

Results

And

Discussion

4.1:- MACROMORPHOLOGY

Standardization of herbal drugs is based on the correct identification of plant and macroscopic characterization. The interpretation of the morphological characters plays important role in drug analysis, its commercial significance and distinction between their adulterants. Thus the targeted endemic plants *Tephrosia jamnagarensis* Santapau and *T. collina* V.S Sharma were studied for morphological characters. The characters observed were compared with the specimens of Santapau (1958), Ahulwalia and Smith (1967) and Sharma (1963). The detail morphological characters are as follows:-

Tephrosia jamnagarensis Sant

Synonym: *Tephrosia axillaris* A.R.Sm.

Habit: Annual herb with height of about 60-82 cm (Fig 4.1A).

Stem: Simple or sparsely branched (Fig 4.1B) covered with densely appressed silky hairs, erect or sub erect cylindrical (Fig 4.2A).

Leaves: Simple, 3.0-6.4 cm long, 6-9.5 mm board, stipules subulate, up to 3mm long, uninerved, pubescent on margin; shortly petiolate up to 2-4 mm with silky appressed hair; lamina oblong –linear or elliptic linear, rounded to attenuate at the base, subacute-obtuse at apex, rarely mucronate, adaxially sub glabrous (Fig 4.2B) and silky villous abaxially (Fig 4.2C), lateral nerve 25-30, margin entire, reticulate venation with alternate phyllotaxy.

Inflorescence: Axillary cyme (flowers in single or pairs or in groups of three) (Fig 4.2D).

Flower: Mauve, 6.5mm long, shortly pedicels (2-3 mm), bracteates (1 mm), ebracteolate, pentamerous, zygomorphic, hermaphrodite (Fig 4.2E).

Calyx: 3.5 mm long, gamosepalous, valvate, campanulate with unequal lobes with silky appressed hairs, persistent in fruits (Fig4.1I).

Corolla: Polypetalous, vexillary aestivation with Standard, wing and keel. **Standard (vexillum)** - 4.5 mm broadly obovate to cordate, emarginate at the apex, punctate hairy toward outer side inner surface is glabrous, retuse at base (Fig 4.2F); **Wing (alae)** - 5.5 mm long, oblong, auriculate above the claw, punctate base, pubescent (Fig 4.2G); **Keel (carina)** - 3.4 mm long, auriculate, punctate, the 2 keel are joined at apical portion covering the staminal tube, mostly glabrous and retuse at base (Fig 4.2H); the claws of the wings and keel as long as their laminae.

Androecium: 4 mm long stamina sheath; anther 0.2 mm. long 10 in 9+1 dialdephous conditions, anthers all fertile, ditheous, introse (Fig 4.2I).

Gynoecium: 4 mm long, ovary subsessile, densely pubescent, monocarpellary, syncarpous, unilocular, superior with marginal placentation, **Style-** short glabrous; **Stigma-** capitate (Fig 4.2J).

Legume: 2 X 0.5 cm long, densely and patently hairy with grayish tinge, oblique at both ends, apiculate, number of seed in each pod is 2-6 and each seed is covered by thin membranous endocarp (Fig 4.2K-M).

Seed: 1-3 mm long, 1.5-3 mm broad, roundish elliptical, muddish brown in colour with black ornamentation near hilum on the seed coat. The hilum is circular and black in colour at the level of seed coat. The seed coat is smooth and shiny in the texture (Fig 4.2N-P).

Table 4.1.1 Gross seed production in *T. jamnagarensis*

Category	Range of Production
No of fruit /plant	60-65
No of seeds/ pod	2-6
No of seeds/ plant	120-390
Weight of 10 seeds	0.0682g
Weight of seeds/ plant	0.84-2.73 g

Flower-fruiting:-August end –October.



A -Habit



B-Branching



C- Flower



D-Pods

Fig 4.1 Habit of *Tephrosia jammagarensis* Sant

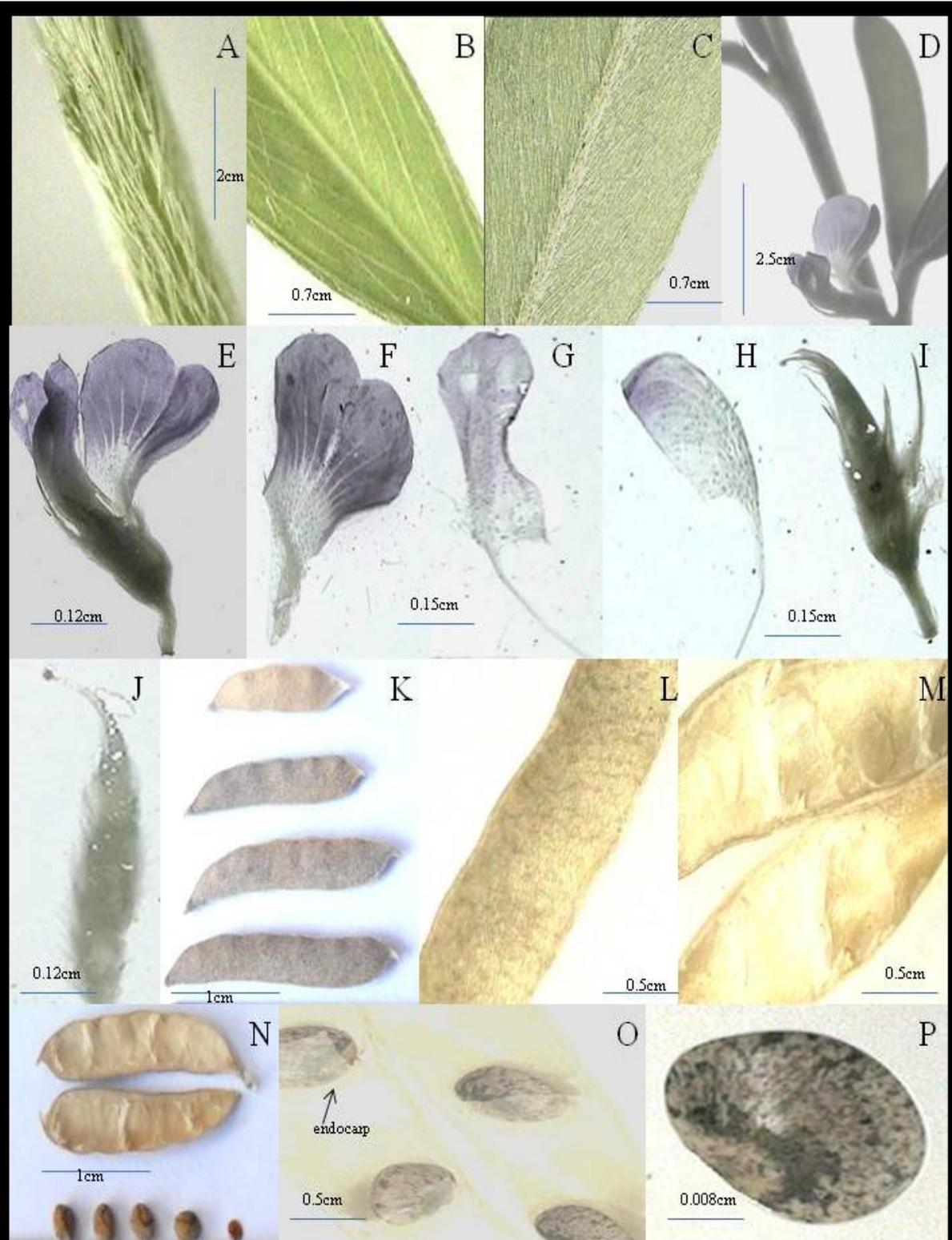


Fig 4.2 Detail Morphological Characters of *Tephrosia jamnagarensis*:-

A- stem, B- adaxial leaves portion, C- abaxial leaves portion, D- inflorescence, E- flower, F- standard, G- wing, H- keel, I- Staminal sheath, J- Gynoecium, K- different size of pods L- outer surface of pod, M- inner surface of pod, N- pod and seeds, O- seed covered by endocarp in pod, P- seed.

Voucher specimen: India, Gujarat, Jamnagar District: Lalpur Taluka: Khadkhambaliya (Grassland Vidi), 23/12/11, GDK549, GDK 560, Gagandeep BSI/AZC/I12012/Tech/2011-12(Pl. Id)/548 (submitted at Botanical Survey of India, Jodhpur) (Fig 4.3 and 4.4)

Specimen examined: India, Gujarat, Jamnagar District: Lalpur Taluka: Khadkhambaliya (Grassland vidi), 17 Oct 2001, P. S. Nagar 1221, 1222, 1234; India. Gujarat. Surat district : wadhana 4.9.69 Jaimin V. Joshi in BARO herbarium Department of Botany, Faculty of Science The Maharaja Sayajirao University of Baroda; India, Gujarat State, Jamnagar District: Victoria Bridge , 30 Sept. 1963, Ahluwalia 3560 (K), 'Sarpankho' (local name); Rosi-Valsura, I Oct. 1964, Ahluwalia 3816 (K, holotype); India, Gujarat State, Jamnagar District: Rozi16 October 1945 Blatter herbarium Bombay, Santapau 7522.

The comparison with the earlier studies showed that there are variations in the morphological characters. Especially, the variations are found in the floral characters (calyx, corolla) and the endocarp of the seeds. The morphological modification observed in present observation which deviated from the earlier documented data is given in Table 4.1.2

Table 4.1.2 Morphological variation observed in *T. jamnagarensis*

Characters	Santapau (1958)	Ahluwalia and Smith (1967)	Nagar <i>et.al.</i> (2003)	Present study
Height (cm)	-	50	-	60-82
Leaves (cm)	3-5.8 x 0.4-0.7	3.1-6.4 x 0.6-0.95	3-5.2 x 5-9	3.1-6.4 x 0.6-0.95
Petiole (mm)	2-3	1.5-3	2-3	2-3
Bract (mm)	Absent	0.5	Absent	1
Calyx (mm)	2-3	2.5	1-3	3.5
Corolla (mm)	Not seen	Standard-3.5 Wings- 3.8 Keel-2.8	Standard-3 Wings- 2.5 Keel-2.5	Standard-4.5 Wings- 5.5 Keel-3.4
Legume (mm)	20 x 5	1.7-2.8 x 4-5	20 x 5	20 x 5
Seeds (No.)	5-6	2-6	5-6	2-6
Seed (mm)	-	2-5 long	-	1-3 long
Endocarp	-	-	-	Present as thin transparent membrane

Comparative account with other common *Tephrosia* species:

Tephrosia jamnagarensis is closely associated with *T. strigosa*, as both have simple leaf. The two species can be distinguished by the leaf size, shape, flowers and pods having long pedicel, its size and shape.

Organoleptic properties:

Visual inspection provides the simplest and quickest means to establish identity, purity and possible quality. The significant organoleptic properties like color, odor, taste and texture were studied. The organoleptic properties of aerial part and root of *T. jamnagarensis* is given below (Table 4.1.3).

Table 4.1.3 Organoleptic properties of *T. jamnagarensis*

Parameters	Aerial part	Root
Colour	Green	Brownish
Taste	Mucilaginous	No specific
Odor	No Characteristic	No odour
Texture	Fibrous	Fibrous

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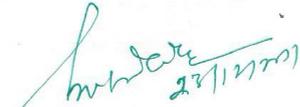
Botanical Survey Of India
Arid Zone Circle
Near Khema ka Kuan
Pal - Basni Canal Link Road
P.O.Nandan Van
JODHPUR-342008 (Rajasthan)

संख्या : भा.व.स./शु.क्षे. परि./
No. BSI/AZC/सं. 12012/Tech./2011-12 (P.I.C.)
सत्यमेव जयते
548

दिनांक/ Date 23.12.11

CERTIFICATE

This is to certify that Plant specimen no. 7 collected by
Dr. P. S. Nagar Assistant Professor, Dept. of Botany, M. S. University of
Baroda (Gujarat) identified as *Tephrosia jamnagarensis* Sant.
belonging to Family: Fabaceae


(Dr. P. M. Padhye)

Scientist "E" & H.O.O

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Fig 4.3 Authentication certificate of *Tephrosia jamnagarensis* Sant.

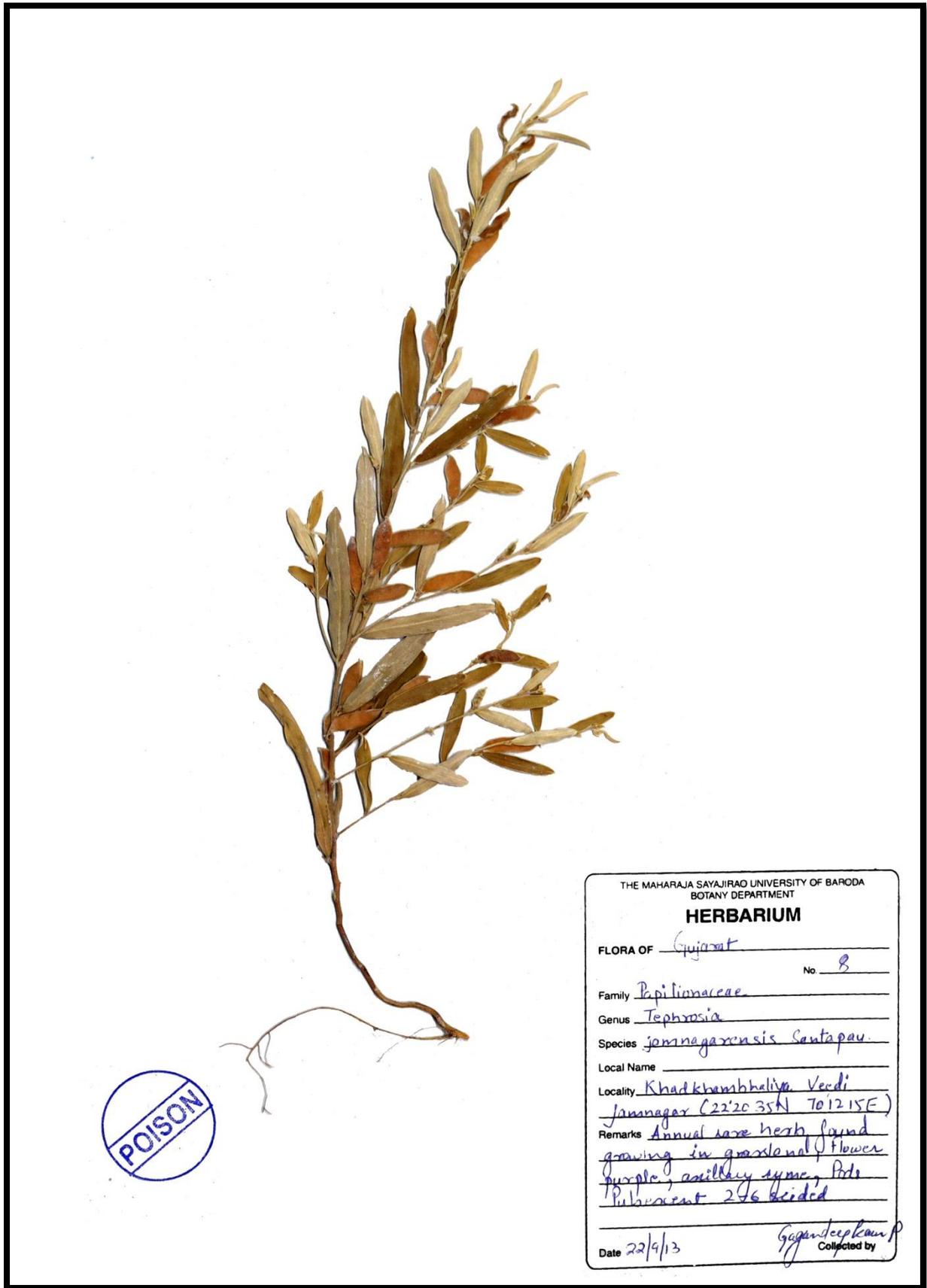


Fig 4.4 Herbarium of *Tephrosia jamnagensis* Sant.

***Tephrosia collina* V.S. Sharma**

Synonym: *Tephrosia collina* var. *lanuginocarpa* V. S. Sharma,

Tephrosia collina var. *collina* V.S. Sharma

Habit: Annual erect herb with height of about 60-100 cm (Fig 4.5 A).

Root: 7-9 cm long, tap roots with many root hairs

Stem: Simple or quite above the base, rarely suffrutescent, terete and thinly pubescent towards base, angled or occasionally subterete, sometime villous and brown/pink striped above, at length sub glabrous (Fig 4.5B).

Leaves: Compound imparipinnate (Fig 4.5B); stipules up to 8mm long, subulate, 3 nerved persistent, leaf-rachis up to 16 cm long. abaxially furrowed, obscurely pulvinate at base; petiolate 1-2.5 cm long, tapering toward apex; leaflets 2-6.5 cm long, 0.5-1.7 cm broad, terminal are usually longer than laterals; 9-11 pairs, opposite or a few casually alternate, the last leaflets shift closure to main axis its distance from other leaflet is 1.3-2.17 cm while that from main axis is 0.2-1.5 cm, lamina of each leaflets is oblong – elliptic, 3-4.5 cm long, 6-9 mm broad, rounded to attenuate at the base, obtuse or somewhat truncately emarginate at apex, rarely mucronate, sericeous abaxially and adaxially glabrous; margin entire, reticulate venation.

Inflorescence: 10-30 cm long, lax terminal racemes, much elongate, pedunculate, 5-9 flowered from usually above the middle or still higher up, 1-2 flowers at each node very rarely at the lowermost node subtended by a small leaf (bract); bracts are smaller than the pedicels, deciduous, similar to stipules (Fig 4.5C).

Flower: Creamy white- sometime with light pink tinch, 1.5-1.7 cm long, bract filiform, bracteolate, shortly pedicel 1.5-2.5 mm densely argenteo-hirsute, zygomorphic, hermaphrodite, pentamerous (Fig 4.5D).

Calyx: 4-5 mm wide, 1.5-3 mm long, gamosepalous, campanulate with unequal lobes appressed with hairs, acuminate, persistent (Fig 4.6B).

Corolla: Polypetalous, vexillary aestivation with Standard, wing and keel. **Standard (vexillum)** -1.5 cm long, 1.2 cm broad, obovate, punctate, unguiculate, abaxially adpressed with silky brown hairs denser and longer in middle, margin ciliolate (Fig 4.6C); **Wing (alae)** -1.4 cm long 4 mm wide, oblong, punctate, eared above claw (Fig 4.6D); **Keel (carina)** -1.5 cm long, 5mm wide, punctate, glabrous, apex retuse with outer straight margin (Fig 4.6E).

Androecium: 1.2 cm long 3 mm broad staminal sheath; filament 2.5-3.5 mm long; **anther** 0.5 mm long 0.2 mm wide 10 in 9+1 diadelphous, fertile, ditheous, introse (Fig 4.6F).

Gynoeceium: 1.3 mm long, ovary sessile, densely pubescent, monocarpellary, unilocular, syncarpous, superior ovary with marginal placentation, **Style-** 2.5 mm long, glabrous; **Stigma** minute, glabrous (Fig 4.6G).

Legume: Light brown, 6.5-8 cm long, 0.6-0.7 cm broad compressed, 8-13 seeded, straight and slightly falcate towards apex, sutures is conspicuously fringed with dull-brown, stiff and almost erect short hairs of uniform length, valves dehiscence twisting completely by 3-4 turns (Fig 4.6H-K).

Seed: Blackish brown in colour with black ornamentation encircling the hilum on the seed coat. The hilum is sub centre positioned on the seed and black in colour. The shape of the hilum was circular and in slight depression on the seed coat. The texture of the seed coat is rough and non-shiny. The shape of seed is oblong with 3 mm- 5 mm in length to 2 mm -3.5 mm in breadth (Fig 4.6L).

Table 4.1.4 Gross seed production of *T. collina*

Category	Range of Production
No of fruits /plant	8-12
No of seeds/ pod	8-13
No of seeds/ plant	64-156
Weight of 10 seeds	0.215g
Weight of seeds/ plant	1.376-3.356 g

Flower-fruiting: August end –November



A - Habit



B – Leaves and leaflet



C –Inflorescences



D – Flower



E –Pods

Fig 4.5 Habit of *Tephrosia collina* Sharma

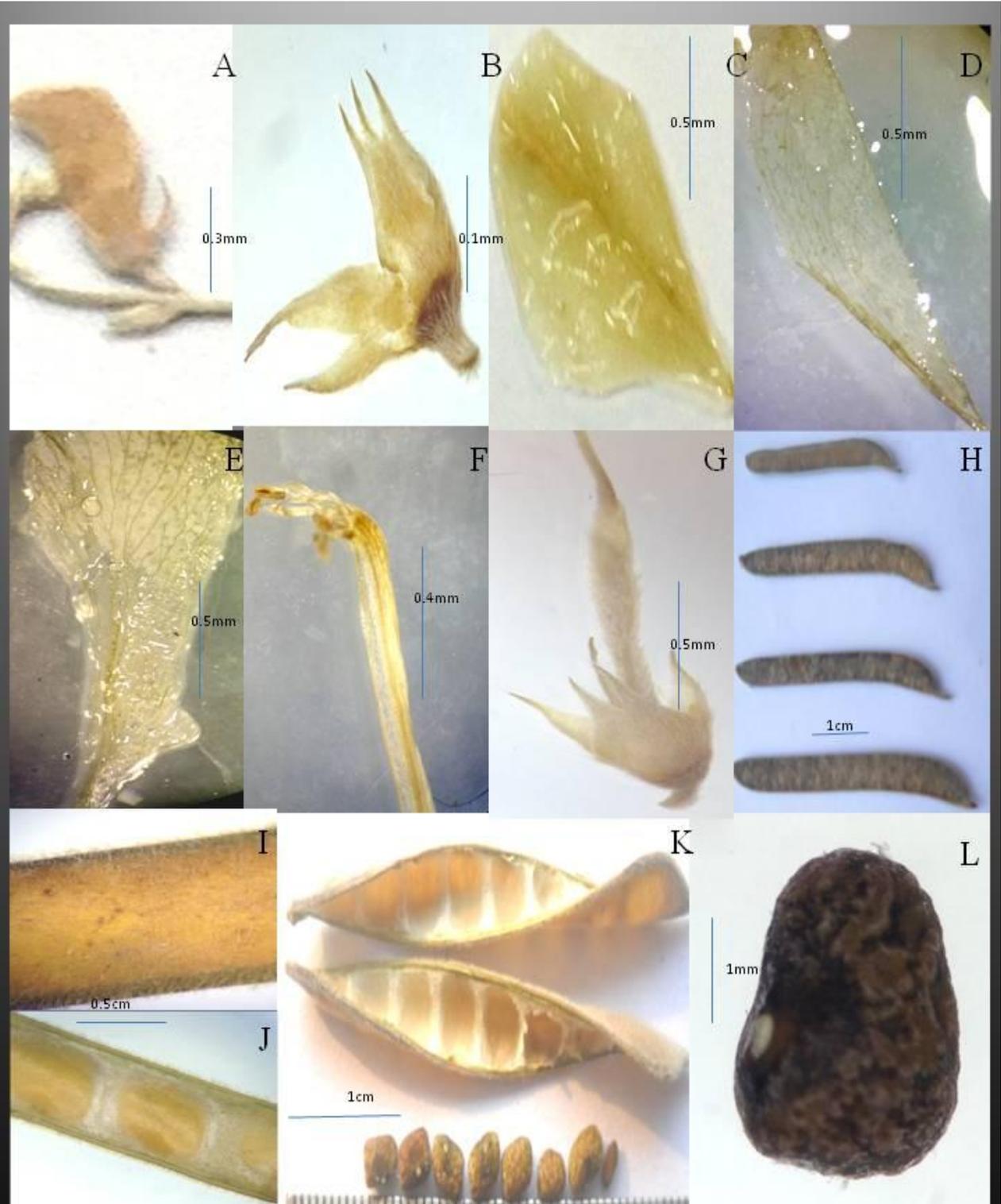


Fig 4.6 Detail Morphological Characters of *Tephrosia collina*:-
 A- flower, B- calyx, C- standard, D- wing, E- keel, F- staminal sheath, G- gynoecium,
 H- different size of pods, I- outer surface of pod, J- inner surface of pod, K- pod and seeds,
 L- seed.

Voucher specimen: India, Gujarat, Jamnagar District: Jamjodhpur Taluka: moti vidi (Grassland vidi), 23/12/11, GDK520, GDK 510, Gagandeep BSI/AZC/I12012/Tech/2011-12(Pl.Id)/550 (submitted at Botanical Survey of India Jodhpur) (Fig 4.7 and 4.8)

Specimen examined: India, Gujarat, Jamnagar District: Jamjodhpur Taluka: Moti Vidi (Grassland vidi), 17 Oct 2001, PSN/BIODIV/MED 1006, 1008, 1009, 1010, 1011, Saurashtra university Rajkot, Gujarat; India, Rajasthan, Ajmer district: Nagpahar Mt. alt. 370-550 m, 4 November 1959, V.S.Sharma1130A(Holotype:NBRI); Nagpahar Mt. alt. 370-550 m, 4 November 1959, V.S.Sharma1130B-C (Isotype of the variety); Ajaysar Gate above Happy Valley 17 September 1958, Sharma 586-B (NBRI).

