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GC-MS studies on variation in the content of major chemical constituents of *Artemisia annua* L. collected from different geographical regions of India

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Abstract

GC-MS analysis of *Artemisia annua* L. leaves samples were done for the samples collected from different geographical in India: A-1 (Uttar Pradesh), A-2 (Jaora, Madhya Pradesh), A-3 (Uttarakhand), A-4 (Baroda, Gujarat), A-5 (Andhra Pradesh), A-6 (Karakhadi, Gujarat). Only five major components, in each geographical area, have been analyzed for their mass-spectrometric fragmentation pattern for identification of major components present in extract samples. Components detected were identified by comparing mass spectral data of samples with those of NIST and NBS standard database hits provided with GC-MS analysis. All the samples differed in their chemical constituents quantitatively. All the samples showed the presence of Artemisinin and deoxyartemisinin. Despite unanimous presence of Artemisinin and deoxyartemisinin, both the compounds differed quantitatively in every sample. Dihydroartemisinic acid, dihydroartemisinic alcohol, artemisinin and deoxyartemisinin were present in extracts of the samples collected from Uttar Pradesh (A-1), Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3), Baroda, Gujarat (A-4) and Kharkhedi, Gujarat (A-6), in addition extract from Uttar Pradesh (A-1) showed the presence of α-amyrin, while extracts collected from Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3) and Baroda, Gujarat (A-4) showed dihyroartemisinic aldehyde, and extract of sample collected Kharkhedi, Gujarat (A-6) showed dihydroartemisinic acid. Comparatively, samples collected from Andhra Pradesh (A-5) and Kharkhedi, Gujarat (A-6) showed clear deviation in chemical constituents from rest of samples. α-amyrin was present in two samples (A-1 and A-5) while β-amyrin is present only in one sample (A-5) which was collected from Andhra Pradesh. Artemisinin content was highest in sample collected from Uttar Pradesh.

Keywords: GC-MS studies, *Artemisia annua*, geographical regions

Introduction

Artemisia (*Artemisia annua* L.) is an annual medicinal and aromatic herb that belongs to the family Asteraceae [1]. It has originated in China and currently distributed in North America, Europe, Africa, Asia, South America and Australia [2, 3]. It is grown for its aromatic and medicinal leaves, which yields artemisinin and essential oil. Artemisinin has been proven to be potent and effective medicine for the treatment of malaria including cerebral malaria and multi drug-resistance Plasmodium falciparum [4, 5]. In addition, medicinal herbal tea can be prepared from dried leaf of A.annua for the treatment of malaria without negative side effect [6]. Malaria is one of the most important tropical diseases, causing great human suffering and loss of life in the affected countries. The conventional anti-malaria drugs such as Chloroquine and Fansidar have become almost ineffective because of the development of resistance by plasmodium species to these drugs [7]. The World Health Organization has thus recommended that all countries experiencing resistance to use combination therapies preferably antimalaria medicines in combination with artemisinin derivatives for Plasmodium falciparum [8]. Not only malaria but also some other medical problems such as haemorrhoids, bronchitis, bilharzias and certain forms of cancer can be treated with A.annua tea and showed good results [6]. Moreover, it is widely used as antibacterial agents and natural pesticides. Apart from artemisinin, essential oils are another active research interests from A. annua as it could be potentially used in perfumery, cosmetics and aromatherapy [9]. The oil has been also reported to possess antimycotic and antimicrobial activities [9]. Artemisinin and essential oil levels in the leaves of A. annua ranges from 0.01 to 1.4 % [10, 11] and 0.04 to 1.9 % [12], respectively. The contents are affected by numerous factors such as geographical conditions, harvesting time, temperature, fertilizer application, population density and age of harvesting [10].

The aim of the present study is to find out how the geographic location influences on the qualitative and quantitative composition of secondary metabolites of n-hexane extracts obtained from defatted *Artemisia annua* L. leaves, collected from different geographical location in India. In this paper, we aimed to monitor geographical area induced changes in the major metabolites profiles of plant A. annua, therefore, we have taken 6 sample of A. annua leaves from six different geographical locations of India and extracted out medium-polar part for GC-MS analysis. Interpretation of five major gas-chromatography detected peaks performed by submitting those peaks to a gas-chromatography coupled with electron impact mass spectrometry equipment.

Materials and methods

1. Plant material authentication and collection of samples

The aerial part (leaves) of *Artemisia annua* L. plants were collected from different locations of India. Green leaves of *Artemisia annua* L. were collected from 10 different geographical places in India: A-1 (Uttar Pradesh), A-2 (Jaora, Madhya Pradesh), A-3 (Uttarakhand), A-4 (Baroda, Gujarat), A-5 (Andhra Pradesh), A-6 (Karakhadi, Gujarat). Field preparation like nursery preparation, transplantation, irrigation, drainage and harvesting time for all the locations were same. Therefore, the phenological stage of the leaves was maintained to be same in all the locations. Hence the variable for the present study was only the geographical location. All the other factors were constant. Collected leaves were shade dried and pulverized to get powder of leaves. The powdered leaves were stored in a close-tight container until used for extraction.

2. Chemicals

All the solvents used in this study were of analytical grade and were purchased from S. D. Fine Chemicals, Mumbai (India).

