1,19- Eicosadiene |  | 15.839 | 278 | $C_{20}H_{38}$ | 0.72 | 14811-95-1 |
| 39. | Methyl tetradecanoate |  | 16.161 | 242 | $C_{15}H_{30}O_2$ | 1.31 | 124-10-7 |
| 40. | Tridecane, 3- methylene- |  | 16.859 | 196 | $C_{14}H_{28}$ | 0.20 | 19780-34-8 |
| 41. | 1- Nonadecanol |  | 16.932 | 284 | $C_{19}H_{40}O$ | 2.53 | 1454-84-8 |
| 42. | Eicosane |  | 17.005 | 282 | $C_{20}H_{42}$ | 1.89 | 112-95-8 |
| 43. | Dodecyl propyl ether |  | 17.074 | 228 | $C_{15}H_{32}O$ | 0.46 | - |
| 44. | Pentadecanoic acid, methyl ester |  | 17.250 | 256 | $C_{16}H_{32}O_2$ | 0.46 | 7132-64-1 |
| 45. | 2- Pentadecanone, 6,10,14-trimethyl- |  | 17.444 | 268 | $C_{18}H_{36}O$ | 0.32 | 502-69-2 |
| 46. | 1,2- Benzenedicarboxylic acid, bis( 2-methyl propyl) ester |  | 17.654 | 278 | $C_{16}H_{22}O_4$ | 0.53 | 84-69-5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 47. | 8- Octadecanone |  | 17.778 | 268 | $C_{18}H_{36}O$ | 2.12 | 79246-41-6 |
| 48. | Methyl hexadec-9-enoate |  | 18.233 | 268 | $C_{17}H_{32}O_2$ | 3.44 | 10030-74-7 |
| 49. | Hexadecanoic acid, methyl ester |  | 18.294 | 270 | $C_{17}H_{34}O_2$ | 20.10 | 112-39-0 |
| 50. | Dibutyl phthalate |  | 18.627 | 278 | $C_{16}H_{22}O_4$ | 1.79 | 17851-53-5 |
| 51. | Muscalure |  | 18.982 | 322 | $C_{23}H_{46}$ | 0.85 | 27519-02-4 |
| 52. | Heptadecanoic acid, methyl ester |  | 19.288 | 284 | $C_{18}H_{36}O_2$ | 0.22 | 1731-92-6 |
| 53. | 10- Nonadecanone |  | 19.776 | 282 | $C_{19}H_{38}O$ | 0.32 | 504-57-4 |
| 54. | 9,12-Octadecadienoic acid, methyl ester |  | 19.948 | 294 | $C_{19}H_{34}O_2$ | 1.96 | 2462-85-3 |
| 55. | Linolenic acid, methyl ester |  | 20.009 | 292 | $C_{19}H_{32}O_2$ | 19.56 | 301-00-8 |
| 56. | Methyl stearate |  | 20.258 | 298 | $C_{19}H_{38}O_2$ | 1.25 | 112-61-8 |



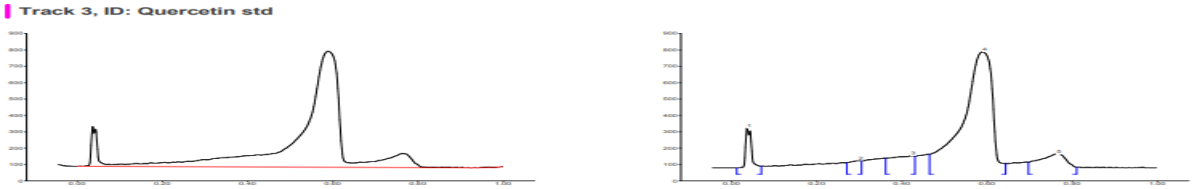

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.02 | 2.3 | 0.04 | 203.4 | 19.86 | 0.08 | 69.0 | 3452.9 | 3.58 | unknown * |
| 2 | 0.13 | 86.3 | 0.42 | 773.7 | 75.53 | 0.47 | 36.8 | 91959.0 | 95.40 | unknown * |
| 3 | 0.61 | 27.0 | 0.61 | 31.7 | 3.10 | 0.66 | 11.8 | 777.4 | 0.81 | unknown * |
| 4 | 0.85 | 3.5 | 0.86 | 15.5 | 1.51 | 0.89 | 2.8 | 198.8 | 0.21 | unknown * |