The morphological variation observed in comparison to earlier studies (Table 4.1.5) showed major differences in the size of rachis and pods.

Table 4.1.5 Morphological variation observed in *T. collina*

Characters	Sharma (1963)	Nagar <i>et.al.</i> (2007)	In study
Height (cm)	30-60	-	60-100
Leaf rachis (cm)	upto15.5	upto 9	upto15.5
Leaflets	9-11	7-9	9-11
Pods (cm)	6.5-8.5 long	3.5-6.5 long	6.5-8 long
Seeds (mm)	3-3.2 broad	2.5-3 broad	2-3.5 broad

Comparative account with other common *Tephrosia* species:

This species of the *Tephrosia* is more similar to *T. villosa* in its morphology. The two species could be differentiated by size of leaves, lax inflorescence, typical white flowers and pubescent pods. Even the number of seeds per pod is 7-11 in *T. collina* where as it is 6-9 in *T. villosa*.

Organoleptic properties:

The organoleptic properties of aerial part and root of *T.collina* is given in table below

Table 4.1.6 Organoleptic character of *T. collina*

Parameters	Aerial part	Root
Colour	Green	Wheatish brown
Taste	No specific	Pungent
Odor	Similar to green tea	No characteristic
Texture	granular	Fibrous

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सत्यमेव जयते
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दिनांक/ Date 23.12.11

CERTIFICATE

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lanuginocarpa Sharma belonging to Family : Fabaceae


Dr. P. M. Padhye

Scientist "E" & H.O.O

FAX No. 91-291-2741736

● Telephone : 2740415, 2747163

● E-mail ID : bsiazc@yahoo.com

Fig. 4.7 Authentication certificate of *Tephrosia collina* Sharma

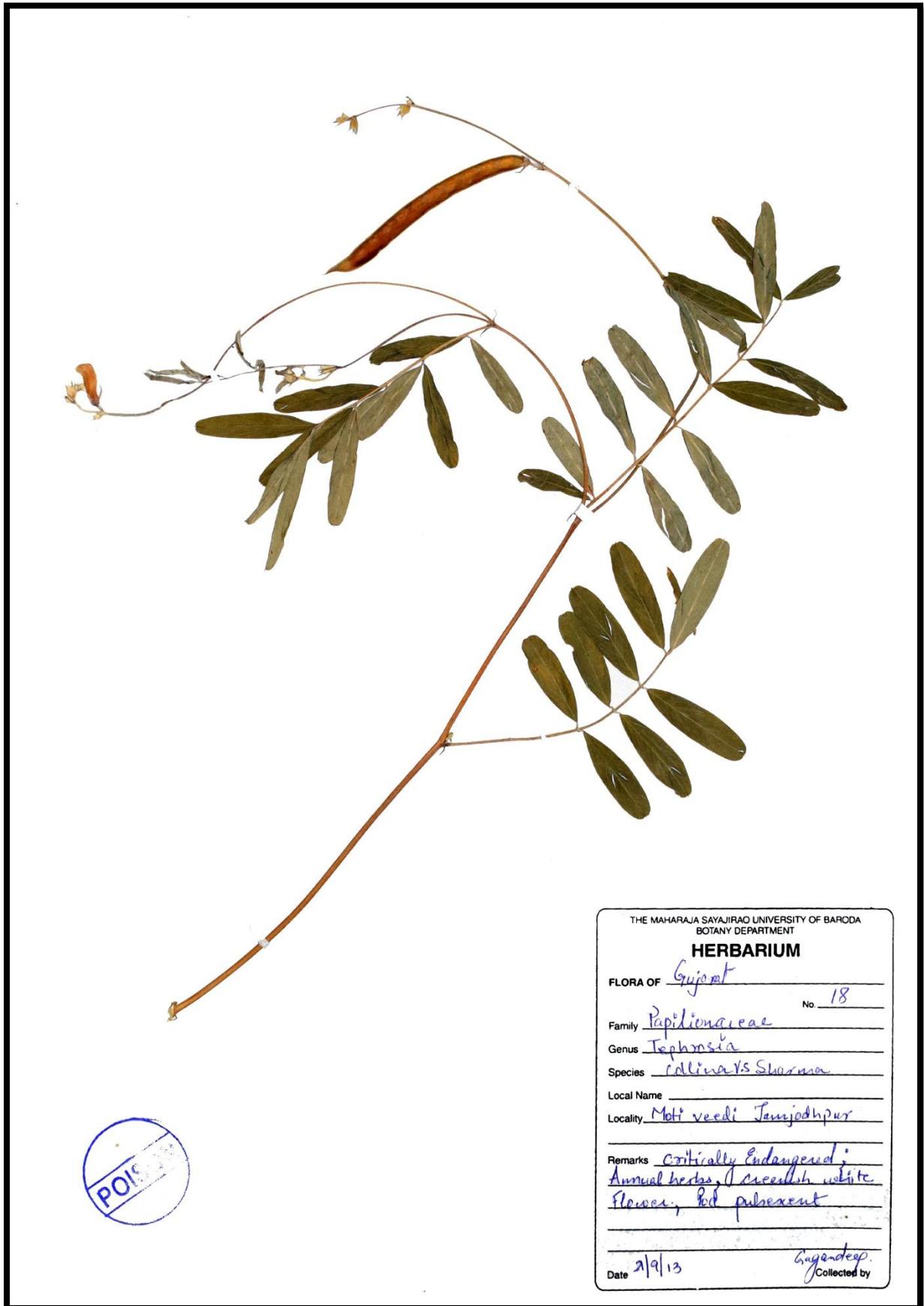


Fig. 4.8 Herbarium of *Tephrosia collina* Sharma

4.2. MOLECULAR CHARACTERIZATION USING *MATK* GENE

The genomic DNAs of two endemic species of *Tephrosia* were isolated and compared with the three commonly occurring species of that region, i.e., *T. strigosa*, *T. purpurea* and *T. villosa*. The DNAs obtained were subjected to Polymerase chain reaction (PCR) analysis. The PCR products amplified with the *matK* primers were analyzed for its purity on gel electrophoresis (Figure 4.9). The sequence of approximately ~600kb in length was obtained and correlated with higher range DNA ruler. The high intensity PCR product of the five *Tephrosia* species with no shrinkage and less amount of the impurities was selected for gene sequencing. The *matK* gene sequences generated were submitted to Genbank, NCBI. After verification, there Accession Numbers were obtained (Table 4.2.1 a).

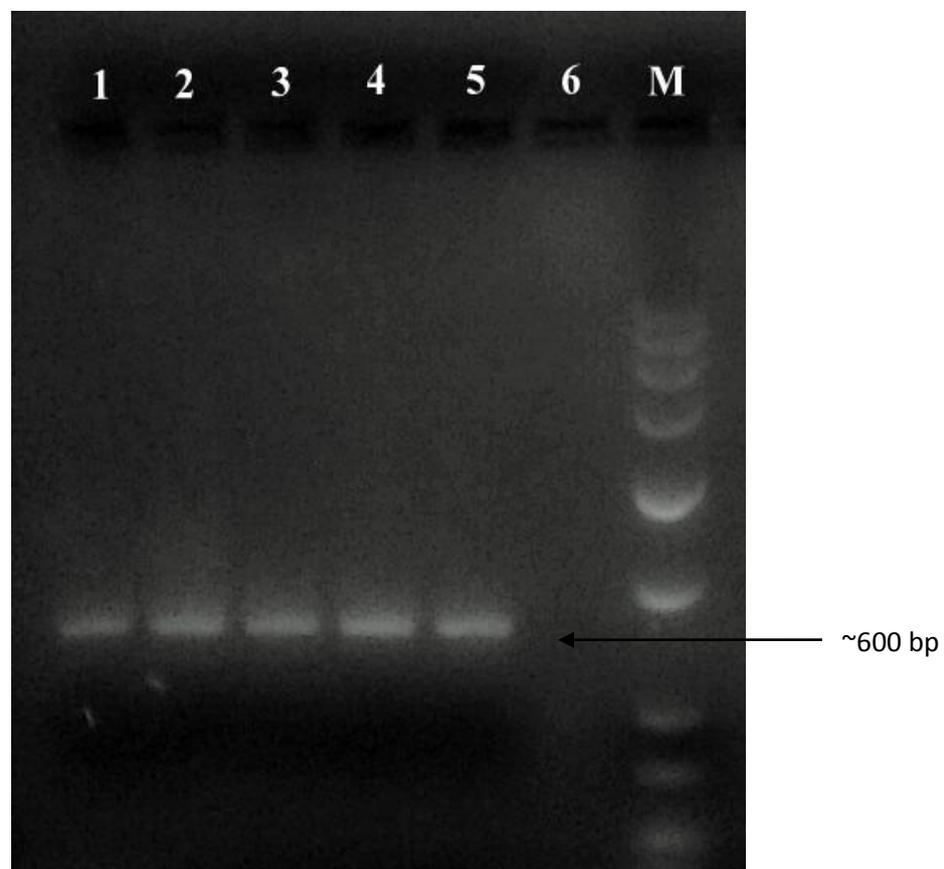


Fig 4.9 Agarose gel electrophoresis profile of *matK* gene

Lane 1: *Tephrosia villosa*, Lane 2: *Tephrosia strigosa*, Lane 3: *Tephrosia jamnagarensis*, Lane 4: *Tephrosia purpurea*, Lane 5: *Tephrosia collina*, Lane 6: Negative control, Lane 7: High range DNA ruler (GeneiTM, India)

Table 4.2.1a. *matK* Sequences generated with Accession no of *Tephrosia* species.

Sr. no.	Accession number of gene submitted	Contig sequence	Contig length	Final genus	Final species
1.	KC594075	<p>1 gatgtggggt cagcaaccac ggggagaagc tgctgatatc atgcacatgc ttctctgtt 61 tgaggatcca ttatattaat gagataattt ctacatatcc gtaaaaatcg atcaagaata 121 tcaaaatcgg atgaatcggg ccaaaccgac ttactaatag gattccccaa tacattacaa 181 aatttctctt taatcaataa tctaattaga aaaagaattg gaactattgt ctcaagcttt 241 ttcatacaa ttcaattag aatgaattt tgtaacattt gactccgtac actgaaagat 301 ttactccata tataaaaata gcccaaaagt gaatgaatgt tcggaaattg atgatatgat 361 cgtcctggtt gaaacaacat caaatgaatg cctaataata aagttttcca ttattctcaa 421 gagggattct gaacagaatg atttctctga tcaaaatagg agaaaggcct taagaaggaa 481 agaataaaaa aaatctttta ag</p>	502	<i>Tephrosia</i>	<i>villosa</i>
2.	KC594076	<p>1 ttgggttccc cgataccag gagcggaggg gttacgcgcg ctttttgtg tcgagcgggg 61 ttttaact ccaaagggg ggggggaccc tcaacctcgg tcagtactat caaatcggg 121 tgaatcggtc ccaaaccgac ttactaatgg gatgccctaa tactttcaa tttctctct 181 ttatccataa attccataaa aaaagaaatg ggacctgggg ccaggtttt ttctaacaa 241 ttccattaa aaaagaattt tggacctttt gcccccccc ccccgaaaa atttaccccc 301 tttttaaaa aaaccccaa aagggaagg aggggtcgaa aaattcttgg gaggggcccc 361 cccgggggga accaaacccc aaatagcttg cccaaaaaaa aaaaaattt tttttttt 421 cccaaagggg gttttgaaa aaaaaaagt tttttgtc cccctgggg gaggggg</p>	477	<i>Tephrosia</i>	<i>strigosa</i>
3.	KC427986	<p>1 tgatggttaa aaaaagacc cgagaggaag cggggggaga gatgtgatac tcttttggg 61 ttgagatcat tattatattg agaaaaaagg cgagatatcc gcaaaatcgg teattactac 121 cccaatcggg gaatcgggtc caaacgact tactaatagg atgccctaat acattacaaa 181 atttctctt atcaataat ttcatagaaa aagaatggga ctagtgtctc agcttttcta 241 acatttcatt agaatgaatt tgtactttga ctccgtacac tgaagattac tctatttaac 301 aatacccaa agtgaagaag ttcggaaatg ctgaaggacg tccggtgaac cacatcaatg 361 acattcggga tataaatgta tcatttctca aaggtgtcgt ggcaagtgg tctgaattca 421 atgagaggac ctgagagtaa gtacaaaate ctacc</p>	455	<i>Tephrosia</i>	<i>jamnagarensis</i>

Sr. no.	Accession number of gene submitted	Contig sequence	Contig length	Final genus	Final species
4.	KC427987	<p>1 atgggttaaa acaggggtgg gagggggggg ggggggtatg gtgtgctctt tcgtttgggg 61 ggcggttacc attgagata aatttctaca tatccgtaaa aatcgatcaa gaatatcaaa 121 atcggatgaa tcggtccaaa gcgacttact aataggatgt cccaatacat tacaaaattt 181 ttcttaate aataatctaa ttagaaaaga attggaacta ttgtctcagc ttttcatac 241 aattcaattg aatgaatttt gtaacatttg actccgtacc ctgaagatta ctctattat 301 aatagccca aagtgaatga tgcgggatat tgatggtatg gacatcctgg tgggacaaca 361 tcaatgatg cctaataata aatagatcca ttattatcaa agagcgagtc ttggaccgaa 421 tgcttttctt gaattacata ggagaaggac ttgaagagga agagaaaaaa atctccgggc 481 ttgt</p>	484	<i>Tephrosia</i>	<i>purpurea</i>
5.	KC422481	<p>1 cttggtcagc attgccggcc aggtgctgcg gtatcatgcg ttacttttgt gtttcgagcg 61 cattatgctc caagaaggat ggtagacata ccctaaatc gatcaataat accccaatcg 121 gatgaatcgg tcaaaccga cttactaata ggatgcccc aacattaca aaatttctct 181 ttaatcaata atctaattag aaaaacaatt ggaactattg tctcaagctt ttctaacat 241 ttcatttaaa agaatttga aactttgacc ccccctgaa aataaccct ttttaaaac 301 cccaagatg aagacggtca gattgatgaa gatccgccgg caaccaatca atcattgaat 361 gcataataat aatgtcttca ttatctcaag aggaatctga acagatgatt ttctgattca 421 actaggagaa gacctgaaat gtaggaaaaa ttttctct</p>	481	<i>Tephrosia</i>	<i>collina</i>

For the cladistic understanding of the phylogenetic trends prevailing in *Tephrosia* genus, *matK* gene sequences of other *Tephrosia* species were obtained from NCBI (Table 4.2.1b).

Table 4.2.1b Accession no of the other *Tephrosia* species sequences extracted from NCBI

Plant name	<i>matK</i> gene sequence Accession no
<i>Tephrosia rhodesia</i>	EU717429
<i>Tephrosia heckmanniana</i>	AF142712
<i>Tephrosia nicaraguensis</i>	JQ587878.1
<i>Tephrosia pentaphylla</i>	KF545843.1
<i>Tephrosia vogelii</i>	KF545842.1
<i>Tephrosia pondoensis</i>	JX517379.1
<i>Cassia abbreviata</i>	JX518172

The *matK* gene sequences of five *Tephrosia* species were correlated with the NCBI database of *Tephrosia* and odd group *Cassia abbreviata* to generate a phylogenetic tree using UPGMA method (Fig 4.10). From the evolutionary aspect of the dendrogram it can be concluded that species belonging to same geographical region has more genetic resemblance and similar *matK* sequences (Yang *et al.*, 2004). In brief the dendrogram is divided into two major clades: - T1 and T2. T1 clade is further branched into sub-clade T1A, T1B and T1C. As it can be depicted from the dendrogram that in Subclade T1A most of the species are of Asian origin, while T1B, T1C and T2 are native to South Africa. The subclade T1A includes the *Tephrosia* from the semiarid region of India i.e., *T. jamnagarensis*, *T. collina*, *T. villosa*, *T. purpurea* and *T. strigosa*. The clade T1B is represented by *T. rhodesia* and Clade T1C includes the *T. pondoensis* both these species are native to South Africa. The Clade T2 consists of *T. nicaraguensis* (native to North America), *T. pentaphylla* (distributed in Somalia, South India, and Arabian countries), *T. heckmanniana* (native to Africa) and *T. vogelii* (native to Africa). Henceforth, there is geographical closeness seen in *T. jamnagarensis* and *T. strigosa*, *T. collina* and *T. villosa* as well as in case of *T. vogelii* and *T. heckmanniana*. There are the chances of their phylogenetic resembles in these species.

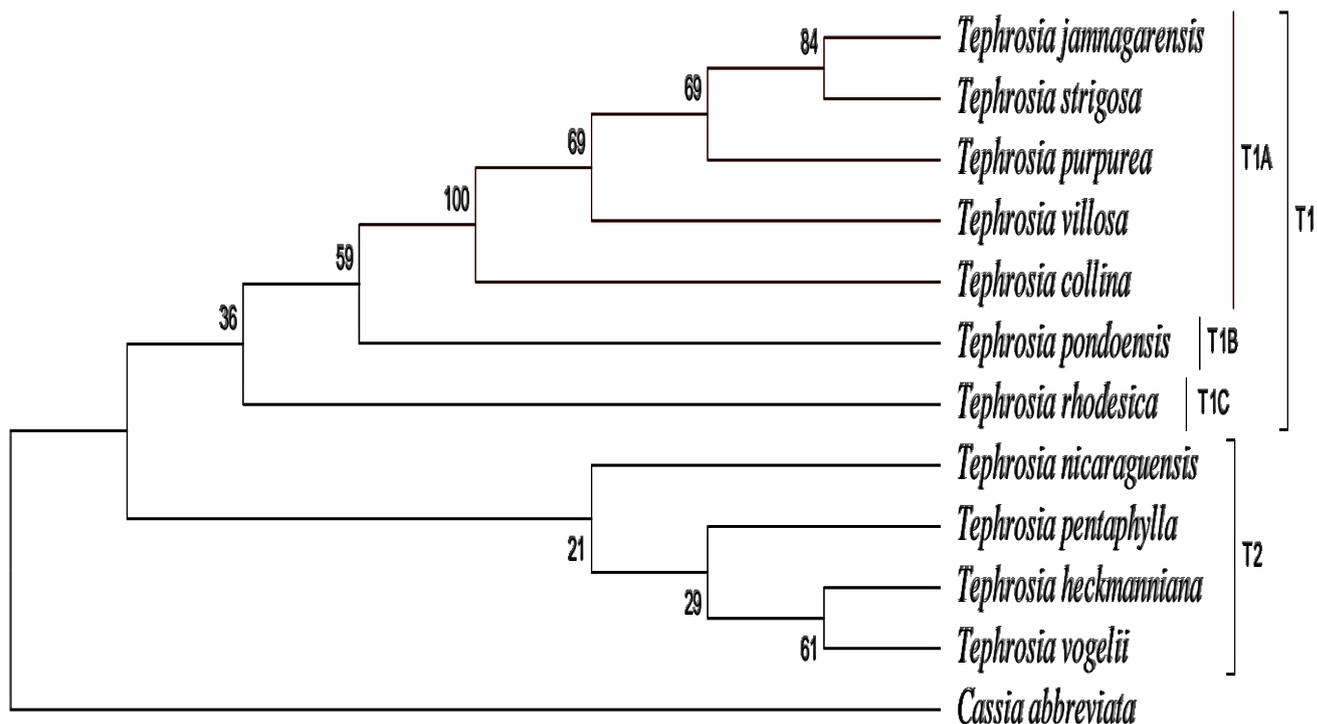


Fig 4.10 Phylogenetic tree of *Tephrosia* species using the *matK* gene

The phylogenetic trees (Fig 4.10) based on *matK* gene sequences showed that there are genetic resemblance and correspondence between *T. jamnagarensis* and *T. strigosa*, *T. collina* and *T. villosa* as well as in case of *T. vogelii* and *T. heckmanniana*. These results are compared with the morphological traits of the species (Table 4.2.2.). It showed that *T. jamnagarensis* in sub clade T1A with primitive morphological traits like simple leaves and axillary cyme inflorescences is morphologically similar to *T. strigosa*. It differs only in having flower and pod on the longer pedicel and its color. This morphological similarity is supported by *matK* gene sequences based on phylogenetic tree.

T. collina which have relatively advance morphological traits like to presence of 9-11 leaflets and creamy white flower on terminal lax raceme is found to be more morphological close to *T. villosa*. They differ in number leaflets present and size of pod and seeds. Similar facts were also depicted by phylogenetic tree. Group T1B and Group T1C consist of solitary species *T. rhodesica* and *T. pondoensis* respectively. Both the species can be segregated based on their morphological traits like flowers which are well spaced on the inflorescences axis and differs only on basis of habit and pod shape.

Table 4.2.2 Morphological traits of *Tephrosia* as per phylogenetic tree.

	SPECIES	LEAF	INFLORESCENCE	PODS
	Group T1A			
	<i>T. jannagarensis</i>	Simple pubescent	Axillary biperous cyme	Densely pubescent and stoutly pedicel
	<i>T. strigosa</i>	Simple pubescent	Axillary cyme with flowers on pedicle with single violet color flower	slightly pubescent with long pedicel
	<i>T. purpurea</i>	Imparipinnate Compound Pubescent	Axillary to terminal raceme with many flowers	Pod glabrous
Group T1	<i>T. villosa</i>	Imparipinnate Compound Pubescent	Axillary to terminal raceme with many flowers	Pod villous
	<i>T. collina</i>	Imparipinnate Compound pubescent	Terminal lax racemes with white flowers	Pod villous to torulose
	Group T1B			
	<i>T. rhodesica</i>	Imparipinnate Compound	Long lax racemes with orange flowers	Pods curved upward and covered in rusty brown hairs
	Group T1C			
	<i>T. pondoensis</i>	Imparipinnate Compound covered with brown rusty hair	Well spaced terminal racemes	Pods flats strongly covered with rusty hairs
	<i>T. nicaraguensis</i>	Imparipinnate Compound	Well spaced terminal racemes	Pods flats strongly covered with rusty hairs
Group T2	<i>T. pentaphylla</i>	Imparipinnate Compound	Axillary cyme red flower	Pubescent and brownish on sutures
	<i>T. heckmanniana</i>	Imparipinnate Compound Pubescent	Axillary to terminal raceme	Pod glabrous
	<i>T. vogelii</i>	Imparipinnate Compound Pubescent	terminal or axillary pseudo-raceme, 8-26 cm long, rusty tomentose	Pod linear, slightly turgid, 5.5-14 x 0.8-1.8 cm. Brown or green, woolly to sericeous

Group T2 consists of *Tephrosia* species which can be assets by distinct morphological traits like, imparipinnately compound leaves (leaflet number varying between 3-14) and the pods covered with brown rusty hairs. In this group *T. heckmanniana* showed resemblance to *T. vogelii* which is supported by cladistic understanding based on DNA Barcoding.

Certain *Tephrosia* species cannot be easily distinguished at morphological level. In such condition correlating morphological data with molecular data and geographical distribution could be helpful tools for differentiating. In earlier ages of taxonomic advancement, De Candolle (1825) had classified genus *Tephrosia* into four sections *Mundulea*, *Brissonia*, *Cracciodes* and *Reineria* of these *Mundulea* and *Reineria* sections represent in India. In the present study of eleven species, *T. purpurea* and *T. villosa* were belongs to *Reineria* section of this genus. Later after gap of one and half century, Brummit (1981) had classified the genus *Tephrosia* into two subgenera based on style, i.e. glabrous style and Barbistyla which is trichiferous style. Substituting this classification in present selected species, *T. purpurea*, *T. pentaphylla*, *T. villosa* and *T. strigosa* belong to glabrous style while *T. nicaraguensis* and *T. vogelii* are Barbistyla. In recent period the classification based on its fruits and seed morphology (Queiroz *et al.*, 2013; Kirkbride *et al.*, 2003; Bhandari *et al.*, 1985).

Today even attempts were made for the better understanding of interrelationships between the various *Tephrosia* species by correlating the morphological and biochemical characters. In this context, Raina *et al.*, (1985) explored this genus for its cytogenetic variation. In 2004 Acharaya *et al.*, had also studied this genus for it evolutionary trends with the member of Tribe Milletieae. RAPD analysis was done by Lakshmi *et. al.*, (2008) to understand phylogenetic interrelationships of *Tephrosia* species. This analysis reveals that *T. villosa* was close to *T. pumila* rather than *T. purpurea* and *T. strigosa* was segregated into separate group with *Sorghum* (Gramineae) and *Crotolaria* (Fabaceae). By extrapolating the above inference in present phylogenetic analysis, it can be assume that *T. collina* can also be genetically associated with *T. pumila* and *T. jamnagarensis* to some members of Gramineae and Fabaceae members.