3. Extraction of dry leaves samples

Extraction of leaves powder (1g) performed by soxhlet extraction by using n-hexane as per the reported method [13], obtained n-hexane extract was concentrated to dryness under vacuum on a rotary evaporator. The same procedure was adopted for the extraction of all the six leaves samples collected from different geographical locations of India. The sample extracts thus prepared were stored in sealed vials at 4°C until GC-MS analysis.

4. Sample preparation for GC-MS analysis

Extract samples, stored under refrigeration condition, were taken out and allowed to get normalized with the atmospheric temperature. A part of extract was dissolved in chloroform and 1µl volume was injected for GC-MS analysis.

5. Instrument and gas-chromatographic-mass spectrometric conditions

GC-MS analysis was carried out using a Perkin-Elmer Auto System XL gas chromatograph coupled to a Turbo Mass spectrometer Detector (MSD). Volatiles were separated on fused silica capillary column (30m length, 250 µm diameter, 0. 5µm thickness). The oven temperature was set at 70°C and hold for 5 minutes, then raised to 280°C at the rate of 10°C/minute and hold at this temperature for 20 minutes. The on-column injector (injection volume 1µl) was heated up to 250°C. The detector's EI source temperature was 220°C and helium gas was used as a carrier gas at rate of 1ml/min. 1µl volumes were injected in split less mode. Peak areas and retention times were measured through electronic integration. The EI-energy was 70 eV. EI-mass spectra were recorded in the 20–620 amu range.

6. Identification and quantification of components

Components detected were identified by comparing mass spectral data of samples with those of NIST and NBS standard database hits provided with GC-MS analysis. The name, molecular weight and structure of the compounds present in the test sample extracts were ascertained by comparing the mass spectra, mass fragmentation pattern with the known compounds either using computer searches on NIST and NBS library or with the help of published literature. The quantitative estimation of each peak obtained in GC was made by computer attached with GC-MS instrument.

7. GC-MS analysis data from different samples of *Artemisia annua* leaves

GC-MS analysis of n-hexane extract samples, of different geographical locations, revealed presence of many components but we have monitored only major components present in the extract samples. Therefore, only five major components, in each geographical area, have been analyzed for their mass-spectrometric fragmentation pattern for identification of major components present in extract samples. Details of GC-MS analysis like, sample I.D., peak area %, retention time and mass fragmentation have been shown in Table 1. Total ion chromatograms of sample1 is shown in Figure 1 (A-1).

Table 1: GC-MS Analysis data of n-hexane extract of *Artemisia annua* (Leaves) collected from different locations of India

Sample I.D.	Major peak no.	Peak area, %	Retention time (minutes)	Mass fragmentation
A-1 (Uttar Pradesh)	1	20.32	22.903	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282, 284, 309, 327, 341, 386 and 399.
	2	13.55	24.260	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 137, 149, 150, 151, 166, 179, 204, 222, 223, 224, 248, 264, 283, 295, 308, 332, 368, 376, 424, 437 and 468.
	3	11.20	21.858	43 (100%), 43, 55, 67, 69, 81, 93, 95, 107, 123, 137, 151, 166, 178, 180, 208, 234, 236, 237, 238, 250, 279, 292, 322, 349, 354, 432, 438.
	4	8.71	22.62	43 (100%), 55, 67, 79, 81, 93, 124, 135, 150, 151, 164, 165, 166, 179, 195, 210, 224, 225, 266, 273, 295, 327, 350, 385, 394, 442, and 490.
	5	6.46	39.77	41, 55, 69, 81, 95, 109, 122, 133, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 298, 313, 315, 339, 368, 381, 409, 424, 425, 426, 474, 501, 536, 579, and 584.
A-2 (Jaora, Madhya Pradesh)	1	14.67	22.903	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282, 284, 309, 327, 341, 386 and 399.
	2	8.40	21.858	41, 43 (100%), 55, 67, 69, 81, 95, 122, 123, 137, 151, 166, 180, 193, 208, 209, 234, 236, 237 and 238.