**Figure 2 Peak report for Gallic acid Standard**



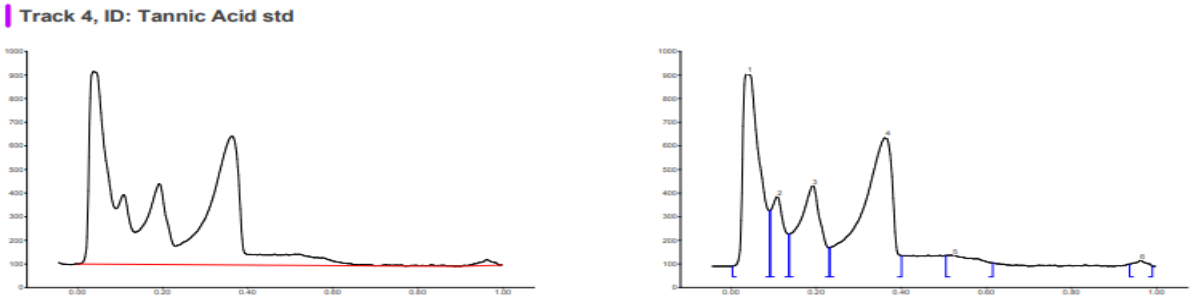

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 3.9 | 0.04 | 765.9 | 74.24 | 0.09 | 17.3 | 11341.0 | 51.89 | unknown * |
| 2 | 0.26 | 35.5 | 0.36 | 71.6 | 6.94 | 0.40 | 62.7 | 5374.8 | 24.59 | unknown * |
| 3 | 0.42 | 58.9 | 0.43 | 65.4 | 6.34 | 0.46 | 51.9 | 1468.9 | 6.72 | unknown * |
| 4 | 0.52 | 47.7 | 0.56 | 91.8 | 8.90 | 0.60 | 16.2 | 3064.7 | 14.02 | unknown * |
| 5 | 0.63 | 9.5 | 0.64 | 20.4 | 1.98 | 0.65 | 14.2 | 218.0 | 1.00 | unknown * |
| 6 | 0.66 | 14.4 | 0.66 | 16.5 | 1.60 | 0.72 | 2.7 | 387.1 | 1.77 | unknown * |

**Figure 2 Peak report for Rutin Standard**



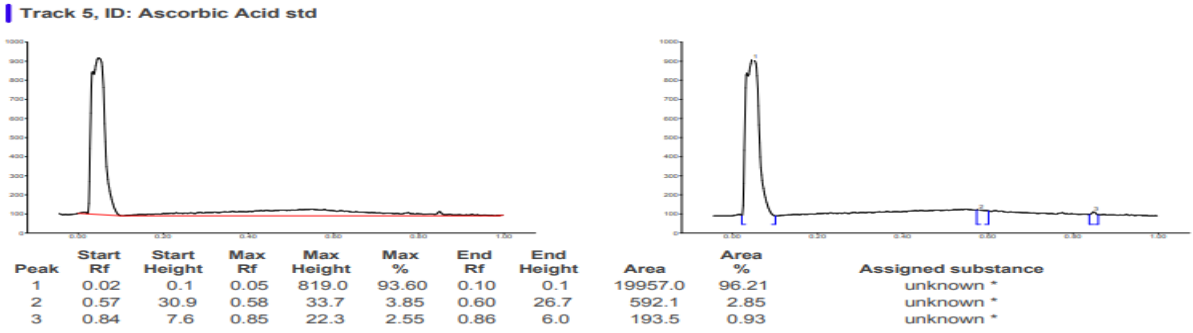

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 0.2 | 0.04 | 241.6 | 20.93 | 0.07 | 11.0 | 2566.4 | 5.44 | unknown * |
| 2 | 0.27 | 32.6 | 0.30 | 44.2 | 3.83 | 0.31 | 43.0 | 948.3 | 2.01 | unknown * |
| 3 | 0.36 | 59.1 | 0.42 | 74.3 | 6.44 | 0.43 | 72.9 | 3152.6 | 6.69 | unknown * |
| 4 | 0.47 | 83.3 | 0.59 | 707.9 | 61.33 | 0.64 | 26.9 | 36379.8 | 77.17 | unknown * |
| 5 | 0.70 | 35.0 | 0.77 | 86.2 | 7.47 | 0.81 | 4.7 | 4093.6 | 8.68 | unknown * |