Cladistic understanding from the phylogenetic tree based on *matK* sequence of the species states that genetic closeness of these species is governed by geographical inhabitant and morphological trait. This could be figure out from the geographical, phylogenetical and morphological closeness between *T. jamnagarensis* and *T. strigosa*, *T. collina* and *T. villosa*, *T. heckmanniana* and *T. vogelii*. In this way attempts can be made to understand evolutionary trends set in at species level.

4.3 ECOLOGY AND DISTRIBUTION PATTERNS

4.3.1 ABIOTIC PARAMETERS

The ecological conditions were analyzed for abiotic and biotic parameters of the distributional site of endemic plants. The distributional site of *T. jamnagarensis* and *T. collina* was Saurashtra peninsular region of Gujarat (Fig 2.1). The climatic parameters were studied based on the available meteorological data from 2002-2013 for Saurashtra region (www.imdahm.gov.in/). The abiotic data of indigenous site was compared with the cultivation site. In order to understand the growth parameters of these endemic species, the microclimatic conditions were studied. The comparative details of these are as follows:

1. Rainfall:

Mean annual rainfall recorded was 500-550 mm during past decade. Maximum average rainfall was recorded in months of July and August. Rainfall in the arboretum (cultivated site) was 200 mm during monsoon in 2012 (Fig 4.11).

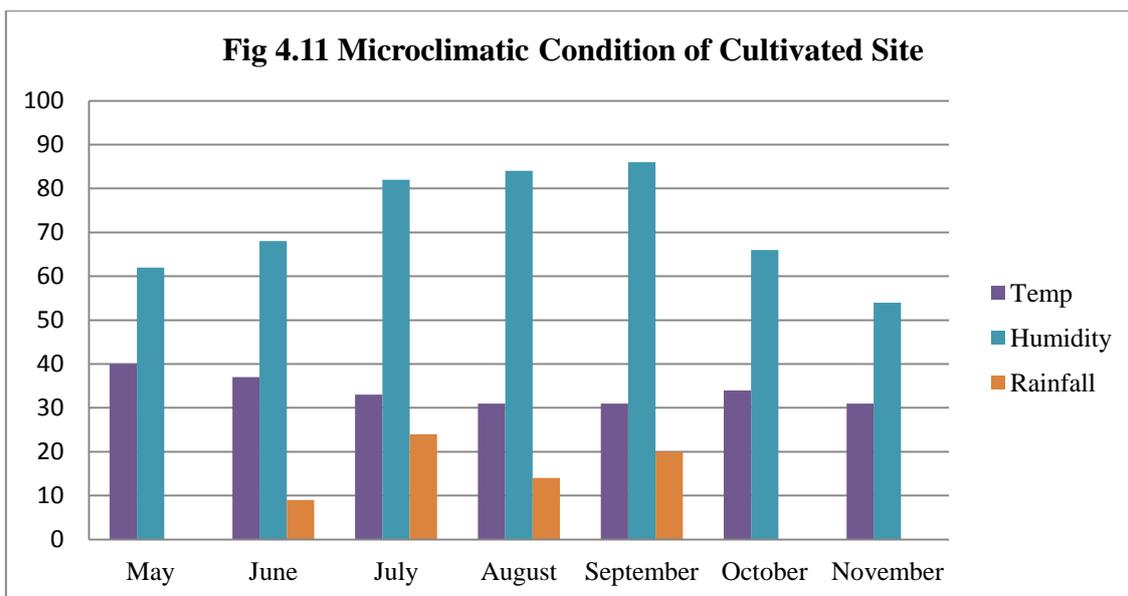
2. Temperature:

An average temperature was 30 – 35°C in Saurashtra, reaching up to 43 – 44°C. The average temperature at the site of cultivation during period (April 2012 to November 2012) of growth and development was 22 – 34°C.

3. Humidity:

Humidity goes to its maximum of 80 – 85% near the coastal tracks of Jamnagar and Porbandar; however, the tracks in which both endemic plants grow have the humidity around 55 – 65% in monsoon. In arboretum, during the cultivation period of these endemic species the humidity ranges between 42 – 64%.

This variation in weather at germination sites might affect the growth and development of these endemic species (Fig. 4.11).



The above graph shows that July, August and September had good rainfall and high humidity at cultivation site. During this duration there is a probability that the plants nourish well and attain maturity.

4. Edaphic properties:

Both the targeted plants are distributed in narrow pockets of the Saurashtra peninsula of Gujarat. The soil color of this region varies from red to brown. It can be alluvial to clay to silt loamy to sand type which further depends on physiognomy of the area. The soil properties of Khadkhambaliya and Moti vidi were analyzed physically and chemically (Table no 4.3.1 and Table 4.3.2). These plants are also cultivated for its phenological studies in arboretum (M. S. University of Baroda). Edaphic conditions of both the sites (cultivation and wild) were compared.

Soil color and texture (c.f. 4.3.1)

The soils of these *vidi* (grassland) showed huge difference in water retention properties. The soil of Khadkhambaliya *vidi* is black and very rich in montmorillonite clay, which has the property of swelling up (when wet) and retaining moisture for long periods. Whereas at moti *vidi* where the hill-locks are less steep the soil is red or red brown clay (gravelly and sandy) with poor water retention properties. The cultivated site soil is brown with water holding capacity in between the black and red soils.

Biochemical analysis of Soil (c.f. Table 4.3.2)

Soil pH: pH of all the three regions was slightly basic ranging from 7.5 – 8 which were considered normal for superior plant growth.

Organic carbon: There are slight variations in the percentage of organic carbon content of both the *vidis*. It's ranging between 0.9 – 1 % which was consider as its high concentration. Half of the organic carbon content of these *vidi* was recorded in the cultivated site. Although, here the organic carbon content was medium but it might not affect the germination and growth.

Available phosphorus: The wild and cultivated sites showed the available phosphorus below the normal level. This condition was appropriate for growth and development of *T. jamnagarensis* and *T. collina* in cultivation site.

Exchangeable potassium: It has been reported that exchangeable potassium decreases in the scrublands and there is a lot of fluctuation in its content in the different Ecozones of Saurashtra peninsula (Nagar, 2007). In the present study both sites are potassium deficit. On the contrary the cultivated site which is located in central Gujarat showed potassium in sufficient quantity for plant growth.

Sulphur content: It was high in both the wild sites while its concentration was medium in the cultivated site.

Micro nutrient: Both the grasslands Khadkhambaliya and Moti *vidi* were analysed for four micronutrients, of which Zinc was in low concentration. In the cultivation site iron was only micronutrient in medium concentration in comparison to others.

Table 4.3.1- Physical Property of Soil

Sr. No.	Plant name	Site name	Soil color
1	<i>T.collina</i>	Jamjodhpur moti vidi	Red, gravelly soil 
2	<i>T. jamnagarensis</i>	Khadkhambaliya	Black clay soil 
3	Arboretum	Cultivation site of <i>T.jamnagarensis</i> and <i>T. collina</i> .	Brown slit soil 

Table 4.3.2- Chemical properties of soil

Site	Jamjodhpur (<i>T. collina</i>)		Khadkambaliya (<i>T. jamnagarensis</i>)		Arboretum	
	Concentration	Quality	Concentration	Quality	Concentration	Quality
Total nitrogen/Or g-anic carbon (%)	0.95	High	1	High	0.51	Medium
Available Phosphorous/Acre	6	Less	6	Less	6	Less
Total Potash/Acre	25	Very Less	28	Very Less	63	Medium
pH	7.83	Normal	7.63	Normal	7.87	Normal
EC	0.19	Normal	0.37	Normal	11	Normal
Sulphur (ppm)	22.7	High	21.2	High	20.1	Medium
Micro-						

Site	Jamjodhpur (<i>T. collina</i>)		Khadkambaliya (<i>T. jamnagarensis</i>)		Arboretum		
Parameter	Concentration	Quality	Concentration	Quality	Concentration	Quality	
nutrient							
Zn	0.26	Less	0.8	Less	3	High	
Fe	31.4	High	16.34	High	8	Medium	
Mn	34.7	High	42.44	High	26.9	High	
Cu	2.9	High	3.02	High	2.06	High	

4.3.2 BIOTIC PARAMETERS

The biotic parameters of selected endemic species were analyzed by studying its associated species, population density and size, Extent of Occurrence (EOO), Area of Occupancy (AOO). The details of these are as follows:

1. Associated plant species of the indigenous sites:

Quadrat study and observations showed that there are 110 species associated with the endemic species. These species belong to 82 genera 24 family of vascular plant (Table 4.3.3). Poaceae among the monocotyledon was the largest families in both the sites where as the Fabaceae are the largest family among the dicotyledon. The monocotyledon includes Poaceae (48 species) and Cyperaceae (8 species). Grasses of the site belongs to Panicoides and Poaceae tribes of family Poaceae. Few common among them are *Apluda mutica*, *Chrysopogon fulvus*, *Aristida adscensionsis* and *Dactyloctenium aegyptium*.

Table 4.3.3 List of associated plant species at indigenous sites of TJ and TC.

Sr. No	Species	Family
MONOCOTYLEDONS		
	Herb	
1.	<i>Aeluropus lagopoides</i>	Poaceae
2.	<i>Alloteropsis cimicina</i>	Poaceae
3.	<i>Apluda mutica</i>	Poaceae
4.	<i>Aristida adscensionsis</i>	Poaceae
5.	<i>Aristida setacea</i>	Poaceae
6.	<i>Arthraxon lanceolatus</i>	Poaceae
7.	<i>Arthraxon lancifolius</i>	Poaceae
8.	<i>Arundinella pumila</i>	Poaceae
9.	<i>Bambusa arundinaceae</i>	Poaceae

Sr. No	Species	Family
10.	<i>Bothriochloa ischaemum</i>	Poaceae
11.	<i>Bothriochloa pertusa</i>	Poaceae
12.	<i>Brachiaria eruciformis</i>	Poaceae
13.	<i>Cenchrus biflorus</i>	Poaceae
14.	<i>Cenchrus ciliaris</i>	Poaceae
15.	<i>Cenchrus setigerus</i>	Poaceae
16.	<i>Chloris barbata</i>	Poaceae
17.	<i>Chrysopogon fulvus</i>	Poaceae
18.	<i>Cymbopogon citratus</i>	Poaceae
19.	<i>Cynodon dactylom</i>	Poaceae
20.	<i>Dactyloctenium indicum</i>	Poaceae
21.	<i>Dactyloctenium aegyptium</i>	Poaceae
22.	<i>Desmostachya bipinnata</i>	Poaceae
23.	<i>Dicanthium annulatum</i>	Poaceae
24.	<i>Digitaria ciliaris</i>	Poaceae
25.	<i>Digitaria sanguinalis</i>	Poaceae
26.	<i>Dinebra retroflexa</i>	Poaceae
27.	<i>Echinochloa colona</i>	Poaceae
28.	<i>Eleusine indica</i>	Poaceae
29.	<i>Eleusine compressa</i>	Poaceae
30.	<i>Eragrostis cilianensis</i>	Poaceae
31.	<i>Eragrostis diarrhena</i>	Poaceae
32.	<i>Eragrostis tenalla</i>	Poaceae
33.	<i>Eragrostis uniolooides</i>	Poaceae
34.	<i>Eremopogon foveolatus</i>	Poaceae
35.	<i>Eriochloa procera</i>	Poaceae
36.	<i>Heteropogon contortus</i>	Poaceae
37.	<i>Heteropogon triticeus</i>	Poaceae
38.	<i>Horedum vulgare</i>	Poaceae
39.	<i>Imperata cylindrical</i>	Poaceae
40.	<i>Ishaemum rugosum</i>	Poaceae
41.	<i>Paspalidium geminatum</i>	Poaceae
42.	<i>Pennisetum typhoides</i>	Poaceae
43.	<i>Phragmitis karka</i>	Poaceae
44.	<i>Saccharum spontaneum</i>	Poaceae
45.	<i>Setaria glauca</i>	Poaceae
46.	<i>Setaria pallidefusa</i>	Poaceae
47.	<i>Setaria verticilata</i>	Poaceae
48.	<i>Sorghum halpense</i>	Poaceae
49.	<i>Cyperus exaltatus</i>	Cyperaceae
50.	<i>Cyperus haspan</i>	Cyperaceae
51.	<i>Cyperus mitis</i>	Cyperaceae
52.	<i>Cyperus rotundus</i>	Cyperaceae
53.	<i>Eleocharis geniculata</i>	Cyperaceae
54.	<i>Fimbristylis littoralis</i>	Cyperaceae
55.	<i>Fuirena ciliaris</i>	Cyperaceae
56.	<i>Pycreus flavidus</i>	Cyperaceae

Sr. No	Species	Family
DICOTYLEDONS		
	Climber	
57.	<i>Atylosia platycarpa</i>	Fabaceae
58.	<i>Cardiospermum halicacabum</i>	Sapindaceae
	Herb	
59.	<i>Acalypha hispida</i>	Euphorbiaceae
60.	<i>Achyranthes aspera</i>	Amaranthaceae
61.	<i>Aerva lanata</i>	Amaranthaceae
62.	<i>Alternanthera sessilis</i>	Amaranthaceae
63.	<i>Alysicarpus bupleurifolius</i>	Fabaceae
64.	<i>Alysicarpus longifolius</i>	Fabaceae
65.	<i>Alysicarpus vaginalis</i>	Fabaceae
66.	<i>Amaranthus viridis</i>	Amaranthaceae
67.	<i>Andrographis echinoides</i>	Acanthaceae
68.	<i>Boerhavia chinensis</i>	Nyctaginaceae
69.	<i>Boerhavia diffusa</i>	Nyctaginaceae
70.	<i>Borreria stricta</i>	Rubiaceae
71.	<i>Cassia pumila</i>	Caesalpinaceae
72.	<i>Cassia tora</i>	Caesalpinaceae
73.	<i>Celosia argentea</i>	Amaranthaceae
74.	<i>Chamaecrista nictitans</i>	Caesalpinaceae
75.	<i>Corchorus trilocularis</i>	Tiliaceae
76.	<i>Enicostemma littorale</i>	Gentianaceae
77.	<i>Euphorbia hirta</i>	Euphorbiaceae
78.	<i>Evolvulus alsinoides</i>	Convolvulaceae
79.	<i>Indigofera cordifolia</i>	Fabaceae
80.	<i>Indigofera linifolia</i>	Fabaceae
81.	<i>Justicia procumbens</i>	Acanthaceae
82.	<i>Leucas aspera</i>	Lamiaceae
83.	<i>Lindenbergia muraria</i>	Scrophulariaceae
84.	<i>Parthenium hysterophorus</i>	Asteraceae
85.	<i>Pulicaria angustifolia</i>	Asteraceae
86.	<i>Senna alata</i>	Caesalpinaceae
87.	<i>Sida acuta</i>	Malvaceae
88.	<i>Tephrosia senticosa</i>	Fabaceae
89.	<i>Tephrosia strigosa</i>	Fabaceae
90.	<i>Tephrosia purpurea</i>	Fabaceae
91.	<i>Trichodesma zeylanicum</i>	Boraginaceae
92.	<i>Triumfetta robusta</i>	Tiliaceae
93.	<i>Vernonia cinerea</i>	Asteraceae
94.	<i>Barleria prattensis</i>	Acanthaceae
95.	<i>Boerhavia boissieri</i>	Nyctaginaceae
	Shrub	
96.	<i>Abelmoschus manihot</i>	Malvaceae
97.	<i>Abutilon indicum</i>	Malvaceae
98.	<i>Calotropis gigantea</i>	Asclepiadaceae
99.	<i>Calotropis procera</i>	Asclepiadaceae

Sr. No	Species	Family
100.	<i>Gossypium herbaceum</i>	Malvaceae
101.	<i>Jatropha curcas</i>	Euphorbiaceae
102.	<i>Lantana camara</i>	Verbenaceae
103.	<i>Martynia annua</i>	Martyniaceae
104.	<i>Mimosa hamata</i>	Mimosaceae
105.	<i>Opuntia elatior</i>	Cactaceae
	Tree	
106.	<i>Acacia nilotica</i>	Mimosaceae
107.	<i>Azadirachta indica</i>	Meliaceae
108.	<i>Butea monosperma</i>	Fabaceae
109.	<i>Peltophorum pterocarpum</i>	Caesalpiaceae
110.	<i>Pithecellobium dulce</i>	Mimosaceae



Fig:-4.12 Associated species of *T. jammagarensis* and *T. collina*
 A-*Justicia procumbens*, B- *Martynia annua*, C- *Senna uniflora*, D- *Atylosia platycarpa*, E- *Pulicaria angustifolia*, F- *Enicostemma littorale*, G- *Trichodesma zeylanicum*, H- *Indigofera cordifolia*, I- *Apluda nutica*

Along with these wild species of fallow lands, vigorous growth of an exotic species, *Senna uniflora* which has invaded the natural habitats of both the endemics (Fig 4.13). It is non-edible to animals and so it completes its life cycle and produces good amount of viable seed. These viable seed flourish in the vacant land of grassland and in this way affect the natural habitat of both the endemics. Habitat destruction due to overgrazing is the main reason of their occupancy of major portion in grassland.



Fig 4.13 Khadkhambilya *vidi* invaded by *Senna uniflora*

2. Population Study:

Population study of these endemic plants was another biotic parameter, which was determined by considering two factors *i.e.*, population size and population density. Population size is studied by counting the plant species in their specific location, while population density is the study of individuals (number) per unit area. Catastrophes of the sudden reduction in population of a species by large fractions are usually caused by extreme climatic conditions and environmental fluctuations (Young 1994). In the present study, alteration of rainfall pattern in semi-arid zone of Saurashtra, leads to extreme climate condition. The selected endemic possesses ephemeral phenology and so their growth is totally dependent on climatic conditions *esp.* rainfall pattern. For instance, during scarcity of rainfall in 2012 population density of the plants showed lagging phase (Table 4.3.4 and

4.3.3.). Hence there is a positive correlation of these species growth with reference to the rainfall (Fig 4.13 & Fig 4.14).

Table 4.3.4 Population census of *T. jamnagarensis* at Khadkhambaliya *vidi*

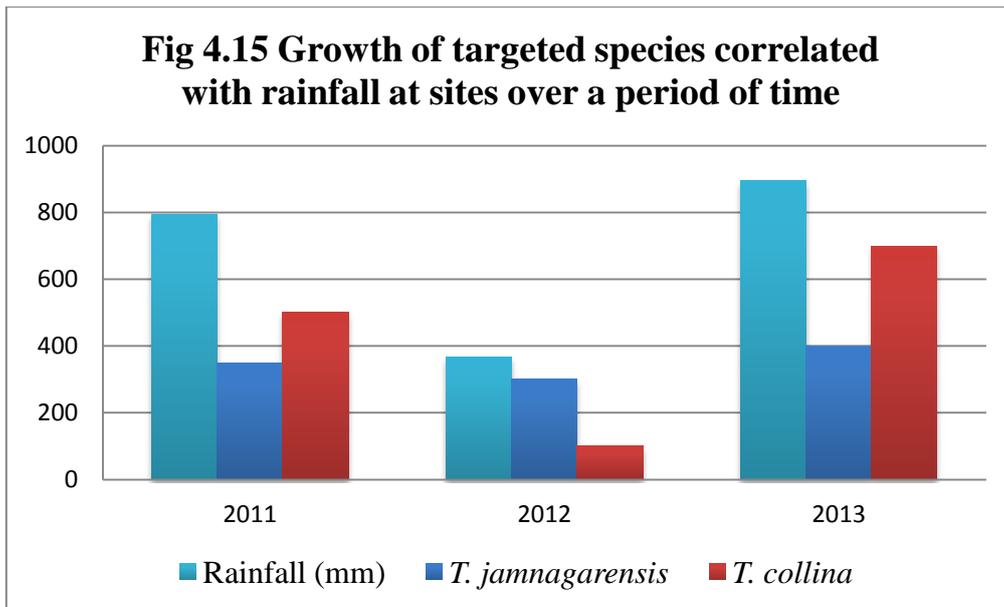
Year	Rainfall (mm)	Population size	Population density
2002 (Nagar , 2002)	Deficit	880	0.24 plant/m ²
2011	794.1	350	0.088 plant/ m ²
2012	366.4	300	0.075plant/m ²
2013	895.5	400	0.1 plant/m ²

Table 4.3.5 Population census of *T. collina* at moti *vidi*, Jamjodhpur

Year	Rainfall (mm)	Population size	Population density
2004 (Nagar, 2004)	Deficit	Common	-
2011	794.1	500	0.1 plant/ m ²
2012	366.4	100	0.02plant/m ²
2013	895.5	700	0.14 plant/m ²

Fig 4.14 Condition of *vidi* (grassland) during scanty rainfall season





The above graph indicates that in 2012 there was scanty rainfall (366.4 mm) and this directly affected the growth of the endemics in that year; moreover, it made these plants to grow in stress condition. For adjusting in such conditions the plant proceeds towards an early flowering stage. In addition, it has been observed that seeds of these species are consumed for nutrition by flies and worms of the *vidis*, so even if the gross production of seeds is high only few of them are successful in germinating next year. These factors further contribute to the declining population in their narrow distributional site.

Over a period of time, the exponential and geometric population growth of *T. jamnagarensis* was 116 and that of *T. collina* was 100. They have patch distribution pattern in grassland, so factors like frequency, abundance and density were studied by sampling across the line transect quadrat. Based on this, frequency of *T. collina* was found to be 60% and that of *T. jamnagarensis* was 50%. Abundance of *T. collina* was 1.99% and its density was 2.35 plants/m² while abundance of *T. jamnagarensis* was 1.2% and its density was 0.6 plant/m².