	3	7.58	22.628	41, 43, 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165 (100%), 166, 179, 195, 210, 224, 225, 266, 275, 295, 306, 327, 355, 413, 484 and 487.
	4	7.32	24.241	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 137, 150, 151, 166, 179, 204, 222, 223, 224.
	5	6.30	20.042	41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218, 236, 250, 294, 309, 346, 381, 394 and 489.
A-3 (Uttarakhand)	1	15.63	22.866	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 165, 166, 167, 178, 192, 193, 211, 222, 223, 239, 282, 284, 306, 343, 346, 379, 414, 428, 472 and 477.
	2	13.62	20.043	41, 43, 55, 67, 77, 79, 91, 107, 121, 133, 147, 162 (100), 163, 175, 190, 218, 219, 236, 264, 294, 297, 331, 363, 367, 382, 432, 464 and 476.
	3	9.85	24.205	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 137, 150, 151, 166, 179, 204, 222, 223, 224.
	4	9.06	22.609	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 195, 210, 224, 225, 266, 273, 312, 319, 369, 394, 396, 417 and 461.
	5	8.59	21.784	41, 43 (100%), 55, 67, 69, 81, 95, 107, 123, 135, 151, 166, 178, 180, 193, 208, 218, 219, 236, 237, 264, 281, 314, 341, 415, 426, 439, 464 and 475.
A-4 (Baroda, Gujarat)	1	17.70	22.866	41, 43, 55, 67, 69, 79, 96, 109, 137 (100%), 165, 166, 167, 192, 193, 211, 222, 239, 254, 282, 295, 305, 413, 467 and 487.
	2	10.02	24.204	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 150, 151, 166, 179, 204, 222, 223, 236, 264, 283, 295, 329, 341, 361, 379, 417, 420, 433, 450 and 477.
	3	8.85	21.784	41, 43 (100%), 55, 67, 69, 81, 93, 95, 107, 123, 135, 151, 166, 178, 180, 193, 208, 218, 219, 236, 237, 250, 281, 297, 334, 346, 376, 400, 426, 435, 466 and 475 and 480.
	4	8.56	22.609	41, 43 (100%), 55, 67, 79, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 177, 195, 210, 224, 225, 238, 266, 282, 296, 318, 344, 364, 387, 446, 450 and 483.
	5	6.66	20.006	41, 53, 55, 67, 77, 79, 91, 93, 107, 121, 133, 147, 162 (100), 163, 164, 175, 218 (M^+-2), 236, 265, 281, 298, 345, 352, 368, 399, 414, 459, 464, 469 and 499.
A-5 (Andhra Pradesh)	1	13.84	22.59	41, 43 (100%), 55, 67, 79, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 177, 195, 210, 224, 225, 238, 266, 283, 303, 319, 345, 364, 394, 412, 450, 466, 469, and 474
	2	10.61	39.68	41, 43, 55, 67, 69, 81, 93, 95, 109, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 311, 313, 314, 341, 381, 406, 409, 425, 426, 465, and 475.
	3	10.52	21.381	41, 43, 55, 60, 71, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 228, 256, 257, 276, 314, 327, 352, 376, 406, 415, 419, 464, 495.
	4	9.08	22.83	41, 43, 55, 67, 69, 81, 96, 109, 137 (100%), 166, 167, 192, 193, 211, 222, 232, 264, 282, 305, 319, 364, 376, 414, 426, 464 and 472.
	5	7.01	38.28	41, 43, 55, 67, 69, 81, 93, 95, 109, 119, 121, 135, 137, 147, 149, 175, 189, 203, 218 (100%), 219, 231, 257, 271, 285, 299, 325, 339, 355, 406, 425, 426, 476, and 484.
A-6 (Kharkhedi, Gujarat)	1	8.85	22.848	41, 43, 55, 67, 69, 81, 95, 96, 109, 137 (100%), 165, 166, 167, 193, 211, 232, 239, 282, 304, 319, 345, 350, 376, 411 and 447.
	2	8.11	21.784	41, 43 (100%), 55, 67, 69, 81, 95, 107, 123, 135, 151, 166, 178, 180, 208, 218, 236, 237, 250, 281, 286, 341, 355, 388, 407, 415, 436, 452 and 477.
	3	7.96	22.609	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 167, 195, 196, 224, 225, 238, 266, 283, 307, 312, 355, 385, 400 and 415.
	4	7.19	24.186	41, 43 (100%), 55, 56, 67, 79, 81, 93, 95, 122, 150, 151, 166, 179, 204, 222, 223, 224, 264, 283, 295, 320, 332, 365, 391, 408, 433, 450 and 489.
	5	6.98	20.024	41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218, 236, 250, 294, 309, 346, 381, 394 and 489.

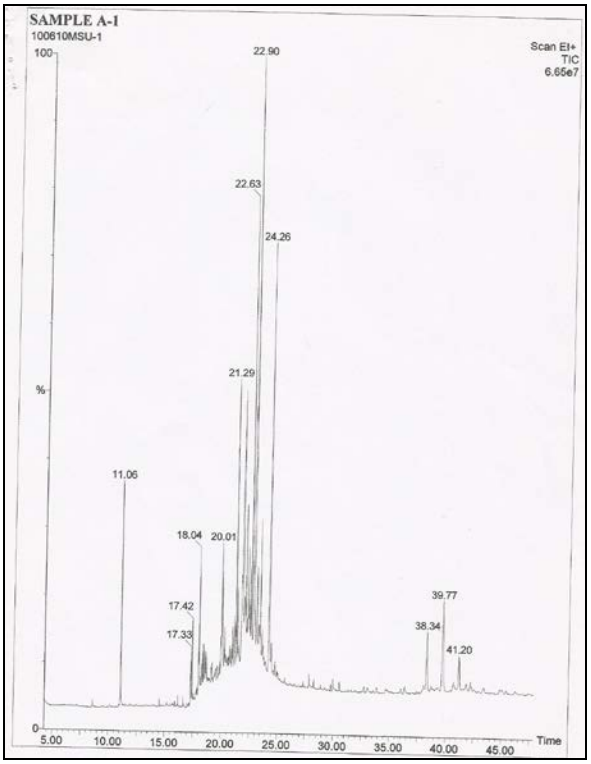


Fig 1: Total ion chromatogram of sample A1

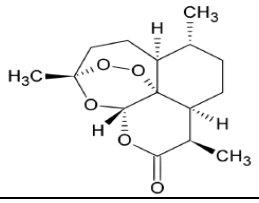
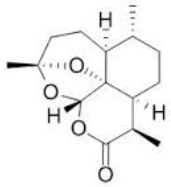
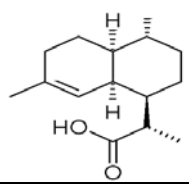
Results and discussion

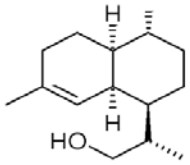
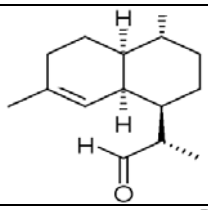
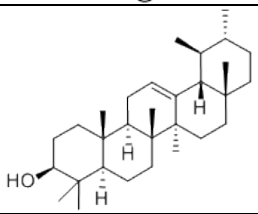
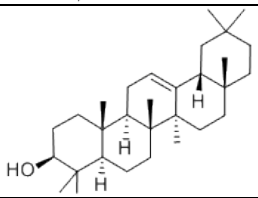
Systematic investigation of mass spectrometric fragmentations of major detected components and their retention times (in minutes) indicated that almost all samples are chemically similar, except A-5, but they differ in the percentage contents of components present.