**Figure 3 Peak report for Quercetin Standard**



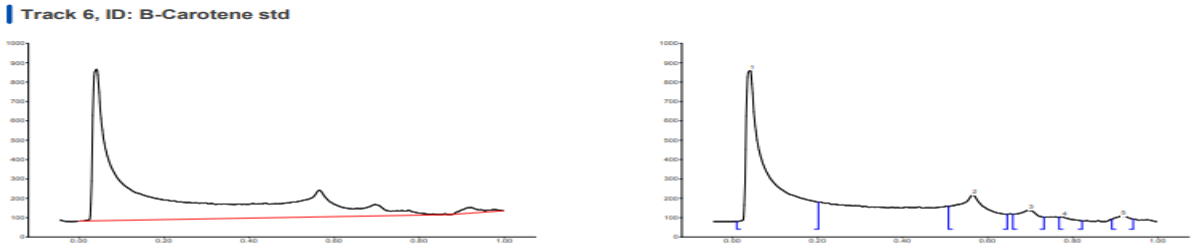

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.1 | 0.04 | 816.5 | 39.39 | 0.09 | 235.9 | 24822.3 | 32.52 | unknown * |
| 2 | 0.09 | 236.9 | 0.11 | 295.4 | 14.25 | 0.14 | 136.1 | 6731.2 | 8.82 | unknown * |
| 3 | 0.14 | 136.4 | 0.19 | 342.4 | 16.52 | 0.23 | 78.4 | 13088.6 | 17.15 | unknown * |
| 4 | 0.23 | 79.2 | 0.36 | 545.4 | 26.31 | 0.40 | 44.5 | 28614.6 | 37.49 | unknown * |
| 5 | 0.51 | 45.8 | 0.52 | 47.6 | 2.30 | 0.62 | 14.7 | 2534.7 | 3.32 | unknown * |
| 6 | 0.94 | 9.4 | 0.96 | 25.6 | 1.23 | 0.99 | 0.3 | 533.7 | 0.70 | unknown * |

**Figure 4 Peak reports for Tannic acid Standard**



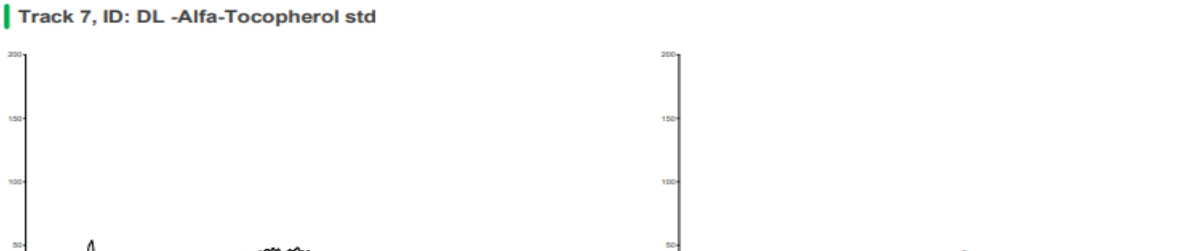

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.02 | 0.1 | 0.05 | 819.0 | 93.60 | 0.10 | 0.1 | 19957.0 | 96.21 | unknown * |
| 2 | 0.57 | 30.9 | 0.58 | 33.7 | 3.85 | 0.60 | 26.7 | 592.1 | 2.85 | unknown * |
| 3 | 0.84 | 7.6 | 0.85 | 22.3 | 2.55 | 0.86 | 6.0 | 193.5 | 0.93 | unknown * |

**Figure 5 Peak reports Ascorbic acid Standard**




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 0.2 | 0.04 | 783.3 | 75.73 | 0.20 | 100.7 | 29123.8 | 72.64 | unknown * |
| 2 | 0.51 | 78.9 | 0.57 | 137.2 | 13.26 | 0.65 | 38.3 | 7532.2 | 18.79 | unknown * |
| 3 | 0.66 | 38.9 | 0.70 | 58.7 | 5.68 | 0.73 | 23.0 | 2148.5 | 5.36 | unknown * |
| 4 | 0.77 | 21.8 | 0.78 | 26.0 | 2.51 | 0.82 | 4.0 | 523.2 | 1.31 | unknown * |
| 5 | 0.89 | 12.1 | 0.92 | 29.2 | 2.82 | 0.94 | 13.4 | 763.1 | 1.90 | unknown * |

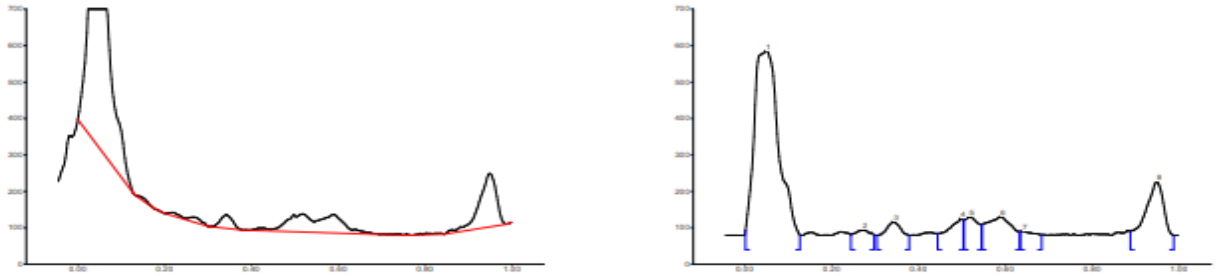
**Figure 6 Peak report for Beta carotene Standard**