3. Area of Occupancy (AOO) and Extent of Occurrence (EOO).

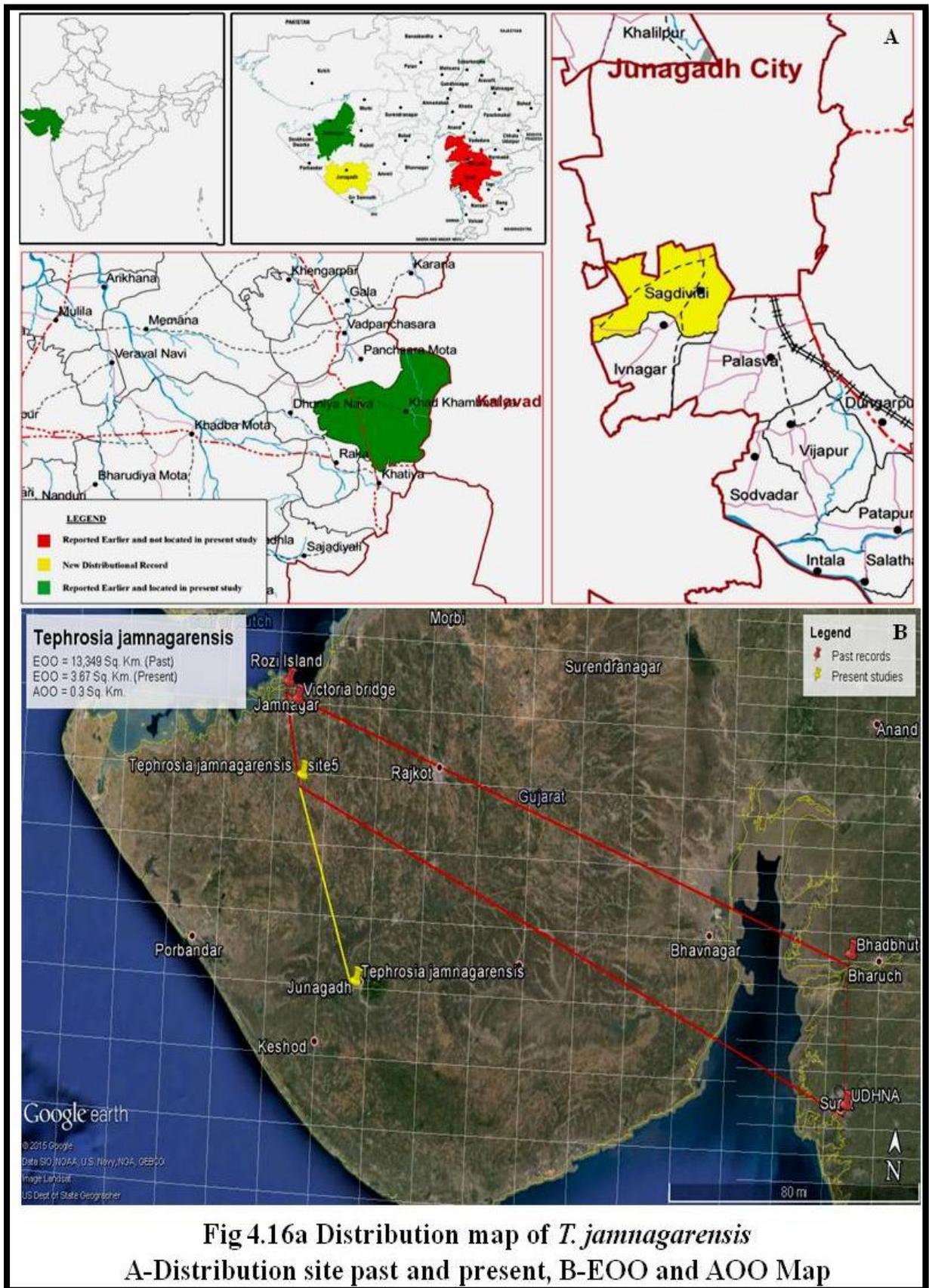
T. jamnagarensis and *T. collina* are, endemics, having restricted localities (Fig 4.16a Aand Fig 4.16bA). *T. jamnagarensis* has been documented from four sites in Gujarat while *T. collina* was from four sites in Gujarat and Rajasthan (Fig 2.1). During preset surveys it was found that *T. jamnagarensis* was relocated only from two localities namely Sagdi *vidi*, Junagadh and Khadakhambaliya *vidi*, Jamnagar. Similarly, *T. collina* was relocated two from its earlier stated sites *i.e.* Junaraj forests, Narmada and Moti *vidi*, Jamjodhpur and with two

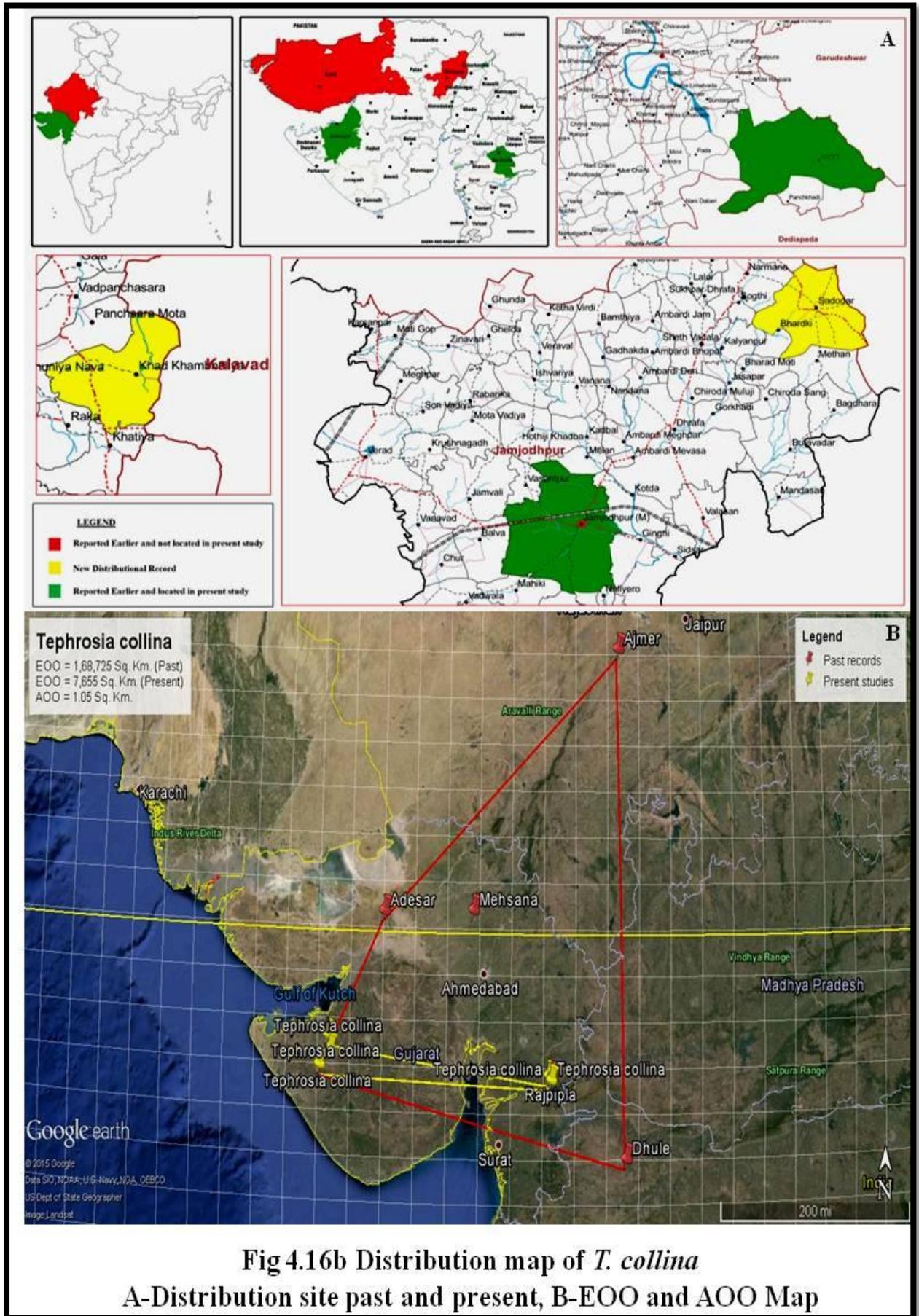
new site Khadkhambaliya *vidi* and Sadodar *vidi* of Jamnagar. Presently, *T. jamnagarensis* could be located in two definite sites and *T. collina* at four sites of Gujarat (Fig 4.16a and Fig 4.16b). Hence based on this survey the EOO and AOO of both these species were calculated (Table 4.3.6).

Table 4.3.6. EOO and AOO of TJ and TC

Parameter Species	Range (km ²) EOO			Area occupied (km ²) AOO	
	Review of Literature	CAMPP (2014)	Present studies	CAMPP (2014)	Present studies
<i>T. jamnagarensis</i>	13,349	2,500-2,700	3.67	2.7-4	0.3
<i>T. collina</i>	35,035	1,000	4,584	1-25	0.45

The results of EOO and AOO clearly highlight the decline in their range (EOO) narrow distribution of this species in wild; moreover, there is an urgent need for their conservation and management plans. Further, destruction of natural habitats of these species is victim of anthropological activities like agriculture activities, overgrazing, and urbanization. This led to land exploitation, habitat loss and habitat fragmentation of these endemics. Henceforth, *Ex-situ* and *In-situ* measures are must to conserve this unique vegetation of grassland ecosystem.





4.4 SEED GERMINATION AND PHENOLOGY

Many desert plant species seeds exhibit some kind of inhibition of immediate germination and remain dormant till the onset of favourable conditions. Dormancy period of arid-semiarid plant species seeds varies from few months to few years. The dormancy exhibited by these seeds could be due to thick seed coat, rudimentary embryos, and physiologically immature embryos and due to presence of inhibitors.

Germination of these seed is also affected to a greater extent by unfavourable environmental conditions. The restricted distribution and small size of populations of few taxa could be reflection of the nature and germination behaviour of seeds and their distribution. Among the different tests that have been designed for determination of seed quality namely, germination test is undoubtedly the most dependable, especially because a number of comparable tests under controlled conditions of media and temperature can be performed, and the test can be repeated with reproducibility, reliability and uniformity.

4.4.1 SEED GERMINATION

T. jamnagarensis and *T. collina* belonging to Fabaceae and possess seed dormancy due to hard seed coat. In present research work the germination of these endemic species with various seed treatments were carried out. The analysis of seed viability showed that 12.5% of *T. jamnagarensis* seeds are viability in comparison to *T. collina* 66.67% viability. Pre-germination seed treatments of *T. jamnagarensis* and *T. collina* with various treatments were done. It showed that the most effective seed treatment was treating seed with concentrated H₂SO₄ treatment for five minutes in both the species (Table 4.4.1, Table 4.4.2 and Fig 4.17). In *T. jamnagarensis* H₂SO₄ treatment showed 90% of seed germination at 15th day wherein all seed swells after second day of treatment only. The other treatments like cold, hot or GA₃ were not at all effective in breaking the seed dormancy till 15 days. The only exception was warm water treatment where the germination percentage was 6.67% and two seed germinates at initially two days of treatment and thereafter no change was seen. However, Babayemi *et al.*, (2003) had shown that warm water treatment was effective in overcoming seed dormancy in *Tephrosia* species Like *T. bracteates*, *T. linearis* and *T. candida*. Similar inference was also observed in *T. vogelii* wherein soaking of seeds in boiling water for 30 seconds was enough to improve the seed germination (Ruppel *et al.*, 1967).

Sr. No.	Seed treatment	Day 1	Day 2	Day 3-15	Total germination %
1	Control	–	–	–	0
2	Cold water	–	–	–	0
3	Hot water	–	–	–	0
4	Warm water	1	1	–	6.67
5	H ₂ SO ₄ treatment (5min)	30	28 ±2.88	–	90
6	GA ₃ 1000 ppm	–	–	–	0
7	GA ₃ 500 ppm	–	–	–	0
8	GA ₃ 250 ppm	–	–	–	0

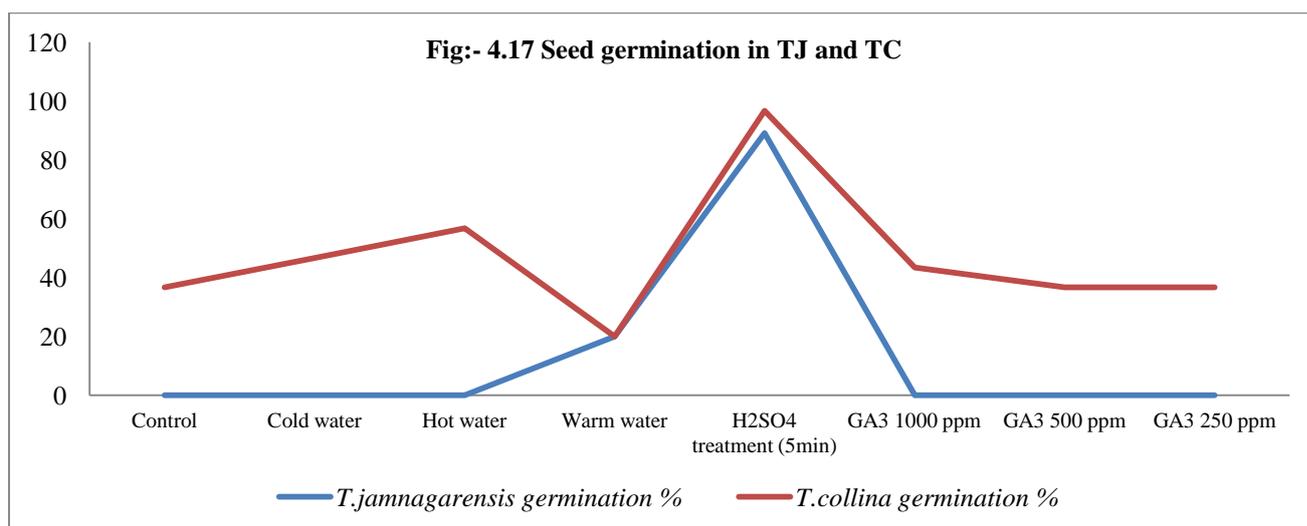
– no effect was observed.

In *T. collina* all the seed germinated on day one of treatment with H₂SO₄ and showed the germination percentage of 96.67. These inferences can be correlated with *T. purpurea* seed germination wherein also treating seed with concentrated H₂SO₄ for five minutes was effective for improve germination (Dharmalingam *et al.*, 1973). The second effective seed germination treatment was Hot water treatment with germination percentage was around 60%. In this treatment more than 50 percent of seed germinated after first day of treatment while two seed germinated after 2^{ed} day and 4th day of treatment respectively. The other treatments like cold water and different GA₃ concentration were moderately effective. In all these treatments less than 50 % of seed germination after first day of treatment and then gradual changes were seen till 15th day. The least effective seed germination treatment was warm water wherein germination percentage was 20%.

Sr. No.	Seed treatment	Seed germination data									Total	Germination %	
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	From 8 -14 Days	Day 15	112/240	46.66	
1	Control	8± 1.53	–	–	1± 0.58	–	1± 0.58	–	–	–	1± 0.58	11 ± 2.3	40
2	Cold water	6	2 ± ±1.15	–	2 ± 0.58	–	1 ± ±0.58	1 ± 0.58	–	–	2 ± 0.58	14 ± 1.53	50
3	Hot water	13 ± 2.08	2 ± 0.58	–	2 ± 0.58	–	–	–	–	–	–	17 ± ±1.53	60

Table 4.4.2. Seed treatment of <i>T. collina</i>											Total	Germination %
Sr. No.	Seed treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	From 8 -14 Days	Day 15	112/240	46.66
4	Warm water	3	–	–	2 ± 0.58	–	1 ± 0.58	–	–	–	6 ± 1	20
5	H ₂ SO ₄ treatment (5min)	29 ± 0.58	–	–	–	–	–	–	–	–	29 ± 0.58	96.67
6	GA ₃ 1000 ppm	8 ± 1.15	1 ± 0.58	–	3 ± 1	–	1 ± 0.58	–	–	–	13 ± 0.58	43
7	GA ₃ 500 ppm	7 ± 1.15	–	–	1 ± 0.58	–	2 ± 0.58	1 ± 0.58	–	1 ± 0.58	11 ± 3.05	40
8	GA ₃ 250 ppm	10 ± 2.3	–	–	1 ± 0.58	–	–	–	–	–	11 ± 2.08	40

– no effect was observed.



Germinated seeds - *T. jamnagarensis*



Germinated seeds - *T. collina* *

* *T. collina* seed swells and develop radical after transplantation in soil.

4.4.2 PHENOLOGY

Phenology is the calendar of events in the life history of plants and indicates the times of seedling appearance, vegetative growth, flowering, fruiting and maturity and seed dispersal of the plants under natural habitat (Misra, 1980). He emphasised on the phenological studies of the plant before starting the research work on uncultivated plants.

As stated in methodology the phenological studies of these endemic species were done in field condition of arboretum. With the phenological studies the climatic parameter like rainfall, temperature, humidity, during the period of experiment from April to November in 2012 were studied and compared with ecology of wild site (*c.f.* pg121).

T. jamnagarensis seeds were transplanted in the experimental site in month of the April 2012. The vegetative phase remains prominent till August. The flowering and fruiting starts quickly in the month of May and continue till July. Later in July and August due to heavy rains mortality of water logged plants takes place. The life cycle of the plants continue till month of September (Table 4.4.3).

➤ **The phenological changes in *T. jamnagarensis*:**

Germination begins on 2/5/12- 2^{ed} day after seed treatment with H₂SO₄ for five minutes

Eophyll: 2, opposite, dark green, orbicular 1 X 0.5 cm, sessile, obtuse at base and apex, margin entire, pubescent adaxially and possess distinct mid rib (Fig 4.18 B).

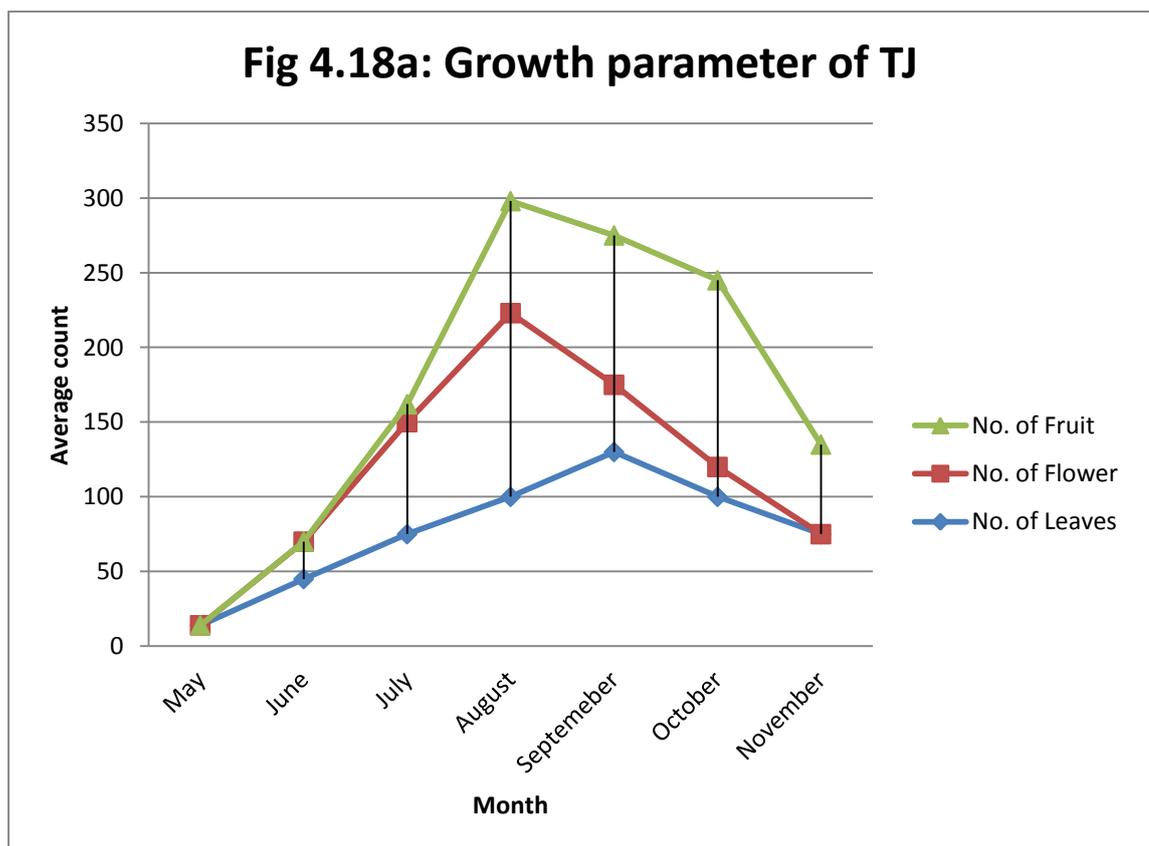
First leaf: simple, alternate, green, linear, 1.8 X 0.5 cm , petiole 2 mm long, apex sub acute, margin entire, adaxially subglabrous and abaxially silky villous, reticulate venation (Fig 4.18 C).

Second leaf: simple, alternate, green, linear, 2.8 X 0.5 cm , petiole 2.5 mm long, apex subacute, margin entire, adaxially subglabrous and abaxially silky villous, reticulate venation (Fig 4.18 D).

Third leaf onwards : Simple, 3.0-6.4 cm long, 6-9.5 mm board, stipules subulate, up to 3 mm long, uninerved, pubescent on margin; shortly petiolate up to 2-4 mm with silky appressed hair; lamina oblong – linear or elliptic linear, rounded to attenuate at the base, subacute-obtuse at apex, rarely mucronate, adaxially sub glabrous (Fig 4.2 B) and silky villous abaxially (Fig 4.2 C), lateral nerve 25-30, margin entire, reticulate venation with alternate phyllotaxy(Fig 4.18 E&F).

Other observation:

- a. Eophyll (cotyledonary leaves) started yellowing on 10th day and remain present till 4th to 6th leaves stage. It sheds fully after 15th day.
- b. Few root nodules are seen from lateral tap roots.
- c. From the axils of 7th to 12th leaves branching of main axis starts after 31th day (Fig 4.18G).
- d. From the axils of 5th leaves flowering bud start to arise after 31th day and flowers opens at 36th day (Fig 4.18H).
- e. Fruiting start after 50th day of germination
- f. The morphological variation seen was formation of compound leaves with three leaflets after 99th day of germination (Fig 4.18I).



Above graph show that the average growth changes. It highlight the fact that exponential growing phase of TJ is high in between month of July to October when monsoon is at its peak.

Phenology chart of *T. jamnagarensis*



Fig 4:18- A-Germinated seed, B- Eophyll, C- First leaves stages, D- Second leaf stage, E- Third leaf stage, F- fifth leaf stage, G- Branching E- Flower buds, I-Variation in leaf formation.

T. collina seedlings were transplanted in the month of May 2012. The vegetative phase of its life cycle remains prominent till the month of August. Toward the end of August flower bud started to come. In September and October, flowering of this plant was at its peak. The fruit formation started towards the end of September and remains till November. In November the plant completes its life cycle (Table 4.4.3).

➤ **The phenological changes in *T. collina*:**

Germination started on 4/5/2012 2nd day after seed treatment.

Eophyll: 2, opposite, dark green, lanceolate, 1.2 X 0.5 cm, rounded at apex, sessile, margin entire (Fig 4.19a B).

First leaf: simple alternate, green 3 X 0.5 cm long, petiolate 0.3 mm, stipules 5 mm, apex emarginate to mucronate, margin entire, pubescent abaxially (Fig 4.19a C)..

Second leaf: imparipinnate compound with 3 leaflets, alternate, green, 5.8 cm long petiolate 0.3 mm, stipules 5 mm, rachis up to 2.8 cm sub glabrous, terminal leaflet 3.8 X 0.6 cm long and side leaflets 3.2 X 0.6 cm long, each leaflet is oblong, apex emarginated to mucronate, margin entire, pubescent abaxially (Fig 4.19a D).

Third leaf: imparipinnate compound with 5 leaflet, alternate, green, 6.8 cm long, petiolate 0.5 mm, stipules 5 mm, rachis up to 3.6 cm sub glabrous, terminal leaflet 3.2 X 0.6 cm long and side leaflets 3 X 0.6 cm long, each leaflet is oblong, apex emarginated to mucronate, margin entire, pubescent abaxially (Fig 4.19a E).

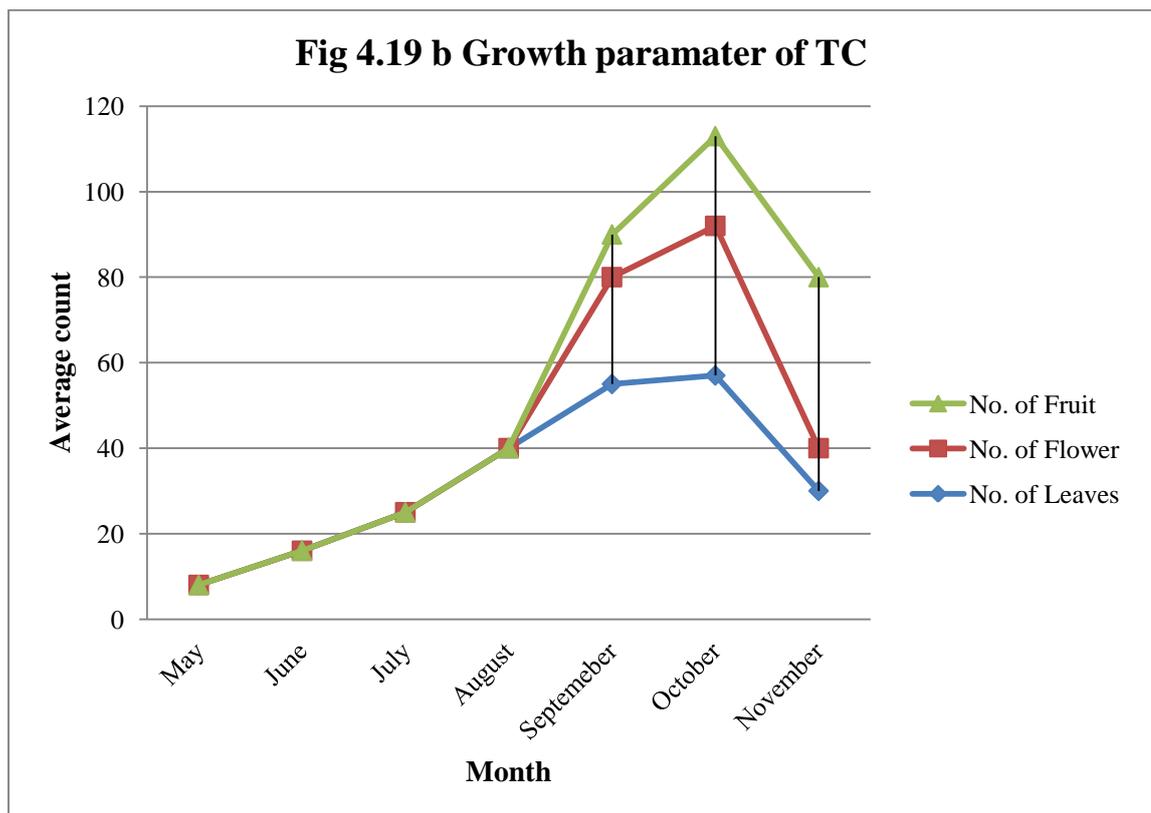
Fourth leaf: imparipinnate compound with 7 leaflet, alternate, green, 8.2 cm long petiolate 0.6 mm, stipules 7 mm, rachis up to 4.8 cm sub glabrous, terminal leaflet 3.8 X 0.6 cm long and side leaflets 3.2 X 0.6 cm long, each leaflet is oblong, apex emarginated to mucronate, margin entire, pubescent abaxially (Fig 4.19a F).

Fifth leaf: imparipinnate compound with 9 leaflet, alternate, green, 10.1 cm long petiolate 1.5 cm, stipules 7mm, rachis up to 7 cm sub glabrous, terminal leaflet 4 X 0.7 cm long and side leaflets 3.6 X 0.6 cm long, each leaflet is oblong, apex emarginated to mucronate, margin entire, pubescent abaxially (Fig 4.19a F).