Total 8 major components present in the leaves were identified, in all six samples collected from different geographical area, with the help of hits (NIST and NBS library) provided with GC-MS analysis, retention times and fragmentation patterns, which are given in Table 2. Identified components in the samples collected from different geographical area and their percentage content in extracts are shown in Figure 2. GC/EI-MS of the identified compounds are shown in Figure 3 (A to H).

Total eight major components (constituents or compounds) were present in all six samples collected from different geographical area. The comparison of samples was based only on the chemical constituents of higher quantity considering their retention time and percentage area. Every sample differed in their chemical constituents quantitatively, as indicated from area percentage value. Sample collected from Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3) and Baroda, Gujarat (A-4) were almost chemically similar. All the samples showed the presence of Artemisinin (average retention time is 22.87 minutes) and deoxyartemisinin (average retention time is 22.61 minutes). In spite of unanimous presence of Artemisinin and deoxyartemisinin, both the compounds differed quantitatively in every sample. Dihydroartemisinic acid, dihydroartemisinic alcohol, artemisinin and deoxyartemisinin were present in extracts of the samples collected from Uttar Pradesh (A-1), Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3), Baroda, Gujarat (A-4) and Kharkhedi, Gujarat (A-6), in addition extract from Uttar Pradesh (A-1) showed the presence of α-amyrin, while extracts collected from Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3) and Baroda, Gujarat (A-4) showed dihyroartemisinic aldehyde, and extract of sample collected Kharkhedi, Gujarat (A-6) showed dihydroartemisinic acid. Comparatively, samples collected from Andhra Pradesh (A-5) and Kharkhedi, Gujarat (A-6) showed clear deviation in chemical constituents from rest of samples. α-amyrin was present in two samples (A-1 and A-5) while β-amyrin is present only in one sample (A-5) which was collected from Andhra Pradesh. Artemisinin content was highest in sample collected from Uttar Pradesh.

Table 2: Major components identified through GC-MS

Components identified	Average peak area, %	Average retention time (minutes)	Compound name and structure	Molecular formula	Molecular weight	Mass fragmentation	Ref.
Artemisinin	14.375	22.87		$C_{15}H_{22}O_5$	282	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282 (M^+), 284, 309, 327, 341, 386 and 399.	14
Deoxyartemisinin	9.29	22.61		$C_{15}H_{22}O_4$	266	43 (100%), 55, 67, 79, 81, 93, 124, 135, 150, 151, 164, 165, 166, 179, 195, 210, 224, 225, 266 (M^+), 273, 295, 327, 350, 385, 394, 442, and 490.	15
Dihydroartemisinic acid	9.03	21.81		$C_{15}H_{24}O_2$	236	43 (100%), 43, 55, 67, 69, 81, 93, 95, 107, 123, 137, 151, 166, 178, 180, 208, 234, 236 (M^+), 237 (M^++1), 238 (M^++2), 250, 279, 292, 322, 349, 354, 432, 438.	16

Dihydroartemisinic alcohol	9.59	24.22		$C_{16}H_{26}O$	222	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 137, 149, 150, 151, 166, 179, 204, 222 (M^+), 223 (M^++1), 224 (M^++2), 248, 264, 283, 295, 308, 332, 368, 376, 424, 437 and 468.	16
Dihydroartemisinic aldehyde	8.39	20.03		$C_{15}H_{24}O$	220	41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218 (M^+-2), 236, 250, 294, 309, 346, 381, 394 and 489.	16
α-Amyrin	8.54	39.72		$C_{30}H_{50}O$	426	41, 43, 55, 67, 69, 81, 93, 95, 109, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 311, 313, 314, 341, 381, 406, 409, 425 (M^+-1), 426 (M^+), 465, and 475.	17
β-Amyrin	7.01	32.29		$C_{30}H_{50}O$	426	41, 43, 55, 67, 69, 81, 93, 95, 109, 119, 121, 135, 137, 147, 149, 175, 189, 203, 218 (100%), 219, 231, 257, 271, 285, 299, 325, 339, 355, 406, 425 (M^+-1), 426 (M^+), 476, and 484.	17
Tritetracontanol	10.52	21.38	CH_3-$(CH_2)_{32}$-CH_2OH	$C_{34}H_{70}O$	494	41, 43, 55, 60, 71, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 228, 256, 257, 276, 314, 327, 352, 376, 406, 415, 419, 464 (M^++H-C_2H_5OH), 495 (M^++H).	--