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 | 0.6 | 0.04 | 28.6 | 48.32 | 0.05 | 10.0 | 375.7 | 26.40 | unknown * |
| 2 | 0.54 | 28.1 | 0.55 | 30.6 | 51.68 | 0.62 | 9.2 | 1047.6 | 73.60 | unknown * |

**Figure 8 Peak report for DL-Alfa- Tocopherol Standard**

Track 9, ID: BTP Methanol



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 13.2 | 0.05 | 506.8 | 58.66 | 0.13 | 1.0 | 21769.7 | 69.02 | unknown * |
| 2 | 0.24 | 2.4 | 0.27 | 14.2 | 1.65 | 0.30 | 0.5 | 315.3 | 1.00 | unknown * |
| 3 | 0.30 | 0.2 | 0.34 | 37.4 | 4.33 | 0.38 | 0.3 | 815.4 | 2.59 | unknown * |
| 4 | 0.44 | 5.0 | 0.50 | 46.9 | 5.43 | 0.50 | 41.0 | 937.0 | 2.97 | unknown * |
| 5 | 0.51 | 41.6 | 0.52 | 49.6 | 5.74 | 0.55 | 29.1 | 1159.9 | 3.68 | unknown * |
| 6 | 0.55 | 29.7 | 0.59 | 50.8 | 5.88 | 0.63 | 9.9 | 2023.4 | 6.42 | unknown * |
| 7 | 0.64 | 10.0 | 0.64 | 12.1 | 1.40 | 0.69 | 1.5 | 221.2 | 0.70 | unknown * |
| 8 | 0.89 | 10.8 | 0.95 | 146.2 | 16.92 | 0.99 | 0.1 | 4297.6 | 13.63 | unknown * |

**Figure 9 Peak report for BTP MeOH**

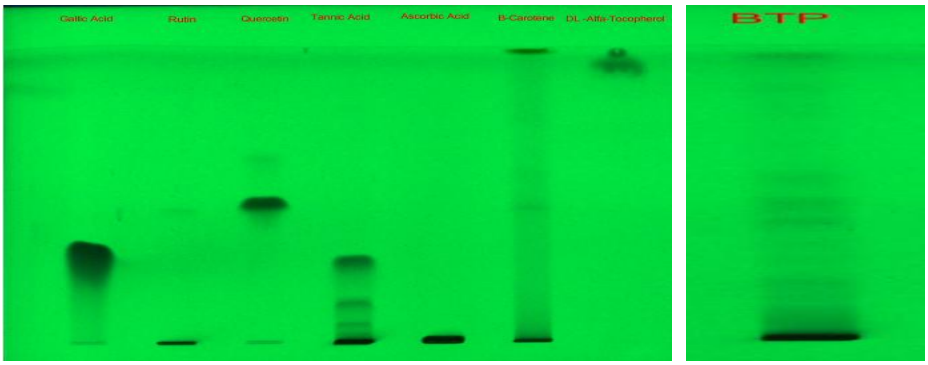



**Figure 10 Image formation at 254 nm**


**Figure 11 Image formation at 366 nm**

## Conclusion

Gas chromatography Mass spectrometry (GCMS) is the most frequently employed analytical technique for detecting organic substances in complex compounds. This study presents a straightforward approach to interpret the results of GC-MS analysis. HPTLC has proven to be a highly effective technique for analyzing and detecting specific phytochemicals in various parts of plants. Understanding the findings from GC-MS and HPTLC analysis is useful for forecasting the chemical formula and structure of multiple compounds within the methanolic extract of *B. tomentosa*. Hence, it is believed that this research article can be considered for justification of phytochemicals in *B. tomentosa*. The identification of numerous bioactive compounds in this plant, conducted for the first time in its pod, signifies its potential as a valuable medicinal component in Ayurveda. It would help in carrying out further research related to isolation, identification, and structure elucidation of pure bioactive compounds like megastigmatrienone, caproic acid, clorius, methyl nicotinate, n-caprylic acid, caryophyllene, cedrene, caprylone, eicosane and muscalure from *B.tomentosa* pods. Analyzing its pharmacological activity would provide encouraging insights for expediting drug development and could serve as a pathway for future studies.

## Acknowledgement

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