Sixth leaf: 11th leaflet (foliolate); 7th leaf: 13th leaflet; 8th leaf 17th leaflet (Fig 4.19a G); 9th leaf: 19th foliolate; 11th-13th leaf: 21- foliolate, 14th leaf onwards: 23th leaflet rachis up to 16 cm.

Other observation:

- a. Eophyll started yellowing on 19/5/2012- 15th day and it full shed on 31/5/2013-27th day.
- b. Root nodules were not seen.
- c. The shoot system grows up to 64 cm on 54th day.
- d. Axillary bud started developing into branches on 54th day from 12th leaf axils.
- e. The pair last leaflet move closure to main axis and also become single.



Above graph show that the average growth changes. It highlight the fact that exponential growing phase of TC is high in its vegetative phase till month of August. Flower and fruiting stage dominate in later stage of development in month between September to November when monsoon is at its retreating phase and their lag phase between monsoons at winter. This period will help in maturation of pods without excess of humidity which might affect the pods and seeds growth.

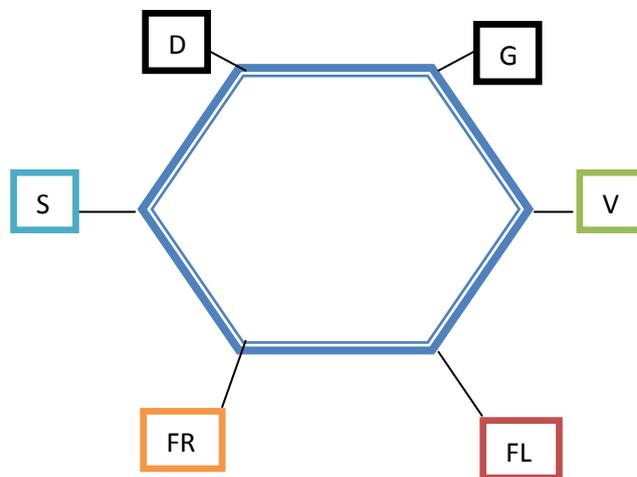
Phenology chart of *T. collina*



Fig 4.19a: A-spouted seed, B- first leaf, C- Second leaves stages, D- Third leaf stage, E- Four leaf stage, F- Six leaf stage, G- ninth leaf stage, E- Flowering and fruiting stage, I-mature plant.

PHENOLOGY AND VARIATION

The benzene structure given below represents the various phenological stages (Sing and Yagava, 1974) and is used to correlate the growth phase both species of same region and same genus in Table 4.4.4 as per experimental site observations.



G: Seed germination, outlet of leaf stage, V: vegetative stage, FL: flowering period, Fr: Fruiting period, S: seed maturation, D: death (Sing and Yagava, 1974).

Table 4.4.3 Phenological Chart of TJ & TC

Species	MONTHS									
	April	May	June	July	Aug	Sep	Oct	Nov	Dec	
<i>T.jamnagarensis</i>										
<i>T. collina</i>										

It was observed that *T. jamnagarensis* complete its life cycle earlier than that *T.collina*. Moreover, *T.collina* has very long vegetative phase as compare to *T. jamnagarensis*.

The phenological studies of these species highlight similarity with other *Tephrosia* species. This includes the time taken for the maturity of the plant. It ranges between six month in *T. jamnagarensis* and seven month in *T. collina*. This fact is also observed in other species like *T. candida*, *T. purpurea* and *T. vogelii* wherein time taken for maturity ranges between 4-7 months (Chadha, 1972). The second character is initial first two growth leaves of both these plant are simple leaf. This character is typical growth features is corresponding

other *Tephrosia* species with imparipinnate compound leaves. This feature was also observed by Augustine (2000) in *Tephrosia* species like *T. purpurea*, *T. maxima*, *T. pumila* and *T. villosa*. As further growth progress *T. jamnagarensis* retain the character of simple leaf while *T. collina* and other *Tephrosia* species develop the compound imparipinnate leaves. In *Tephrosia* species mature compound leaves develops gradually with increases in number of leaflets from 3 to 7/9 leaflets stage. This growth pattern was seen during phenological observation of *T. collina*. Height of the targeted plant showed increases in length at cultivated site, in *T. jamnagarensis* it was 60 cm while in *T. collina* 140 cm which is varies from natural fallow land (c.f. habit pg 90 & 98). Targeted plants also showed the development of a few root nodules which is also a phenological events common with of *Tephrosia* species like *T. purpurea*, *T. maxima*, *T. pumila* and *T. villosa* (Augustine, 2000); *T. vogelii* (Rutunga *et al.*, 1999).

One of the key characters in *Tephrosia collina* is that the last leaflet shifts closer to the main stem axis at their maturity. This character has been also observed in common species like *T. purpurea* and *T. villosa* (Fig 4.19c). However, occasionally in *T. collina* the last leaflet pair becomes unpaired.



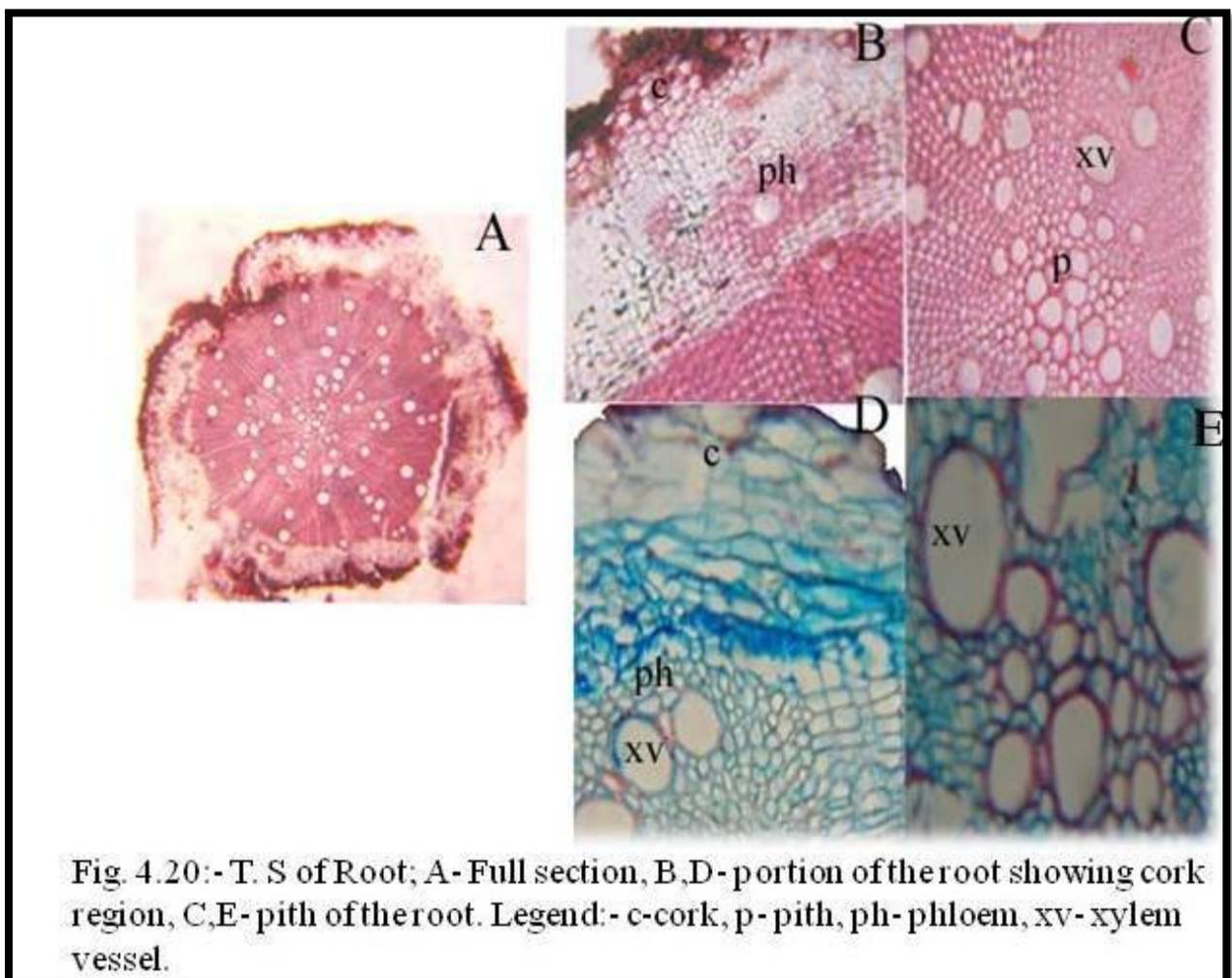
Fig: 4.19c Variation in arrangement of last leaflets of *T. collina* in comparison with *T. purpurea* and *T. villosa*

A- *T. collina*, B- *T. purpurea*, C- *T. villosa*

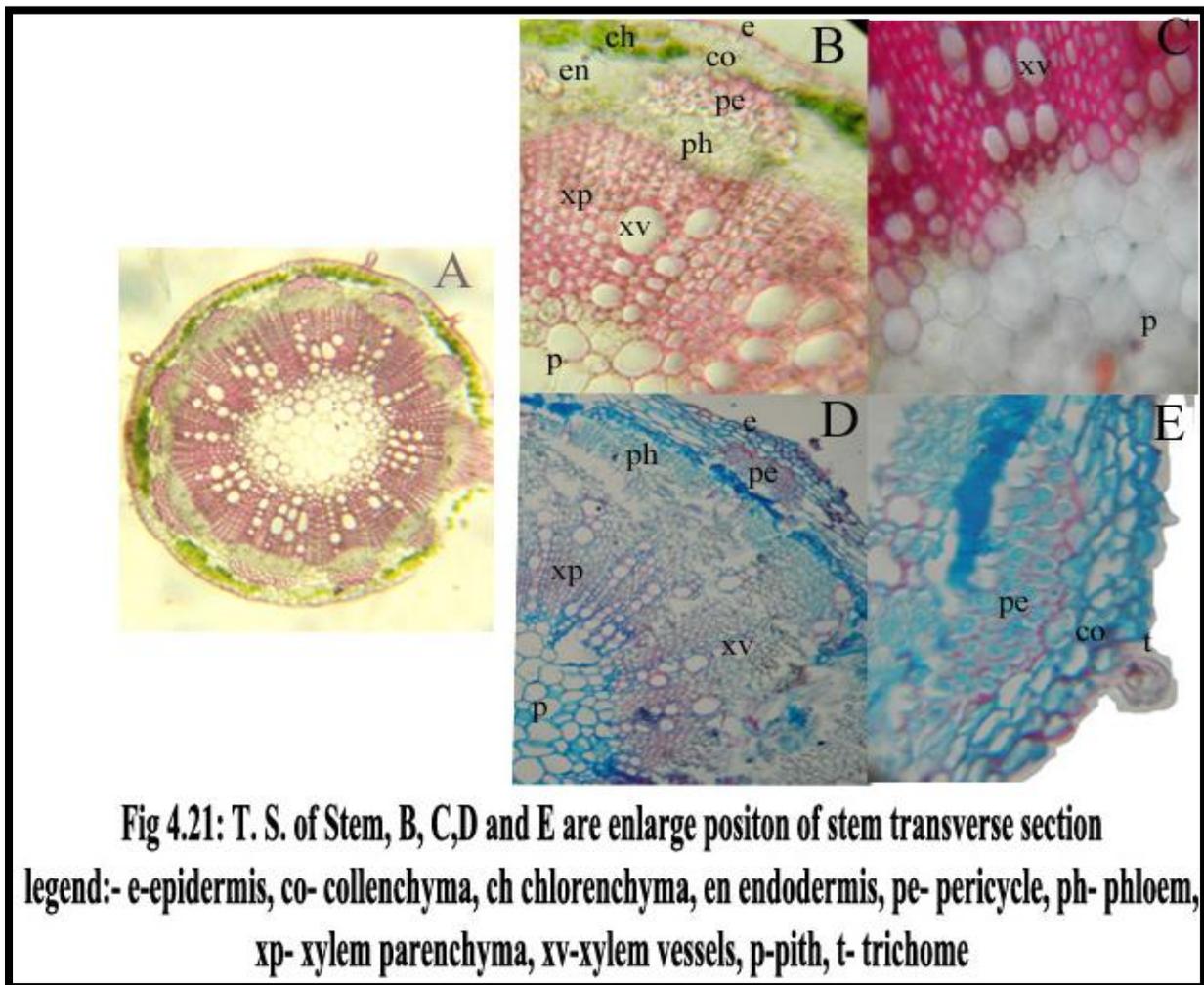
4.5 PHARMACOGNOSY

4.5.1 ANATOMY

The transverse section of root of *T. jammagarensis* is circular in outline with 0.45 - 0.54 mm in diameter (Fig 4.20A). The outer most layers shows 7-10 layered dark brown cork cell. The cortex is narrow composed of 7-8 layers parenchymatous cells (Fig 4.20 B and D). The stellar region shows absence of the endodermis and pericycle. The cortex is interrupted by the phloem (125-85 μ m) which shows prominent phloem fibre patches (25-62.5 μ m). The xylem tissue composed of xylem vessels, xylem fibres and xylem parenchyma which stores rhomboidal calcium oxalate crystals. Pith is absent (Fig 4.20 C and E).



The transverse section of *T. jamnagarensis* stem is circular in outline of diameter 1 - 0.81mm (Fig 4.21A). The epidermis is single layered shows barrel shaped cells lined by thin cuticle and warty unicellular trichomes (375 X18.75 μ m) (Fig 4.21 E). Hypodermis consists of one layer of collenchyma (27.5 μ m) followed by cortex of 2-3 layers of chlorenchyma (26.67 μ m) patches alternating with parenchyma patches. Endodermis barrel shape cell is distinct above pericycle. Pericycle is composed of interrupted ring of sclerenchyma patch containing of calcium oxalate crystal. Phloem is found in patches separated by large medullar cells (Fig 4.21 B and D). Xylem consists of angular vessels (23.8 μ m), paedomorphic ray with calcium oxalated crystals and xylem fibres. Medullary phloem patches are seen towards the pith. Pith is large, round and parenchymatous.



The transverse section of *T. jamnagarensis* leaf shows lamina segregated into upper epidermis, palisade, spongy and lower epidermis (Fig 4.22A). The upper and lower epidermis composed of large barrel shape cells covered by thick cuticle with anisocytic stomata. The trichomes present on the lower epidermis are warty unicellular trichome (343.5 X 65 μ m). The mid rib shows the presence of large vascular bundle (28.8 μ m) having pericycle on the either side of vascular bundle. The pericycle composed of the sclerenchyma cells containing rhomboidal calcium oxalate crystal. The vascular bundle composed of xylem and phloem. Mesophyll consists of three to four layered palisades (89.6 μ m) and closely packed spongy tissues (22 μ m).

Micromorphology

The leaves and stem of the *T. jamnagarensis* show the presence of the anisocytic stomata (Fig 4.22 B) and warty unicellular trichomes (Fig 4.22 C). The values of the stomatal index, vein termination no, vein islet no and palisade ratio are given in Table 4.5.1.

Stomatal index	18.4 \pm 3.13
Palisade ratio	4.4 \pm 0.5
Vein islet no.	3.6
Vein termination no.	1 or 2

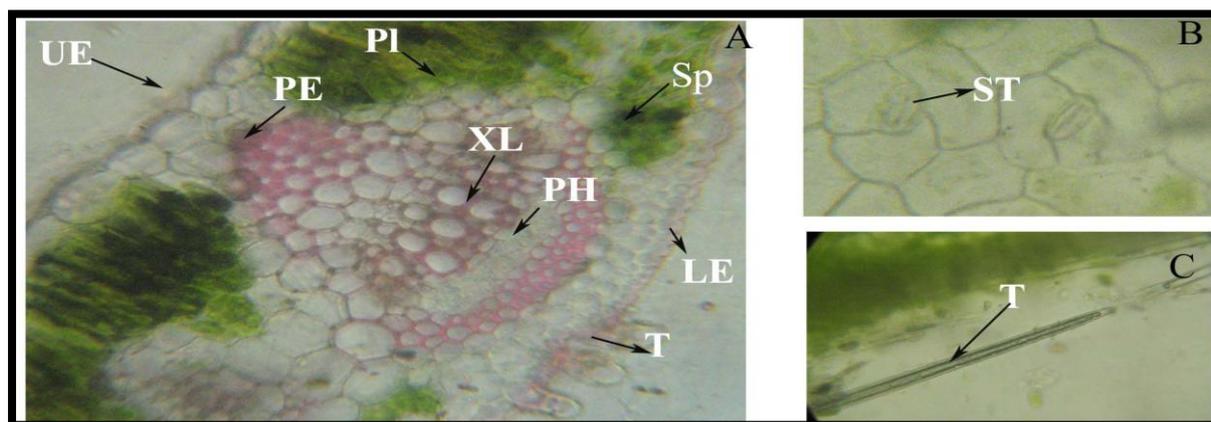
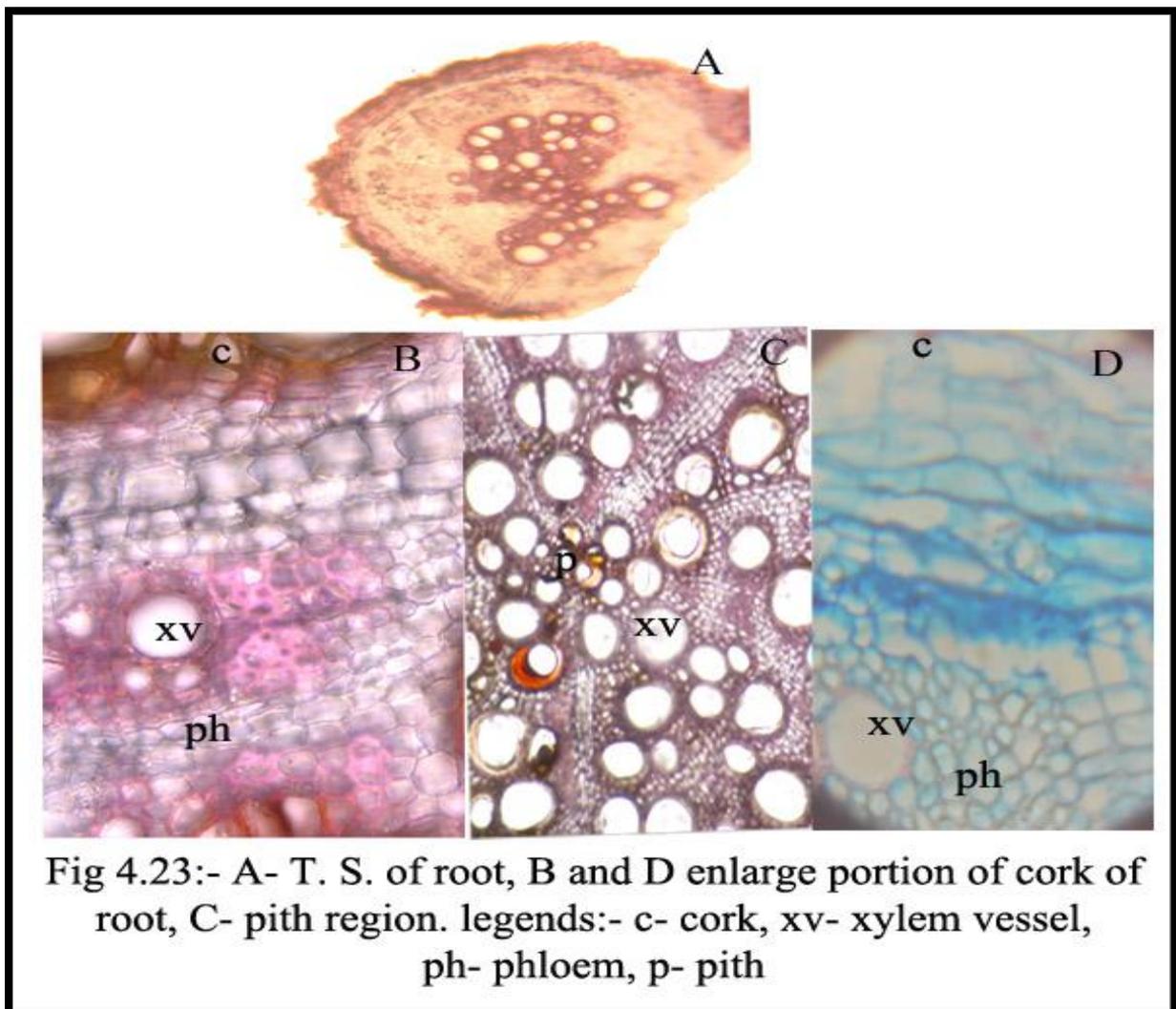


Fig 4.22: A-T. S. of leaf, B- anisocytic stomata(st), C-trichome(T); legends- ue- upper epidermis, pl – palisade, pe- pericycle, x- xylem, Ph- phloem, Sp- spongy tissues, le- lower epidermis.

The transverse section of *T. collina* root is 1-10 mm thick and circular in outline (Fig 23A). The epidermis one layer is replaced by many layer phellem. Phellem arises from outer layer of cortex (Fig 23 B and D). The cork is 198-365 μm composed of small squares cell of 31-36 X 67-79 μm . The ground tissues of cortex four-five layer composed of parenchyma (81.3-114 X 53-59 μm). The phloem (36-94 X 46-127 μm) is composed of sieve tube and phloem fibre. The 2/3 of the area of root is occupied by secondary xylem composed of xylem fibre, trachieds and xylem vessel of 38-120 X 27-141 μm size. The pith was not observed (Fig 4.23 C).



The transverse section of the stem of *T. collina* is slightly angular in outline with 1-10 mm thickness (Fig 4.24A). The epidermis is single layered (15-29 X 25-59 μm), covered by thick cuticle and uniseriate warty trichomes (241-373 X 12-60 μm) with narrow lumen. The hypodermis consist of collenchymas (29-70 X 15-50 μm) patches alternating with chlorenchyma (117 X 80 μm) patches. The endodermis is single layered, encircling the sclerenchymatous pericycle 3-4 layered (216.4 X 111.1 μm). Pericycle shows presence of prim shape calcium oxalate crystals (28X 49 μm) (Fig 4.24B and D). Secondary phloem (21-44 X 23-45 μm) encircles secondary xylem composed of sieve tube and phloem parenchyma (Fig 4.24 C). The secondary xylem consists of medullary ray, xylem vessel (81.3X 83.36 μm) and trachied (Fig 4.24 E). Pith is parenchymatous show presence of starch grain and centre portion is voids.

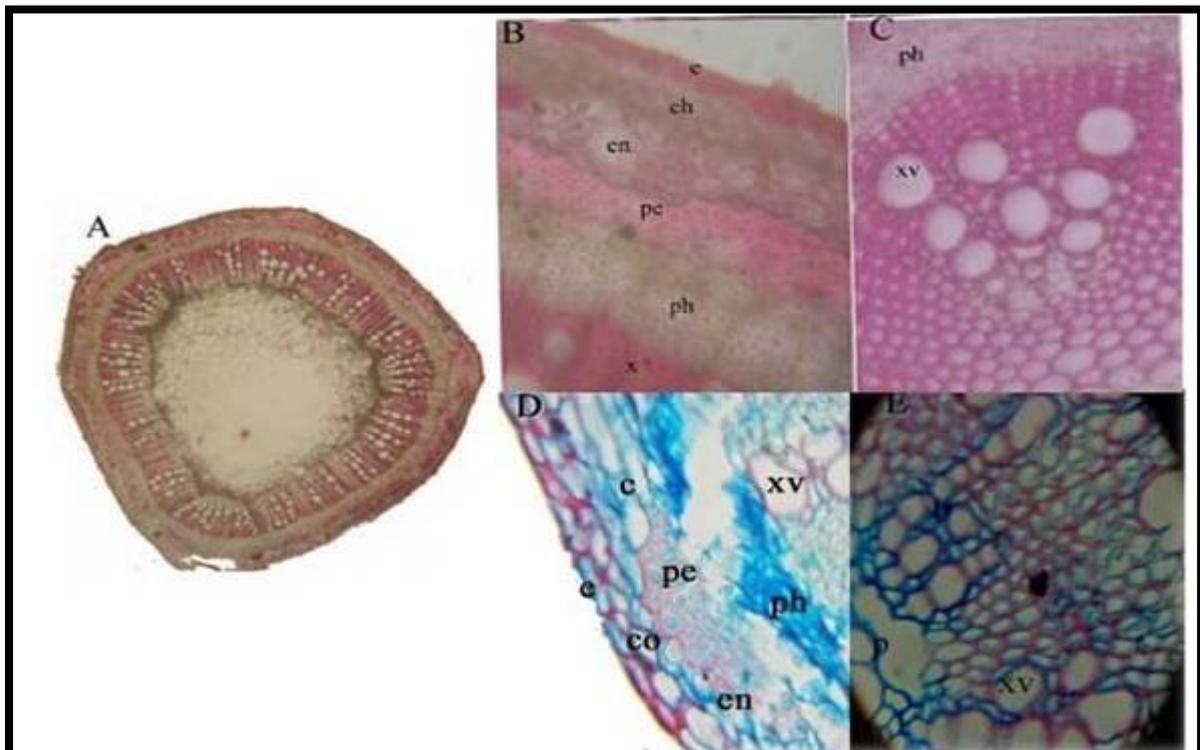


Fig. 4.24:- A-T.S. Of Stem; B,D- Enlarge cortex of stem, C,E- pith of the stem. Legends:- c-cortex, ch-chlorenchyma, co-collenchyma, e-epidermis, en-endodermis, p-pith, pa-parenchyma, pe-pericycle, ph-phloem, x-xylem, xv-xylem vessel.