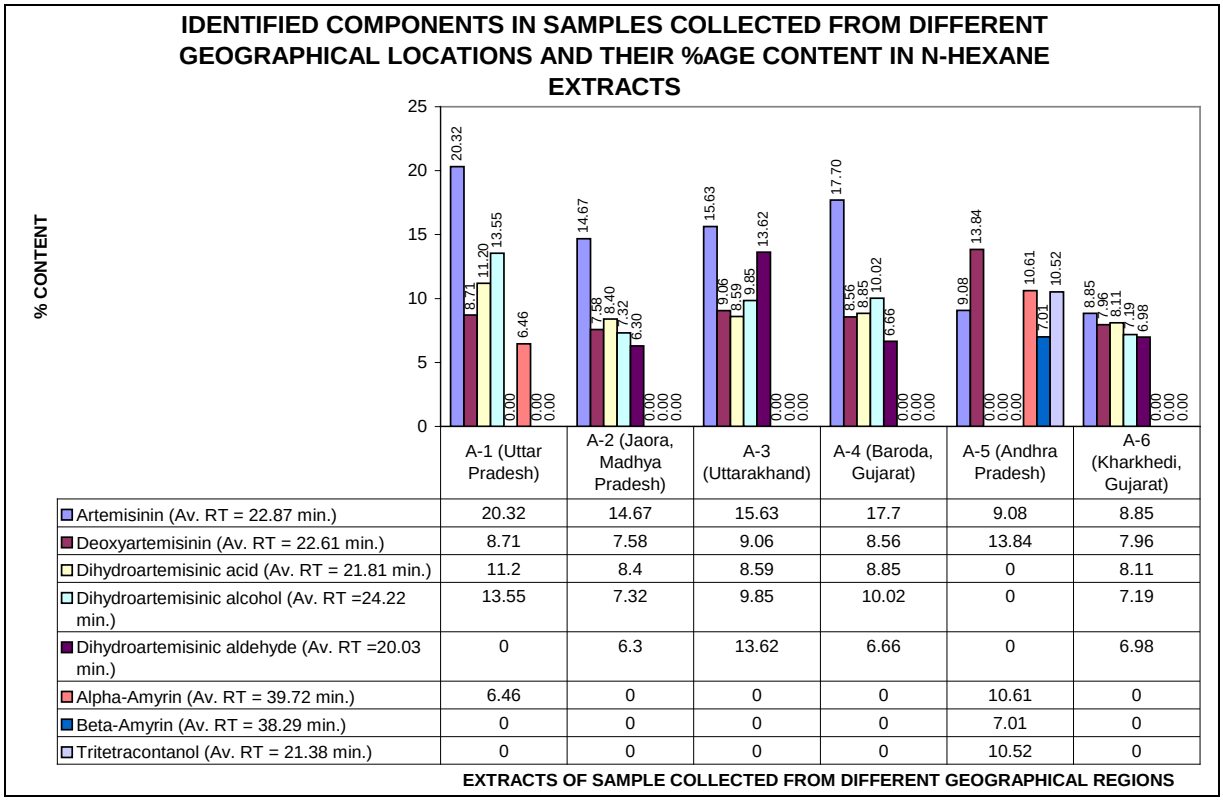


Fig 2: Identified components in the samples collected from different geographical area and their percentage content in extracts