Transverse section of *T. collina* leaf is dorsiventrally flat. It shows presences of upper and lower epidermis (21-39 X 18-35 μm) covered by thick cuticle (13-28 μm) and along with trichomes and stomata (Fig 4.25). Stomata (47 X 77 μm) are anisocytic type and trichomes are unicellular warty (241-372 X 12-60 μm). The mesophyll tissue is differentiated into palisade and spongy. Palisade composed of elongated, linearly arranged rows of 2-3 cells. Spongy tissue is made up of three layers of rounded loosely arranged cells with little air space. The midrib portion of leaf contains vascular bundles with sclerenchymatous pericycle (97-123 μm) encircling on either side of it. Pericycle shows the presence of prim shape calcium oxalate crystal (73 X 32 μm). Phloem composed of few thin walled cells. Xylem composed of 4-5 smaller size vessels (50-98 X 46-87 μm) and few trachieds with protoxylem pointed upwards. The ground tissue is parenchymatous (36-121 X 46-107 μm) and toward either side epidermis there is double layered of collenchymas (36-50 X 39-54 μm) hypodermis.

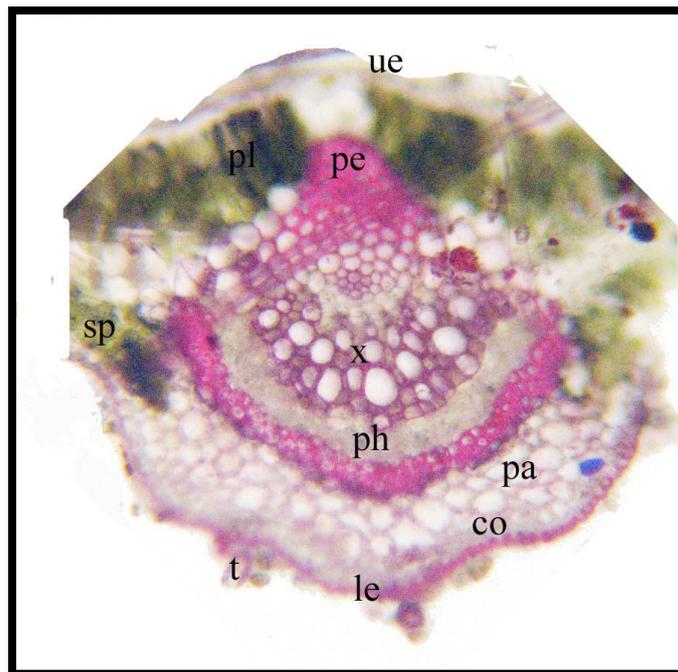


Fig 4.25:- T. S. of leaf ;legends- ue- upper epidermis, pl – palisade, pe- pericycle, x- xylem, Ph- phloem, Sp- spongy tissues, le- lower epidermis.

The leaves and stem of the *T. collina* show the presence of the anisocytic stomata (Fig 4.26A) and warty unicellular trichomes (Fig 4.26B). The values of the stomatal index, vein termination no, vein islet no and palisade ratio are given in Table 4.5.2.

Table 4.5.2: Micromorphology analysis of *T. collina* of leaves.

Parameters	Values
Stomatal index	14 ±0. 02
Palisade ratio	3.57 ±0.83
Vein islet No	2.95
Vein termination No.	1or 2

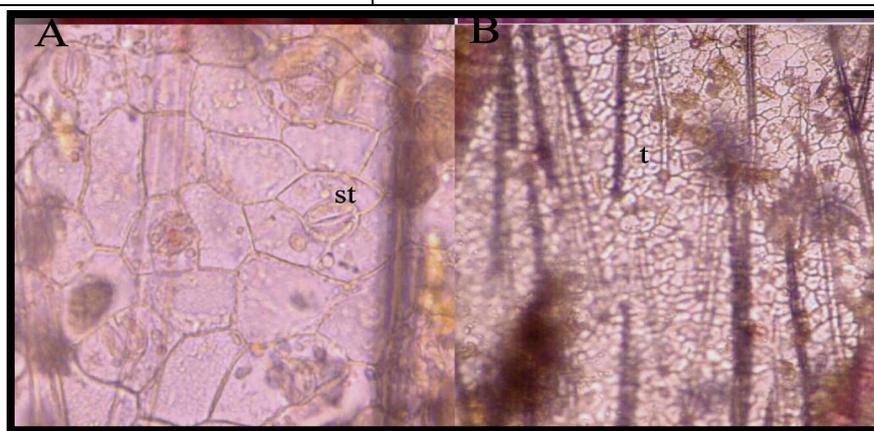
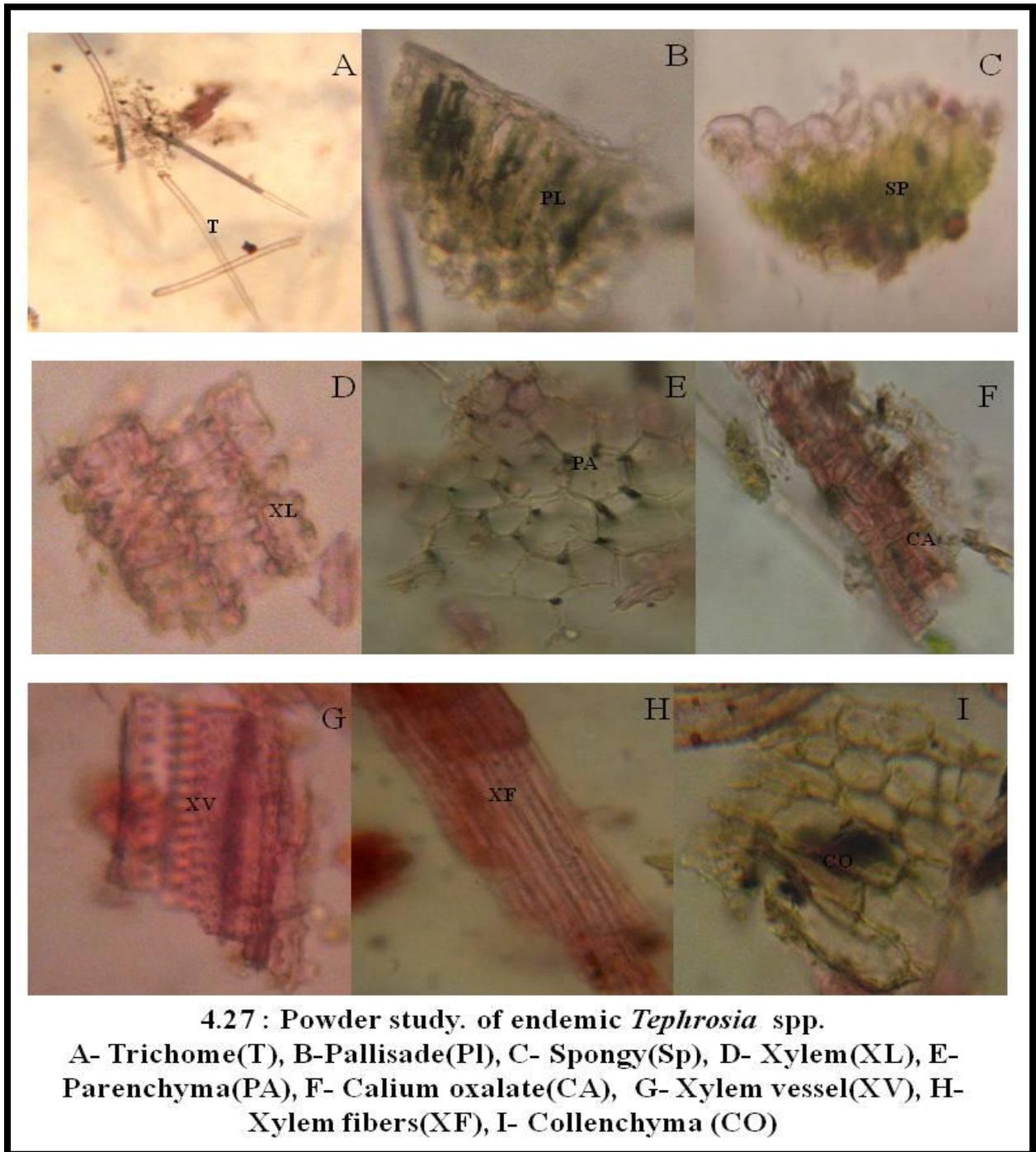


Fig 4.26:- A- stomata (St), B- Trichomes (T)

Pharmacognostic study provides the simplest and quickest means of establishing the identity and purity of herbal medicines. Microscopic analysis of plant is based on the observation of specific anatomical marker present in transverse section and powder study. *T. jamnagarensis* discerning anatomical features are warty unicellular trichomes, rhomboidal calcium oxalate crystal and anisocytic stomata while paedomorphic ray in stem are integral part for identifying and quality of a crude drug. The microscopic features of *T. collina* are presence of warty unicellular trichomes, rhomboidal calcium oxalate crystal and anisocytic stomata. In *T. purpurea* has stem and root with two cylinder of secondary growth. *T. purpurea*'s root transverse section xylem parenchyma occurs in thick targential bends. They are paratracheal and bended. Xylem rays are fairly wide and straight (Gopalakrishnan *et al.*, 2009). In transactional view of *T. maxima* secondary xylem appeared as a circular cylinder with three wide lobes cleaved by three dilated xylem rays. The rays were narrow in the centre and gradually wider towards the periphery (Sandhya *et al.*, 2011).

4.5.2 POWDER STUDY

The microscopic observations of all three plants parts of both endemic plants showed prominent characteristic. The warty unicellular elongated trichome, anisocytic stomata, palisade, spongy, parenchyma, collenchyma in powder of leaves and stem, chlorenchyma in stem, cork cell in root powder, xylem vessel showing large rhomboidal calcium oxalate crystals, xylem vessel with annular and pitted thickening are the characteristic features (Fig 4.27).



4.5.3 HISTOCHEMICAL TEST

The results of histochemical detection are furnished in Table 4.5.3.

Table 4.5.3: Histochemical analysis of <i>T. jamnagarensis</i>				
Cell content	Reagent used	Root .T.S.	Stem T.S.	Leaves T.S.
Tannin	Ferric chloride	-	-	-
Calcium oxalate	Concentrated Hydrochloride	+	+	+
Starch grain	Iodine	-	-	-
Alkaloid	Dragendroff	+	+	ND
Mucilage	Ruthenium red	+	+	+
Cellulose	Iodine and sulphuric acid	+	+	+
Alueron grain	Iodine	-	-	-

+: mean test is positive, -: mean test is negative, ND: mean not determine.

Table 4.5.4: Histochemical analysis of <i>T. collina</i>				
Cell content	Reagent used	Root T.S.	Stem T.S.	Leaves T.S.
Lignified cell wall	Phloroglucinol and Hydrochloride	+	+	+
Calcium oxalate	Concentrated Hydrochloride	+	+	+
Starch grain	Iodine	-	+	-
Tannin	Ferric chloride	ND	ND	ND
Cellulose	Iodine and sulphuric acid	+	+	+
Alkaloids	Dragendorff	ND	ND	ND
Aleurone grains	Iodine	-	+	-

+: mean test is positive, -: mean test is negative, ND: mean not determine.

The histochemical analysis in both these endemic species showed that tannin, alkaloids were absent while calcium oxalate and cellulose were present.

4.6 PHYTOCHEMISTRY

4.6.1 PHYSICOCHEMICAL STANDARDIZATION

Physicochemical standardization of crude drug was done by determination of various parameters like foreign matter, solvent extractive values, ash values (total ash, acid insoluble ash) and loss on drying (Mukherjee, 2007). The values of aerial parts and root of *T. jamnagarensis* (TJA, TJR) and *T. collina* (TCA, TCR) were compared with *Tephrosia purpurea* aerial parts (TPA) and root (TPR) observation furnished by Gopalakrishnan *et al.*, (2009).

Table 4.6.1 Physicochemical properties of TJ and TC

Sr. no.	Parameters	Aerial parts			Roots		
		TJA	TCA	TPA*	TJR	TCR	TPR*
1	Foreign organic matter %	0.016	0.024	-	0.0189	0.0026	-
2	Loss on drying %	6	7.2	3.96	10.2	7.6	6.28
3	Total ash%	4.15	9.4	5.64	5.84	2.16	3.75
4	Water soluble ash%	0.83	2.58	1.18	1.45	1.09	1.63
5	Acid insoluble ash%	0.37	1.02	2.69	0.59	0.23	2.45
6	Water extractive	11.5	12.75	15.26	7.36	0.65	12.69
7	Alcohol extractive	6.75	5.43	20.25	1.75	3.75	16.18
8	Swelling index	Absent	Absent	-	Absent	Absent	-
9	Foaming index	<1	<1	-	<1	<1	-
10	Heavy Metal Analysis						
	Lead (ppm)	ND	ND	-	ND	ND	-
	Cadmium (ppm)	ND	ND	-	ND	ND	-
	Arsenic (ppm)	1.596	0.826	-	1.023	ND	-
	Mercury (ppm)	0.051	0.055	-	0.064	0.060	-

• * sign indicate that the values are taken from Gopalakrishnan *et al.*, 2009.

- The above analysis showed that the amount of the foreign organic matter in aerial parts and roots of both the endemic species were within the permissible limit of W.H.O. (Anonymous, 2002).

- The moisture content percentage was high in the roots of *T. collina* and *T. jamnagarensis* in comparison to their aerial parts. However, such similarity was seen in *Tephrosia purpurea* where in root's moisture content was double of its aerial part (Gopalakrishnan *et al.*, 2009).
- The total ash content percentage was higher in aerial parts in comparison to their root of targeted species as well as reference species. The maximum value of total ash content was found in *T. collina* aerial parts.
- The water soluble ash value was high in *T. collina* aerial part however, in their roots samples it was ranging between 1-1.65%.
- The acid insoluble ash value was double in *T. purpurea* aerial parts and roots in comparison to the endemic plants. In these plant, acid insoluble ash values was high in aerial part of *T. collina*.
- The water extractive values were high in the aerial part of *T. jamnagarensis* and *T. collina*. However in *T. purpurea* alcohol extractive values were four times high in aerials parts and roots in comparison to endemic species values (Gopalakrishnan *et al.*, 2009).
- Both the endemic plant showed negative inference for the parameter like swelling index and foaming index indicate absence of saponin.
- The heavy metal analysis of aerial and roots of *T. jamnagarensis* and *T. collina* showed that metals like lead and cadmium were totally absent while metal like arsenic and mercury were detected. The content of arsenic metal was found above the WHO permissible limit in *T. jamnagarensis* and *T. collina* aerial and roots parts.
- Various physicochemical parameters showed that the biomaterial was of appropriate quality for further analysis with exception of Arsenic.

4.6.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis was done qualitatively and quantitatively for total carbohydrate content, total protein content, total flavonoids content and total phenolic content. The aerial parts, roots and seeds extracts of *T. jamnagarensis* and *T. collina* were analysed.

4.6.2.1. QUALITATIVE ANALYSIS

The preliminary phytochemical analysis of the crude extracts of different plant parts of TJ and TC gave the clues regarding the presence and absence of phytochemicals in crude extract of plants.

- From the preliminary phytochemical analysis it was found that phytochemicals like carbohydrates, protein, flavonoids, steroids and phenolic acids were present in all the plant parts of TJ and TC while alkaloid, tannin, iridoides and saponins were absent (Table 4.6.2.1). However, from preliminary phytochemical analysis of *Tephrosia purpurea* the phytochemicals present were flavonoids, reducing sugars, phenolic compounds and alkaloids in all parts. Steroids and saponin were present only in aerial parts. The other components like terpenoids and xanthoproteins were absent in parts of *T. purpurea* (Gopalakrishnan *et al.*, 2009).
- The phytochemical terpenoid was present in the extracts of aerials as well as root of both the species.
- Lignans were present in aerial parts and roots of *T. jamnagarensis* and *T. collina*.
- Anthocyanin was present in *T. collina* aerial parts and roots extracts of both the plants.
- The phytochemical quinone was present in all the different plant parts of both the species except in the *T. jamnagarensis* aerial parts and *T. collina* seed.

Table 4.6.2.1 Preliminary phytochemical analysis of TC and TJ

Phytocomponent group	Aerials Parts		Roots		Seeds	
	TJA	TCA	TJR	TCR	TJSE	TCSE
Carbohydrates	+	+	+	+	+	+
Protein	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Tannin	-	-	-	-	-	-
Terpenoids	+	+	+	+	-	-
Steroids	+	+	+	+	+	+
Saponins	-	-	-	-	-	-
Lignans	+	+	-	+	-	-
Anthocyanins	-	+	+	+	-	-
Iridiodes	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Quinones	-	+	+	+	+	-
Phenolic acids	+	+	+	+	+	+

*The + sign indicates positive inference for the biochemical test for corresponding the Phytocomponent stated and similarly – sign for the negative inference. The details

Abbreviations: *T. jamnagarensis* (TJA), *T. jamnagarensis* Root (TJR), *T. jamnagarensis* seed (TJSE), *T. collina* aerial parts (TCA), *T. collina* root (TCR) and *T. collina* seed (TCSE)

4.6.2.2 QUANTITATIVE ANALYSIS

The Quantitative analysis (Table 4.6.2.2) showed the following results

- Total carbohydrate content as well as the total phenolic content was high in the aerial part of *T. jamnagarensis* and *T. collina*.
- Total protein content was high in the *T. jamnagarensis* root and *T. collina* seeds.
- Total flavonoids content was high in *T. jamnagarensis* root and *T. collina* aerial parts. On contrary, in *T. purpurea* total phenolic and total flavonoids content was high in roots methanolic extract instead of aerial parts methanoic extracts (Nile and Khobragade, 2011).

Table 4.6.2.2 Quantitative analysis of phytochemicals in TJ and TC extracts

Sr. no.	Parameters	Aerial Parts		Roots		Seeds	
		TJA	TCA	TJR	TCR	TJSE	TCSE
		(in mg)					
1	Total Carbohydrate Content (mg of glucose)	99	99	42	25	66	23
2	Total Protein Content (mg of BSA)	0.6	30	36	24	30	34
3	Total Phenolic Content (mg of gallic acid)	80	92	9	59	74	44
4	Total Flavonoids Content (mg of quercetin)	0.98	1.32	1.45	1.05	1.02	1.05

4.6.3 PHYTOCHEMICAL MARKER STANDARDIZATION

From the preliminary phytochemical analysis of TJ and TC it was found the polyphenols component like flavonoids, steroids, phenols and terpenoids were the major constituents. Thus the further analysis of these phytocomponents were done using the chromatography technique like planar chromatography that includes Paper chromatography (PC), Thin layer chromatography (TLC), Column chromatography (HPLC, GC-MS, LC-MS/MS) and NMR. The isoflavonoid Rotenone which is the key phytocomponent in *Tephrosia* genus was also analysed in the various parts of these plants.

CARBOHYDRATES

- Carbohydrates analysis was indicative of presences of different reducing (Glucose, Mannose, Maltose, Arabinose Fructose, Ribose, Xylose and lactose) and non reducing sugars (Sucrose) in the plant material. In the present study TLC analysis of carbohydrate was done with the standard procured from Sigma Aldrich along in aerials parts and root of *T. jamnagarensis* and *T. collina* (Fig 4.6.1). The results showed that the
- Aerial parts of both the plant showed presence of Glucose, Mannose, Maltose, Arabinose and lactose. *T. collina* aerial parts also showed presence of Fructose, Ribose and Xylose.
- Roots of both plant showed presence of Fructose, Glucose, Maltose, Ribose and Xylose. Along with this sugars *T. jamnagarensis* root extracts also showed presence of Sucrose and Mannose.
- **Galactose was only sugar absent in all plant parts of both species (Table 4.6.3.1).** Although, galactose and mannose had been reported in *Tephrosia tinctoria* (Kumar and Prasad, 2011).
- **Although the sugars detected from *T. collina* aerial parts and root, the GC MS analysis of its seed extract showed the presence of sugar alcohol D- Mannitol (Fig 4.6.2).** *Tephrosia vogelii* had metabolic pathways for D mannose degradation occurs to utilize the extracellular sugars (<http://ensembl-sigenae.jouy.inra.fr/META/NEW-AGE?type=ORGANISM&object=TAX-1157238>).

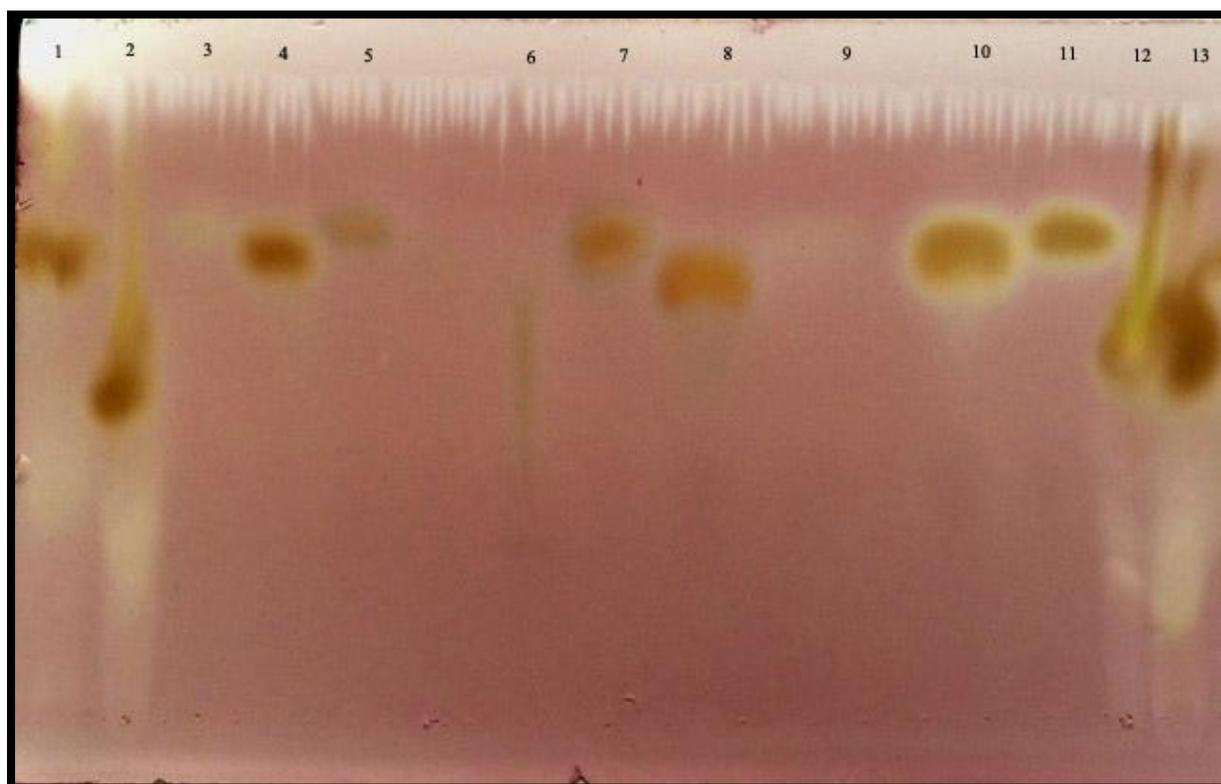


Fig 4.6.1: TLC of sugar Standard with samples.

Details of tracks on TLC plate: Track 1- TJR, Track 2- TJA Track 3- Arabinose, Track 4- Fructose, Track 5- Glucose, Track 6- Galactose, Track 7- Lactose, Track 8- Mannose, Track 9- Maltose monohydrate, Track 10- Sucrose, Track 11- Ribose, Track 12- Xylose, Track 12 – TCA, Track 13- TCR.

Table 4.6.3.1 Sugar analysis in TJ and TC

Std of Sugars	Aerial parts		Roots	
	TJA	TCA	TJR	TCR
Arabinose	+	+	-	-
Fructose	-	+	+	+
Glucose	+	+	+	+
Galactose	-	-	-	-
Lactose	+	+	-	-
Mannose	+	+	+	-
Maltose Monohydrate	+	+	+	+
Sucrose	-	+	+	-
Ribose	-	+	+	+
Xylose	-	+	+	+

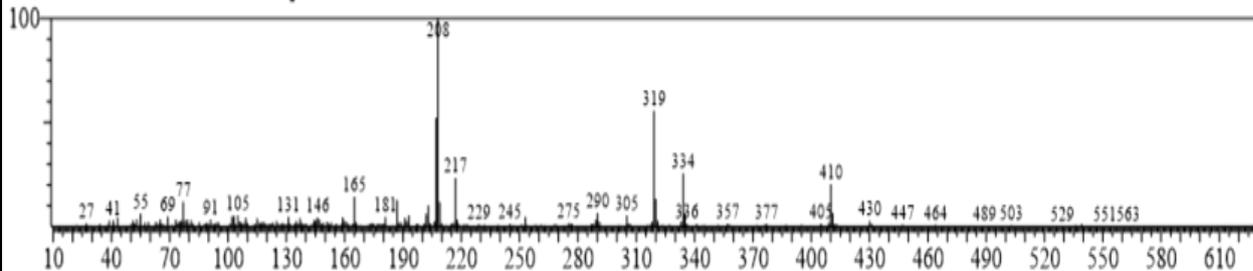
*The + sign indicates positive inference and – sign for the negative inference.

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CompName:D-Mannitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- \$\$ Mannitol, hexakis-O-(trimethylsilyl)-, D- \$\$ 1,2,3,4,5,6-Hexakis-O-(trimethylsilyl)-

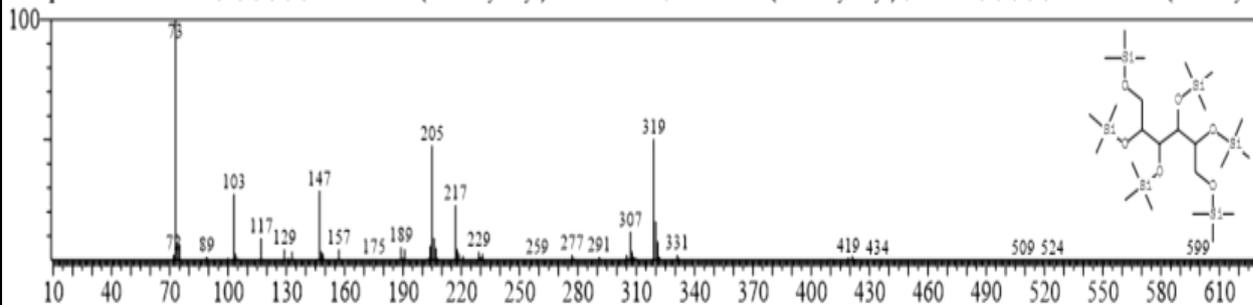


Fig 4.6.2: GC MS of D-mannitol (trimethylsilyl ester)

CARDIAC GLYCOSIDE

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants, but also in some animals. **Strophanthidin (a cardiac glycoside) was detected in the GCMS analysis of *T. collina* aerial part (Fig 4.6.3).** Its mechanism of action is similar to Digitalis, Ouabain and digitoxin. It specifically inhibits the membrane protein Na⁺/ K⁺ ATPase in muscle tissue (heart) which can lead to Ca²⁺ overload, diastolic dysfunction, arrhythmias and ultimately to heart failure and death. Strophanthidin is also present in plant like *Apocynum venetum*, *Acokanthera* sp. and *Corchorus olitorius* which are useful for increasing myocardial contractility and lowers blood pressure. It has been reported from Ranunculaceae, Moraceae, Tiliaceae and Apocynaceae (ESFA, 2009). There are no such records of its presence earlier in family of Fabaceae.

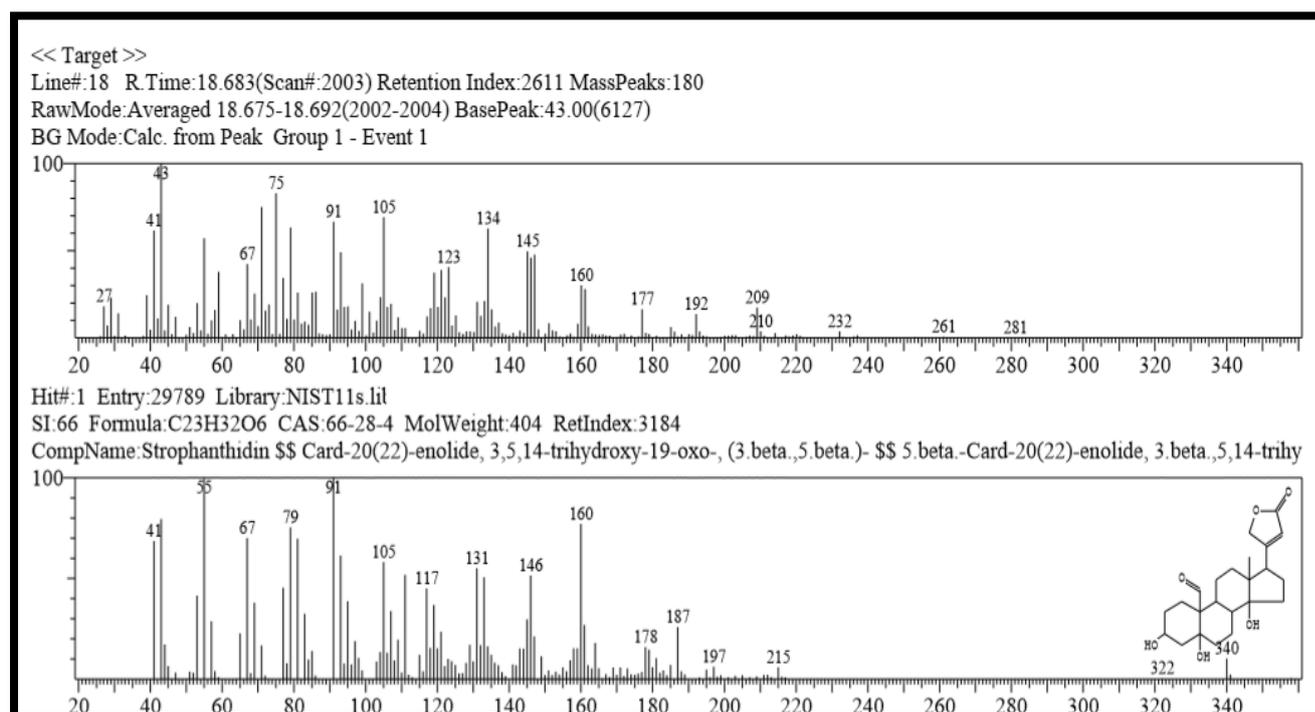


Fig 4.6.3: GC MS of Strophanthidin

AMINO ACID

Amino acids are the basic unit for protein synthesis. The presence of different amino acids in aerial and roots of *T. jamnagarensis* and *T. collina* was done by thin layer chromatography technique with standard of amino acid procured from Sigma Aldrich (Fig 4.6.4). The following were the major observations (Table 4.6.3.2).

- *T. jamnagarensis* aerial parts showed presence of 12 amino acids out of 20 which were Asparatic acid, Glutamine, Glutamic acid, Lysine, Phenlyalanine, Tryptophan, Tryosine, Methonine, Alanine, Leucine, Isoleucine and Serine, While *T. collina* aerial parts showed presence of 13 amino acid with addition of Arginine and Proline while Tryptophan was not detected.
- Similarly root of *T. jamnagarensis* showed presence of 14 amino acids where as in *T. collina* (TCR) about 15 amino acids were detected.
- Of those 20 amino acids studies one essential amino acid **Histidine** and one non essential amino acid **Asparagine** were not detected in the extracts of both the species.
- *T. purpurea* aerial parts showed high content of amino acid, Proline in comparison to its roots. It is helpful for maintaining the water potential and osmoregulation in plant growing in stress condition. The accumulation of this amino acid might helpful in survival of plant in deserts (Earkar and Murumkar, 1995). In present study Proline was detected in roots of both plant and in aerial part of *T. collina* which is indicative both plant are adaptable to semi arid stress.
- The probable amino acid ester detected from the *T. collina* seed was 3,5-pyrimedicarboxylic acid in GC MS analysis (Fig 4.6.5).

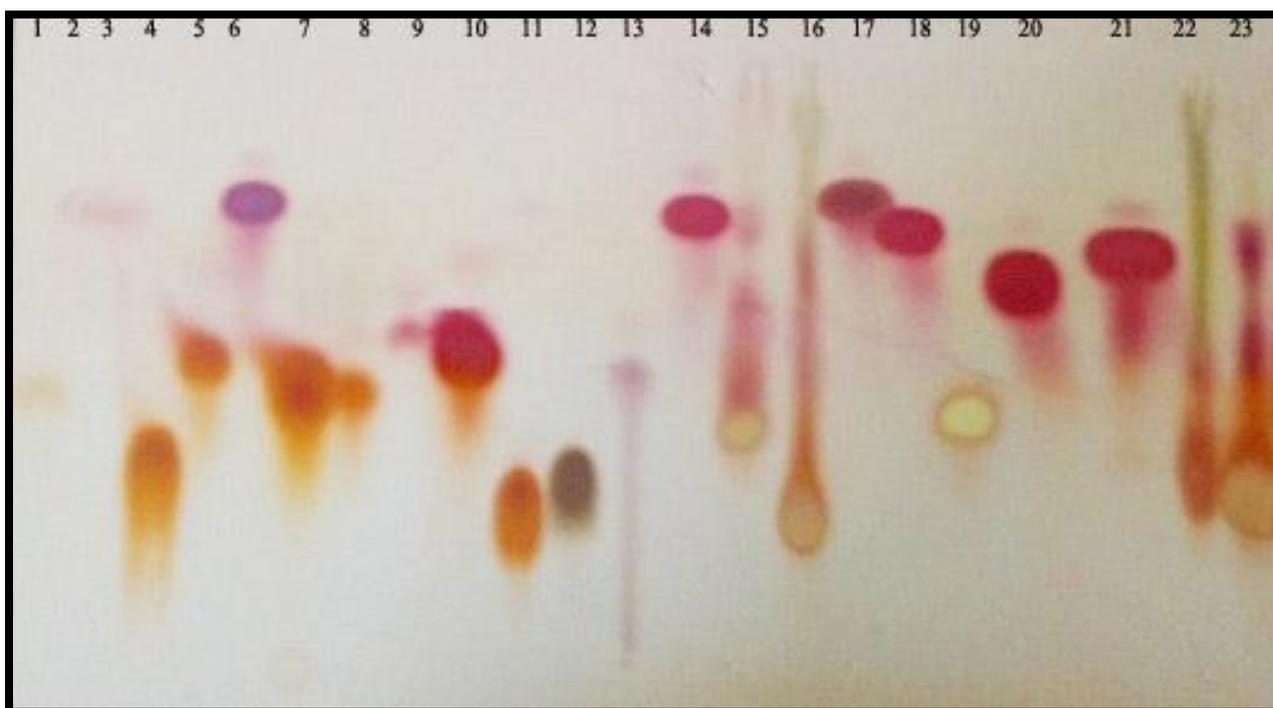


Fig 4.6.4: TLC of Amino acid Standard with samples.

Details of tracks on TLC plate:- Track 1-Asparagine, Track 2-Tyrosine, Track 3-Arginine, Track 4- Theonine, Track 5- Phenylalanine, Track 6- Serine, Track 7-Glutamine, Track 8- Glutamic, track 9- Alanine, Track 10 – Lysine, Track 11- Histidine, Track 12- Aspartic acid, Track 13- Leucine, Track 14- Tryptophan, Track 15- TJR, Track 16- TJA, Track 17- Isoleucine, track 18- Proline, Track 19- Valine, Track 20- Cystine, Track 21- Methonine, Track 22-TCA, Track 23- TCR

Table 4.6.3.2 Amino acids analysis in TJ and TC

Amino acid	Aerial parts		Roots	
	TJA	TCA	TJR	TCR
Asparatic acid	+	+	+	-
Asparagine	-	-	-	-
Glutamine	+	+	-	-
Glutamic acid	+	+	+	+
Histidine	-	-	-	-
Lysine	+	+	-	+
Arginine	-	+	-	-
Phenlyalanine	+	+	+	+
Tryptophan	+	-	-	+

Amino acid	Aerial parts		Roots	
	TJA	TCA	TJR	TCR
Tryosine	+	+	+	+
Cysteine	-	-	+	+
Methonine	+	+	+	+
Proline	-	+	+	+
Glycine	-	-	+	+
Alanine	+	+	+	+
Valine	-	-	+	+
Leucine	+	+	+	+
Isoleucine	+	+	+	+
Serine	+	+	+	+
Theonine	-	-	+	+

The + sign indicates positive inference and – sign for the negative inference.

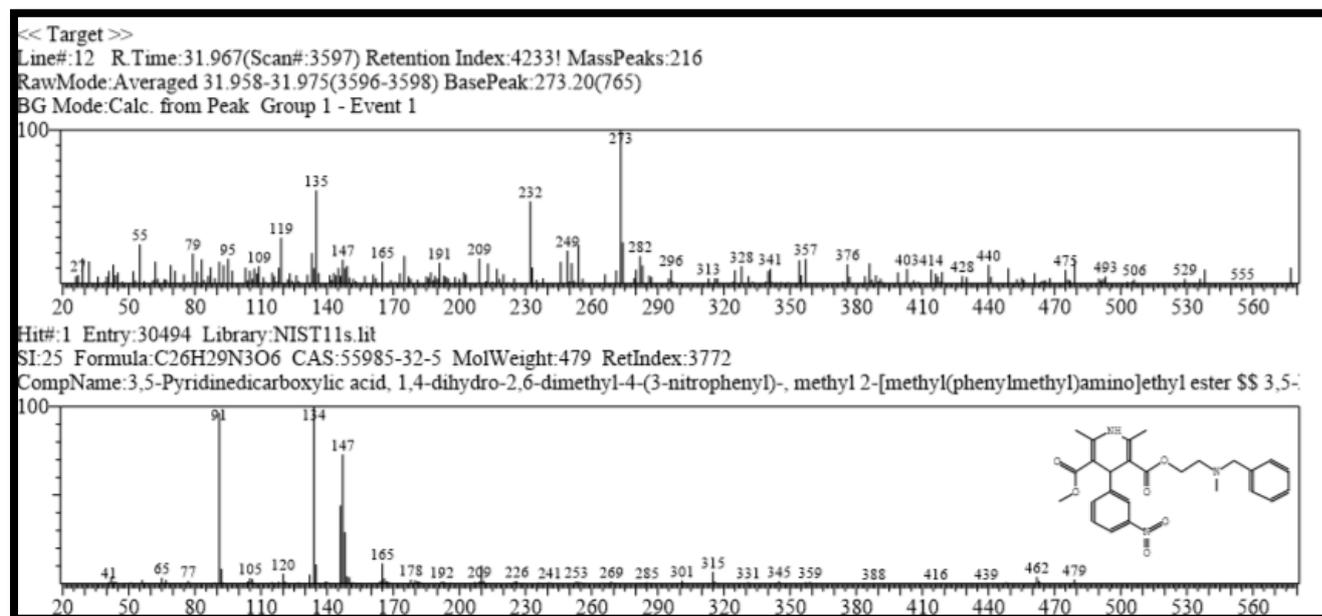


Fig 4.6.5: GC MS of 3,5-pyrimedicarboxylic acid

FATTY ACIDS

Fatty acids are the lubricant present in plant and animal. The GCMS analysis of aerial and seed extracts showed following results;

- *T. jamnagarensis* aerial part showed lipid percentage of 2.766% while in *T. collina* aerial part it was 3.578%.
- *T. jamnagarensis* seed extract lipid percentage was around 3.5% while in *T. collina* seeds was 4%
- The study showed that the lipid composition of the aerial part of the *T. jamnagarensis* composed mainly of saturated fatty acid while that of seed showed presence of the seven fatty acids including both saturated and unsaturated fatty acid, of which 66.73 % were unsaturated fatty acid while 10.87% were saturated fatty acid while about 22.4% were other substances.
- In case of *T. collina* aerial part and seeds lipid composition consist of 70.29% of unsaturated fatty acid and 23.64% of saturated fatty acids.
- *T. jamnagarensis* aerial parts showed presence of pentadecanoic acid, 14 methyl and methyl stearate while aerial part of *T. collina* from their GC MS (Fig 4.6.6-4.6.8) analysis show the presence of fatty acid like Octadecanoic acid, decanedioic acid (Sebacic acid), Trans-2-undecenoic acid and 9,12,15 octadecatrienoic acid(α -Linolenic acid).
- *T. jamnagarensis* seed showed presence of fatty acids like n-Hexadecanoic acid, Decanoic acid, 9,11-Octadecadienoic acid (linoleic acid), 9-Octadecadienoic acid (oleic acid), Oleic acid, 1-hexadecene, octadecanedioic acid and Cis-9-hexadecenal.
- The fatty acids *T. collina* seeds were 9-Octadecanoic acid (49.06%), 9,12-Octadecadienoic acid (13.81%), Octadecanoic acid (5.92%), hexadecanoic acid (2.53%), 11-octadecenoic acid methyl ester and pentadecanoic acid 14 methyl (21.11%) detected by the GCMS analysis.
- The other species of *Tephrosia* like *T. purpurea* had Hexadecanoic acid dominating Fatty acid in the oil of its stem (69.61%) and root (46.97%) while Other common components in the oil were linoleic acid and bulnesol and epiglobulol (Arriaga *et al.*, 2005). *Tephrosia vogelii* seed consisted more amount of hexdecanoic acid (palmitic acid,18.70%) along with tetradecanoic acid, pentadecanoic acid, heptanoic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosanoic acid, heneicosanoic

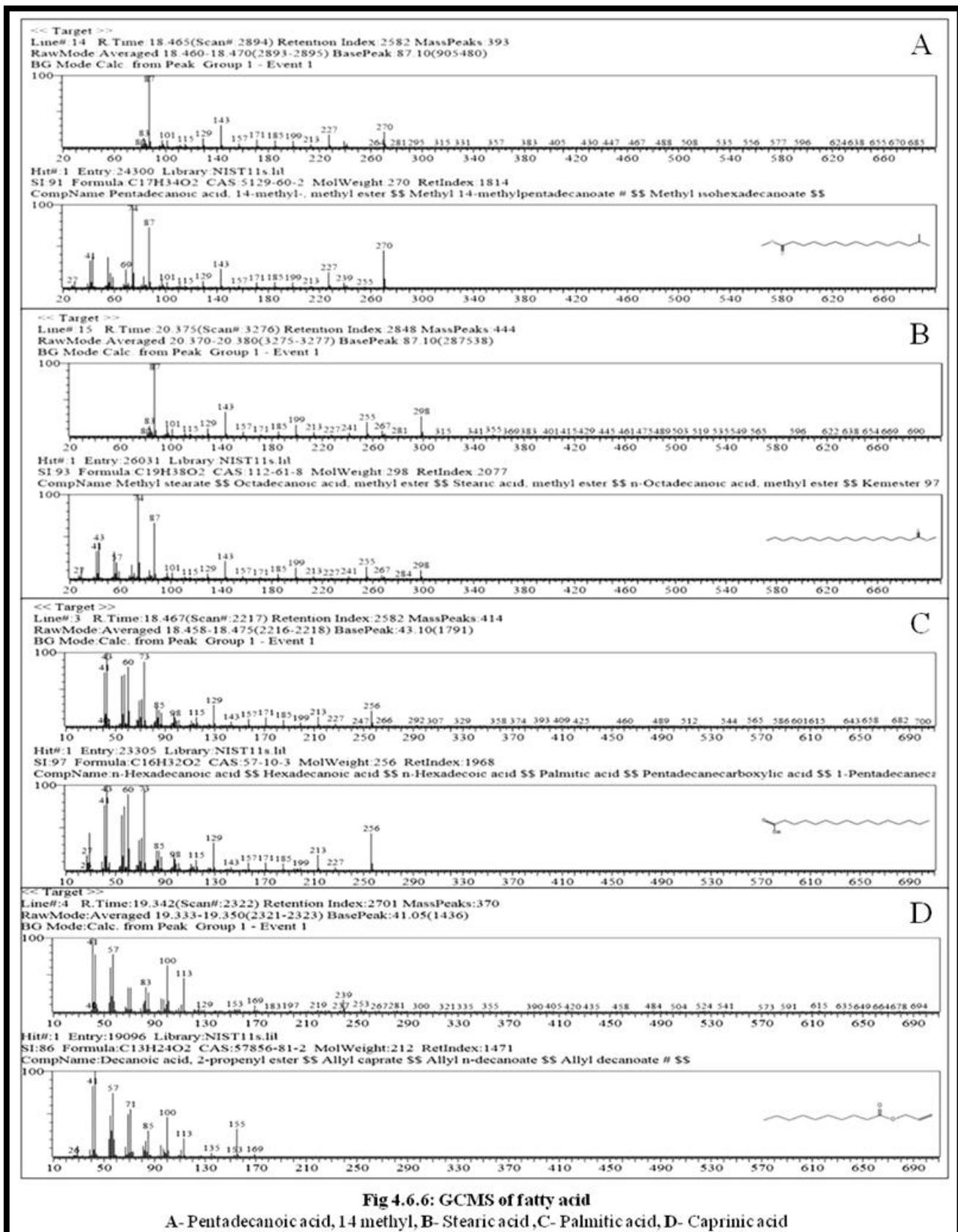
acid, docosanoic acid, tricosanoic acid and tetracosanoic acid (Sahayaraj *et al.* 2014; Yan *et al.*, 2009).

Table 4.6.3.3 Fatty acid profile of *T. jamnagarensis* and *T. collina*

Fatty Acid	Oil %			
	<i>T. jamnagarensis</i>		<i>T. collina</i>	
	Aerial parts	Seed	Aerial parts	Seed
Saturated fatty acid (SFA)		10.87%		23.64%
Palmitic acid (Hexadecanoic acid)		0.47		2.53
Caprinic acid (Decanoic acid)		1.56		
Stearic acid (Octadecanoic acid)	1.16	1.90		
Sebacic acid (Decanedioic acid)			1.41	
methyl 14 methyl pentadecanoate (Pentadecanoic acid, 14 methyl)	3.49			21.11%
Cetene (1 hexadecene)		6.94		
Trans-2-undecenoic acid			3.09	
Unsaturated Fatty acid (UFA)		66.73%		70.29%
Vaccenic acid (9, 11-Octadecadienoic acid).		1.60		13.81
Oleic acid (9, Octadecadienoic acid Z-)		63.11		49.06
Octadec11-enoic acid (11-Octadecenoic acid)				1.50%
linolenic acid (9,12,15-Octadecatrienoic acid, (Z,Z,Z)-)			1.52	
<i>cis</i> 9 hexedecenal (Z-9-		2.02		

Fatty Acid	Oil %			
	<i>T. jamnagarensis</i>		<i>T. collina</i>	
	Aerial parts	Seed	Aerial parts	Seed
hexadecenal)				
Octadecanedioic acid (Hexadecanedicarboxyli acid)				5.92
Fatty acid alcohol				
Behenic alcohol			2.13	

Similarly, palmitic acid, oleic acid and linoleic acid that are detected in *T. jamnagarensis* and *T. collina* were also found presence in *T. villosa* seed oil (Mukarram *et al.*, 1987). Hence it can be said that oil composition of the both these endemic species show some similarity with other species of same genus. Vaccenic acid, is an omega-7 fatty acid, is found in Sea Buckthorn (*Hippophae rhamnoides*) oil. The seed oil of TJ and TC show presence of oleic acid more than 50% on in comparison soyabean oil wherein oil acid value was 20 %. In Soyabean oil there is presence of 54.2% of Linoleic acid where as among both these plant TC aerial part only showed it.