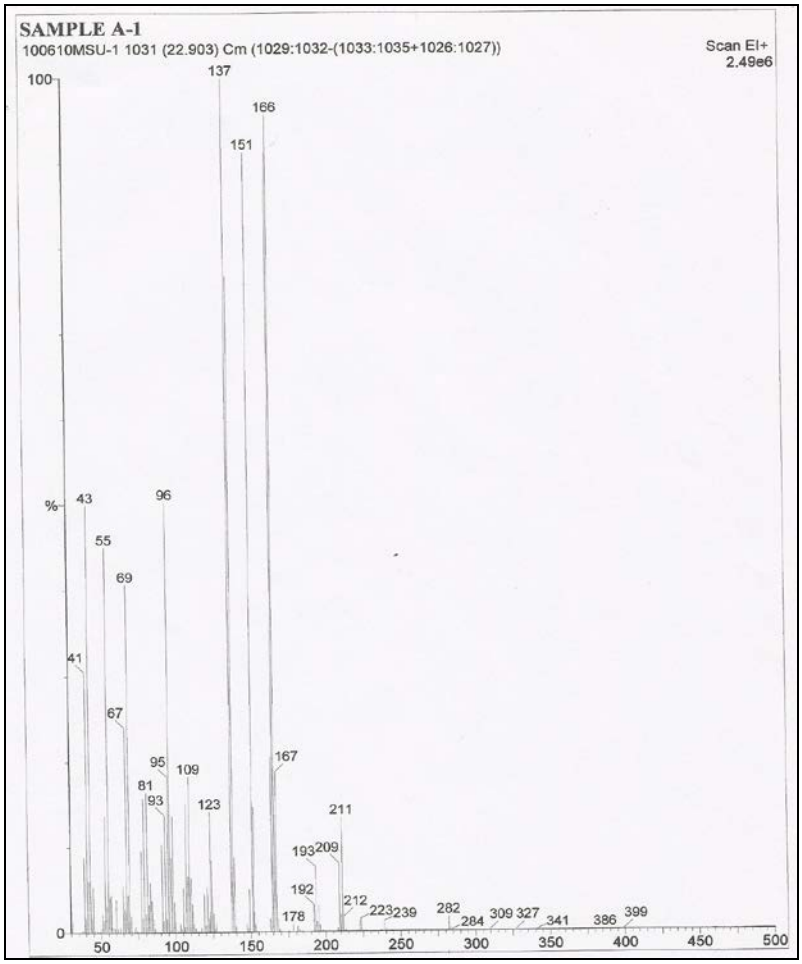


Fig 3 (A): GC/EI-MS of Artemisinin

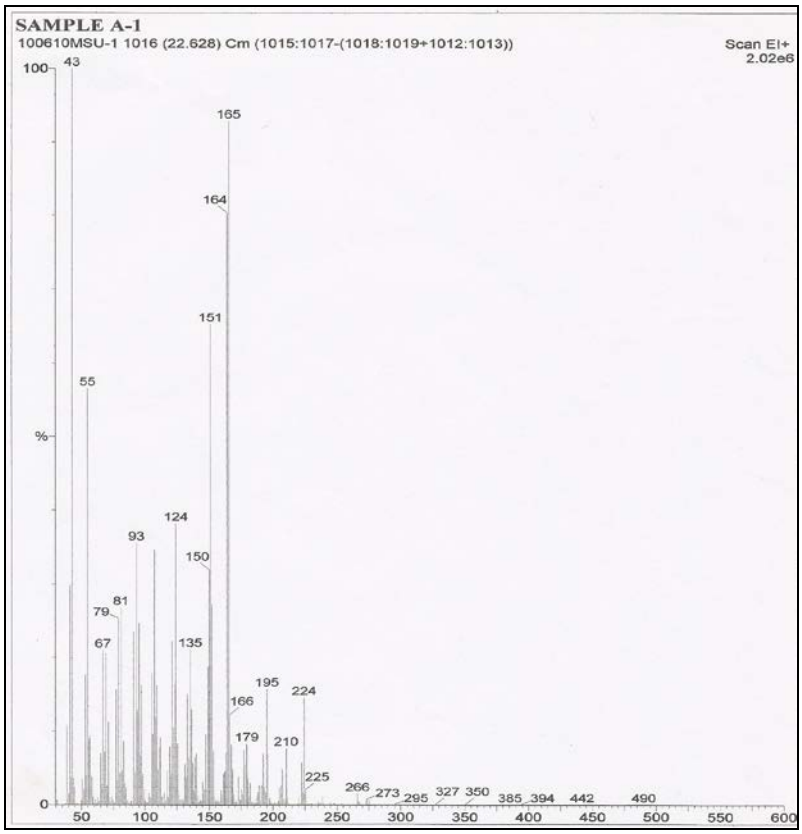


Fig 3(B): GC/EI-MS of Deoxyartemisinin

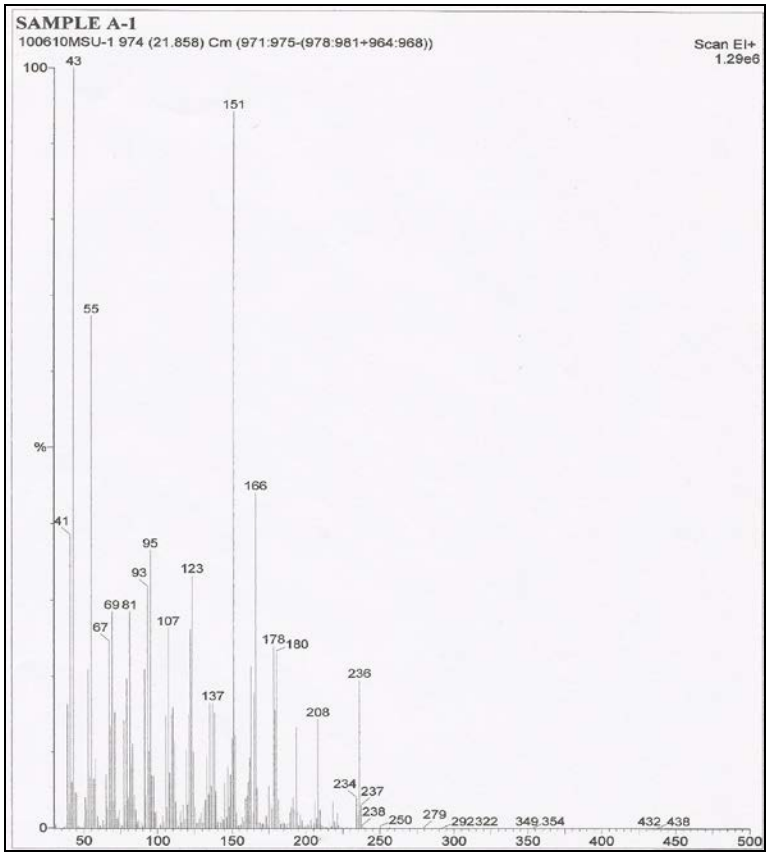


Fig 3 (C): GC/EI-MS of Dihydroartemisinic acid

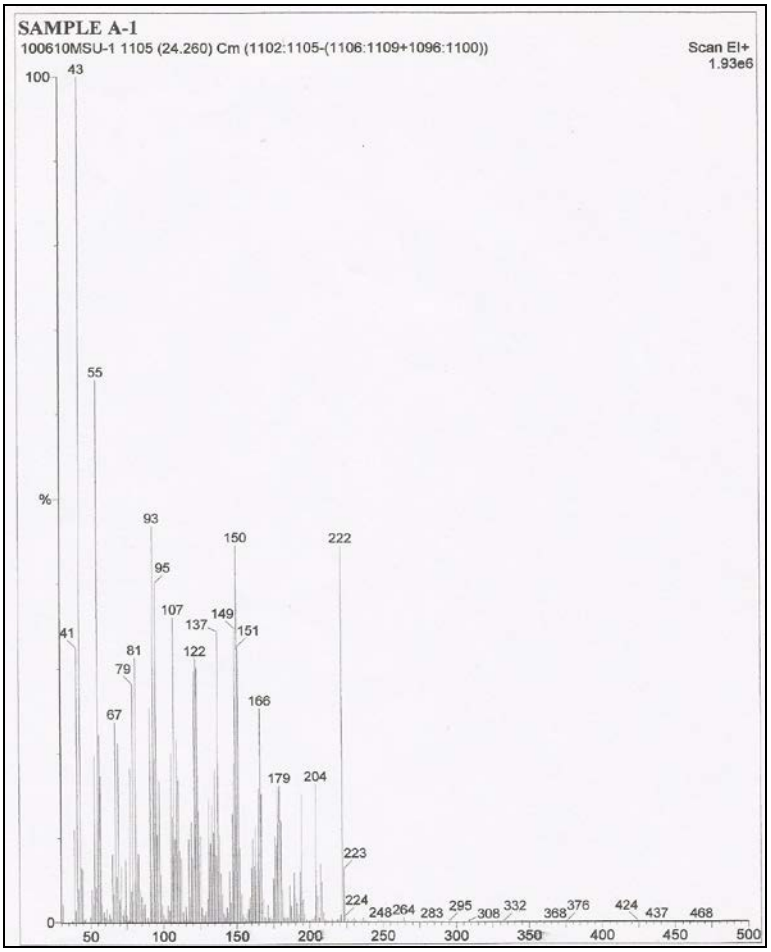


Fig 3 (D): GC/EI-MS of Dihydroartemisinic alcohol

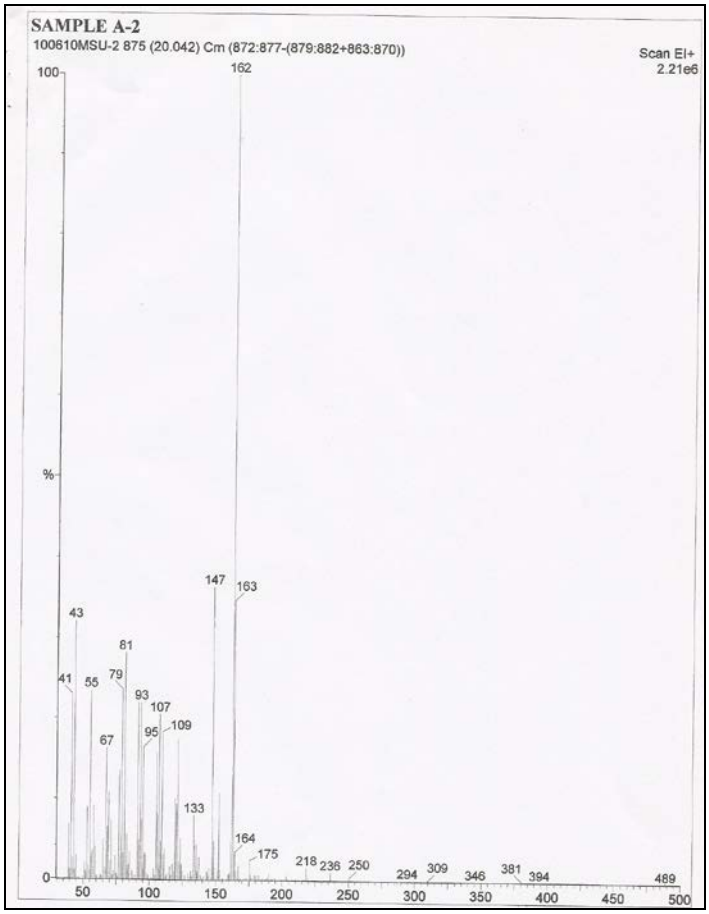


Fig 3 (E): GC/EI-MS of Dihydroartemisinic aldehyde

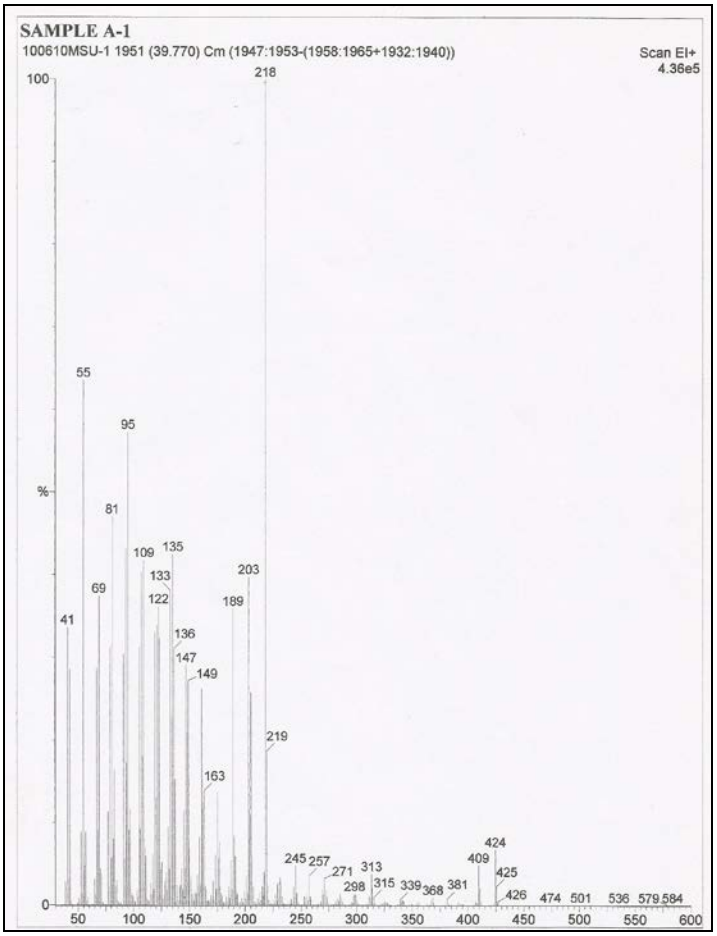


Fig 3 (F): GC/EI-MS of α-Amyrin

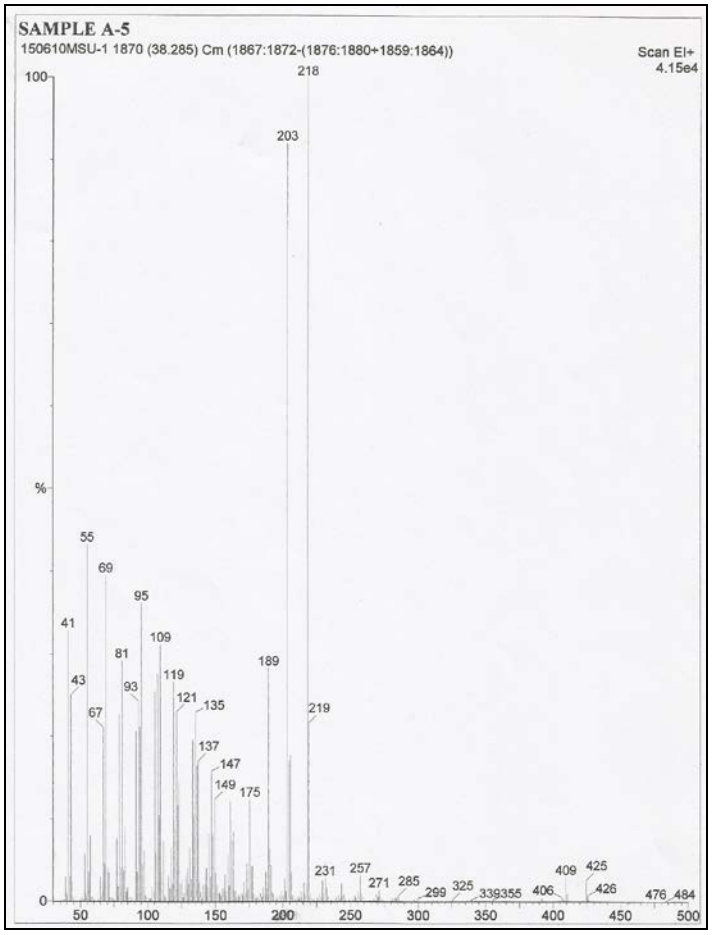


Fig 3 (G): GC/EI-MS of β-Amyrin

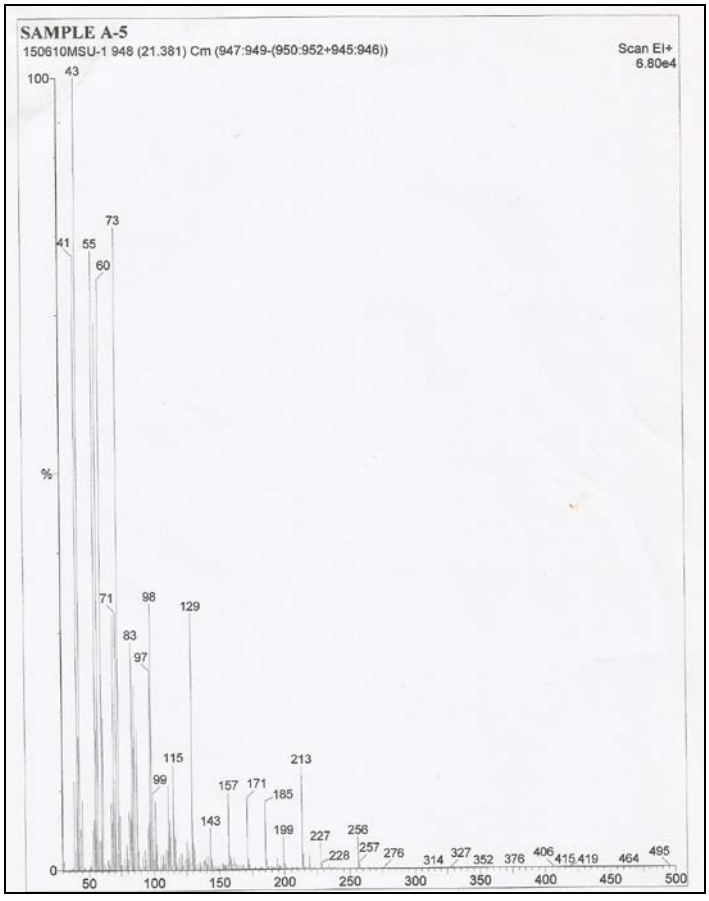


Fig 3 (H): GC/EI-MS of Tritetracontanol

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