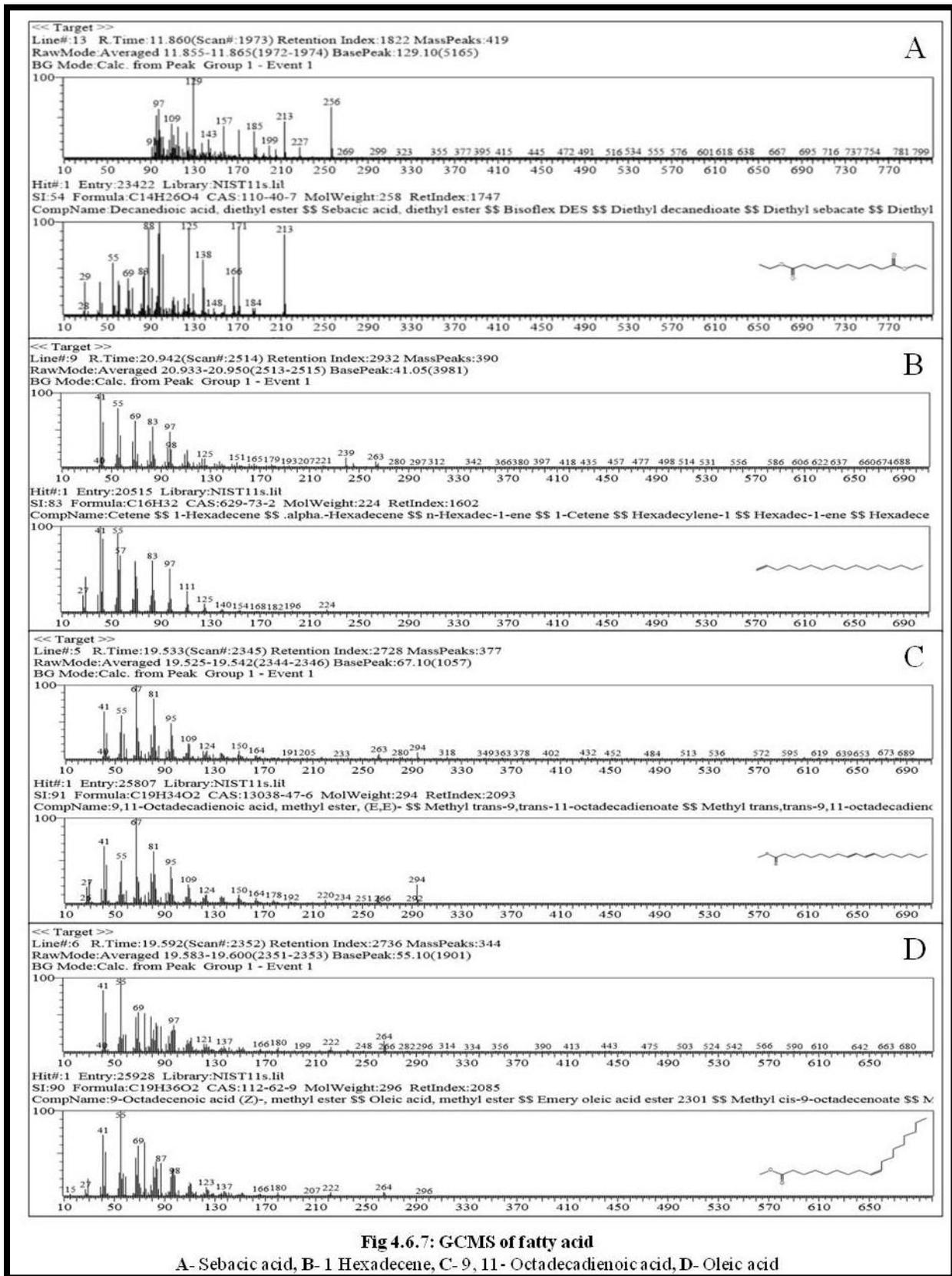


Fig 4.6.7: GCMS of fatty acid

A- Sebacic acid, B- 1 Hexadecene, C- 9, 11- Octadecadienoic acid, D- Oleic acid

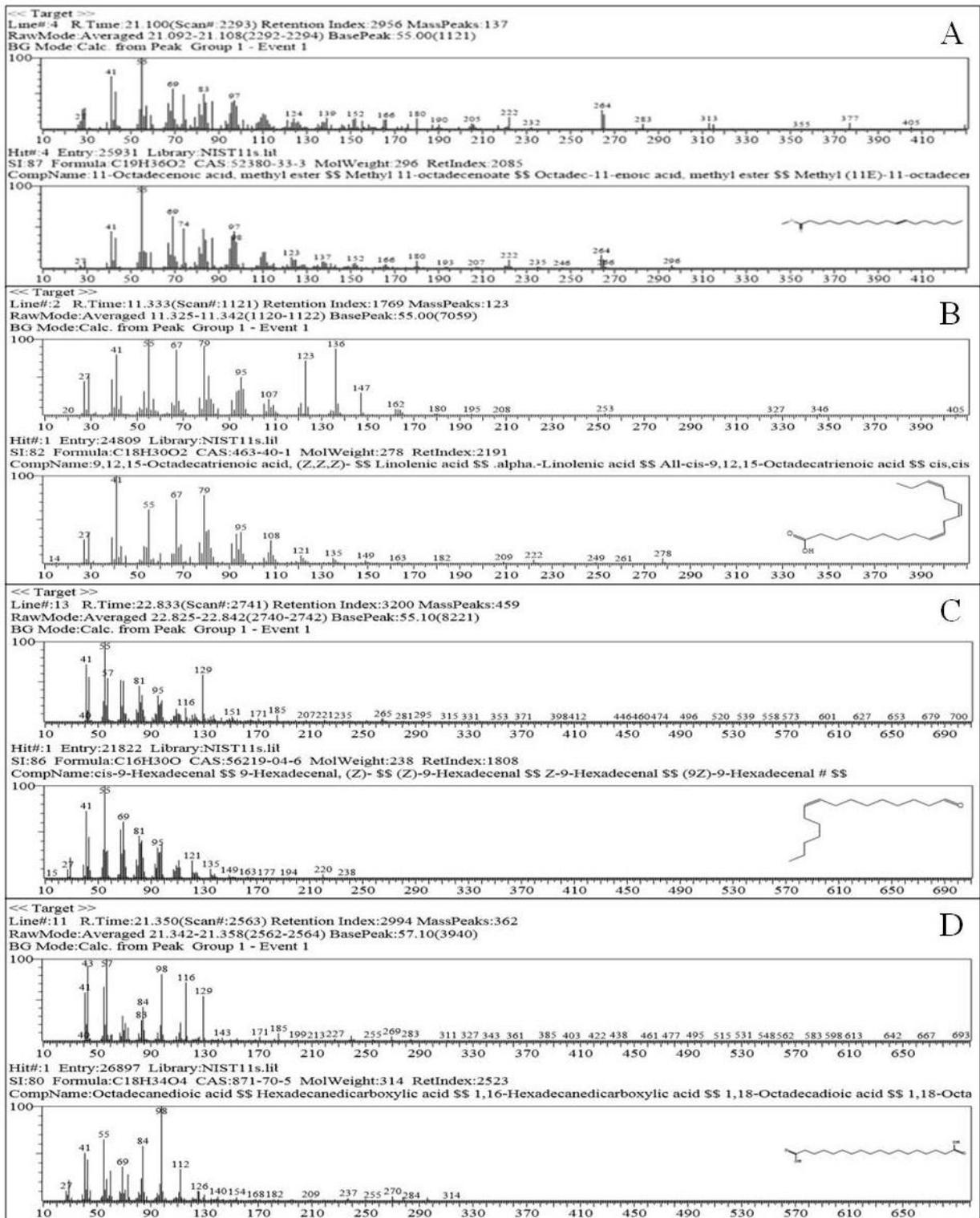


Fig 4.6.8: GCMS of fatty acid

A- 11- Octadecenoic acid, B- linolenic acid, C- cis 9 hexedecenal, D- Octadecanedioic acid

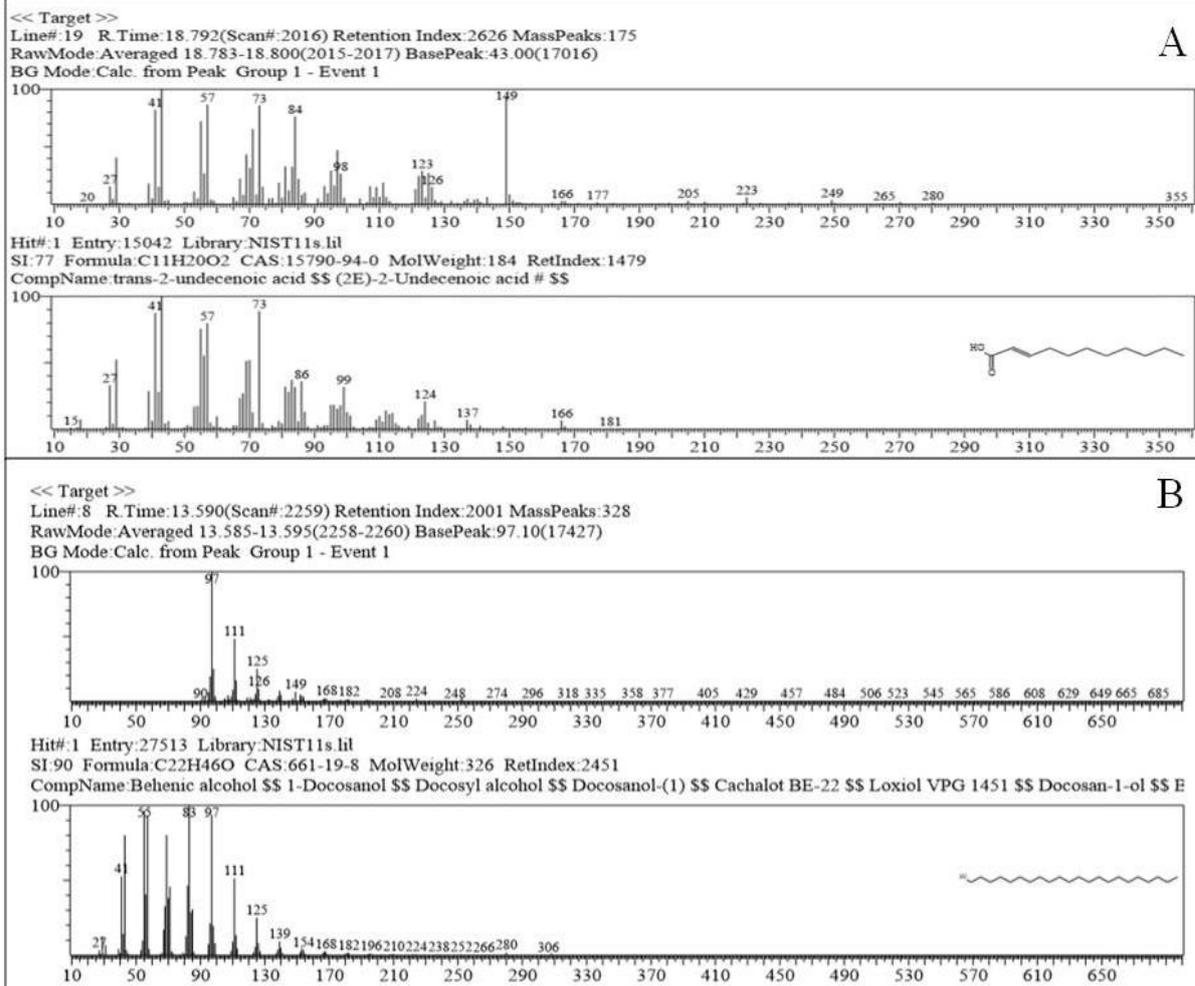


Fig 4.6.9: GCMS of Fatty acid and fatty acid alcohol

A- Trans -2 undecenoic acid , B- Behenic alcohol

ALKALOIDS

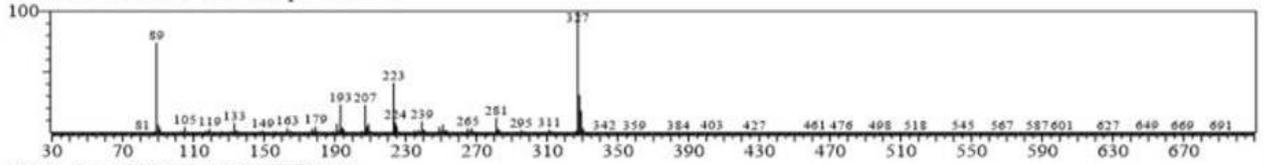
Although during preliminary analysis alkaloids were absent (*c.f.* pg158-159). The GCMS analysis of both the species showed the presence of the alkaloids.

- In *T. jamnagarensis* aerial part, the presence of alkaloid Diethyl 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate (Fig 4.6.10a) was found which is penta-substituted pyridine alkaloid earlier also isolated from the rhizome of *Jatropha elliptica* (Pohl) Muell. Arg. It has molluscicidal activities against *Schistosoma mansoni* and *Biomphalaria glabrata* (Santos *et al.*, 2014). This alkaloid is first report from this family and genus. Further extensive studies will confirm its presence.
- It also showed presence of toxic alkaloid (-)-**Norephedrine, detected by MS, NMR (Fig 4.6.10b)**. This an *Ephedra* herb alkaloid earlier not recorded from *Tephrosia* genus however it was recorded from members of Celastraceae, Papaveraceae, Ranunculaceae, Taxaceae and Malvaceae like *Taxus baccata*, *Catha edulis*, *Roemeria refracta*, *Aconitum napellus*, *Sida acuta* and *Sida cordifolia* (EFSA, 2013).
- In *T. collina* seed extract showed the presence of the alkaloid (-)-Quebrachidin (Fig 4.6.10aB). (-)-Quebrachidin is rare indole alkaloid and its biological sources are Apocynaceae members like *Aspidosperma quebracho*, *Vinca erecta*, *V. herbacea* and *V. major*. Its major action is for hypotensive and sedative action (Azimova and Yunusov, 2013).
- Alkaloids also occur in aerial parts of *Tephrosia* species like *Tephrosia candida*, *T. coriacea*, *T. macropoda*, *T. purpurea* and *T. virginiana* (Willaman *et al.*, 1961).

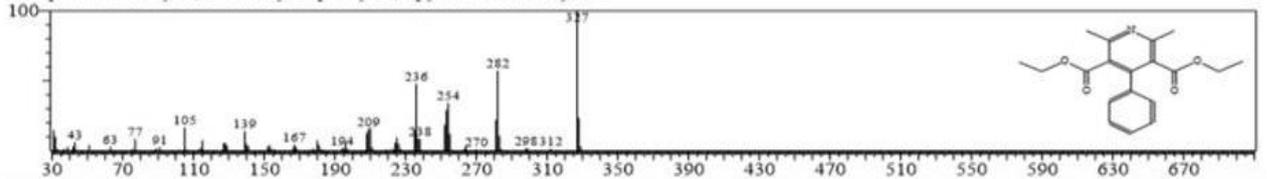
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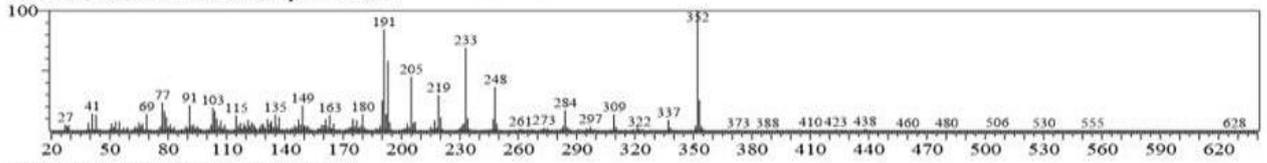
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SI: 50 Formula: C19H21NO4 CAS: 1539-44-2 MolWeight: 327 RetIndex: 2546
CompName: Diethyl 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate



<< Target >>

Line#: 11 R.Time: 30.700(Scan#: 3445) Retention Index: 4089! MassPeaks: 337
RawMode: Averaged 30.692-30.708(3444-3446) BasePeak: 352.15(12707)
BG Mode: Calc. from Peak Group 1 - Event 1

B



Hit#: 1 Entry: 28445 Library: NIST11s.lit
SI: 41 Formula: C21H24N2O3 CAS: 4835-69-2 MolWeight: 352 RetIndex: 2654
CompName: Ajmalan-16-carboxylic acid, 19,20-didehydro-1-demethyl-17-hydroxy-, methyl ester, (2.alpha.,17S,19E)-

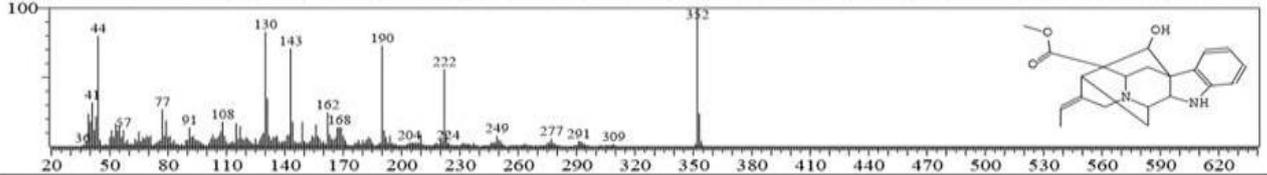
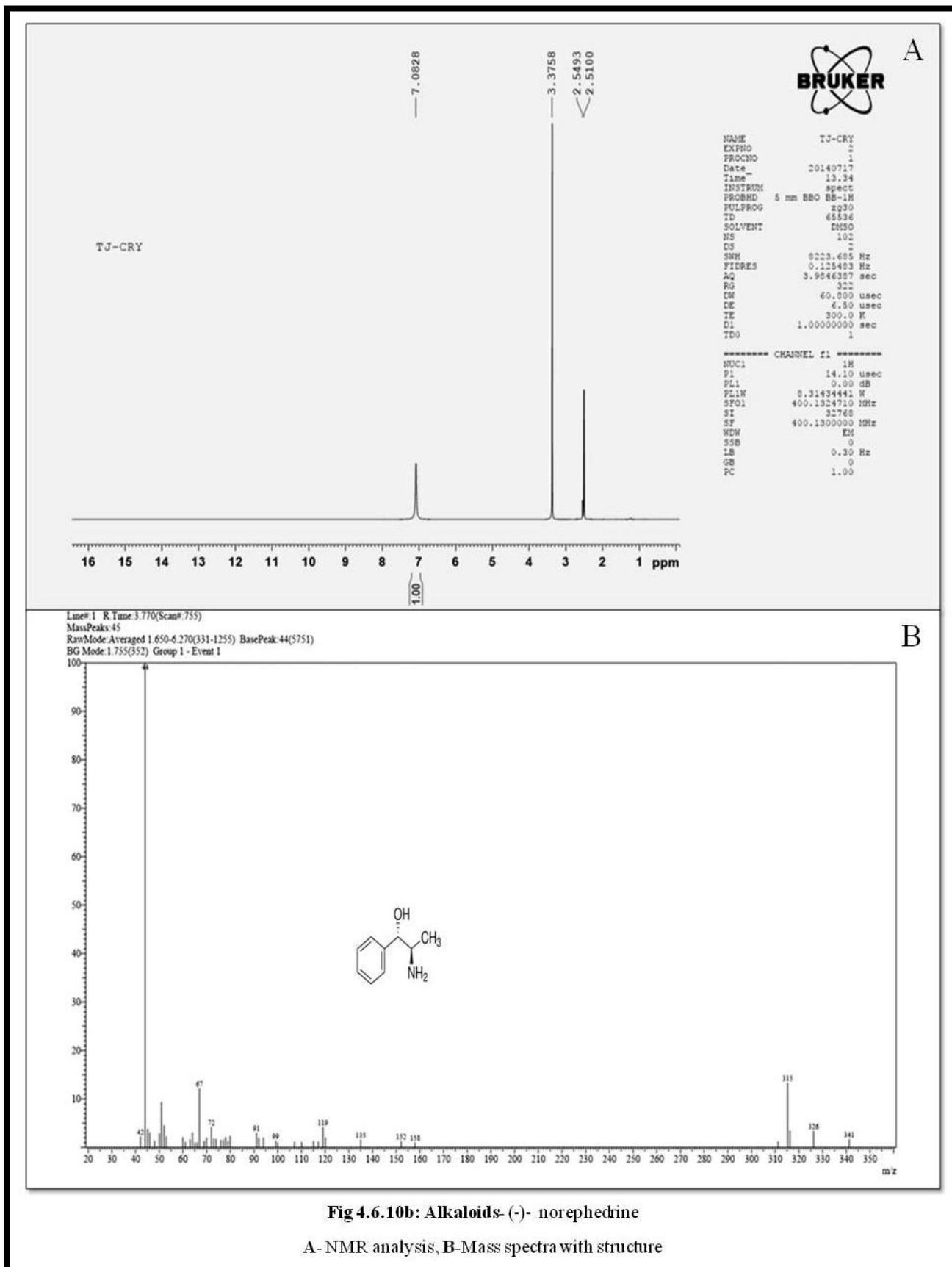


Fig 4.6.10a: GCMS of Alkaloids

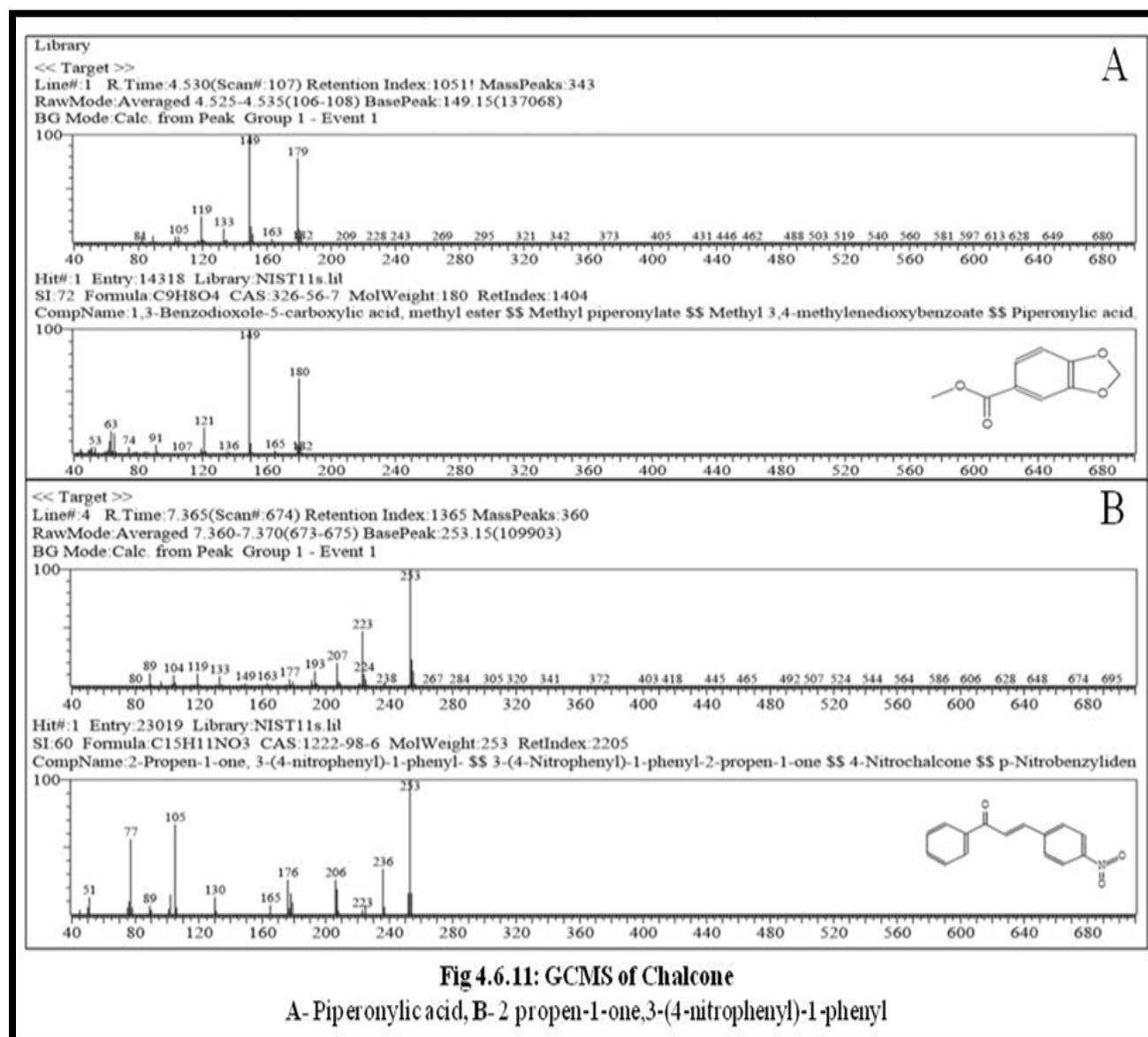
A- Diethyl 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate, B- (-)-Quebrachidin



CHALCONES

Chalcones were earlier reported from other *Tephrosia* species i.e. *T. candida*, *T. elata*, *T. major*, *T. crassifolia*, *T. obovata*, *T. carrollii* and *T. spinosa* (Chen *et al.*, 2014).

- The GCMS analysis of *T. jamnagaerensis* aerial part reveals the presence of the two chalcones - Piperonylic acid (Fig 4.6.11A) and 2-propen-1-one,3-(4-nitrophenyl)-1-phenyl (Fig 4.6.11B). Piperonylic acid is a chalcone that mimics that activity of phenolic acid cinnamate 4-hydroxylase (Bubna *et al.*, 2011) and it is reported from barks of Paracoto tree (Lauraceae) and *Glycine max* (Fabaceae) (Schalk *et al.*, 1998). 2-propen-1-one, 3-(4-nitrophenyl)-1-phenyl possess anti-inflammatory effect (Rivera *et al.*, 2013).
- However chalcones were not detected in phytochemical analysis of *T. collina*.



FLAVONOIDS

Flavonoids are the polyphenols incredibly known phytonutrients. This group is responsible for rich colour diversity in plants (Harborne, 1998). There are many flavonoids reported from the genus *Tephrosia* (Chen *et al.*, 2014).

In present research work it was also found that both the endemic plants also have good composition of flavonoids. **In *T. jamnagarensis* aerial part the presence of Kaempferol and Quercetin was detected via UV spectroscopy (Fig 4.6.12) where *T. collina* root, stem and leaves showed presence only Quercetin (Fig 4.6.13). This fact was reconfirmed via HPTLC (Fig 4.6.14) with corresponding standards.**

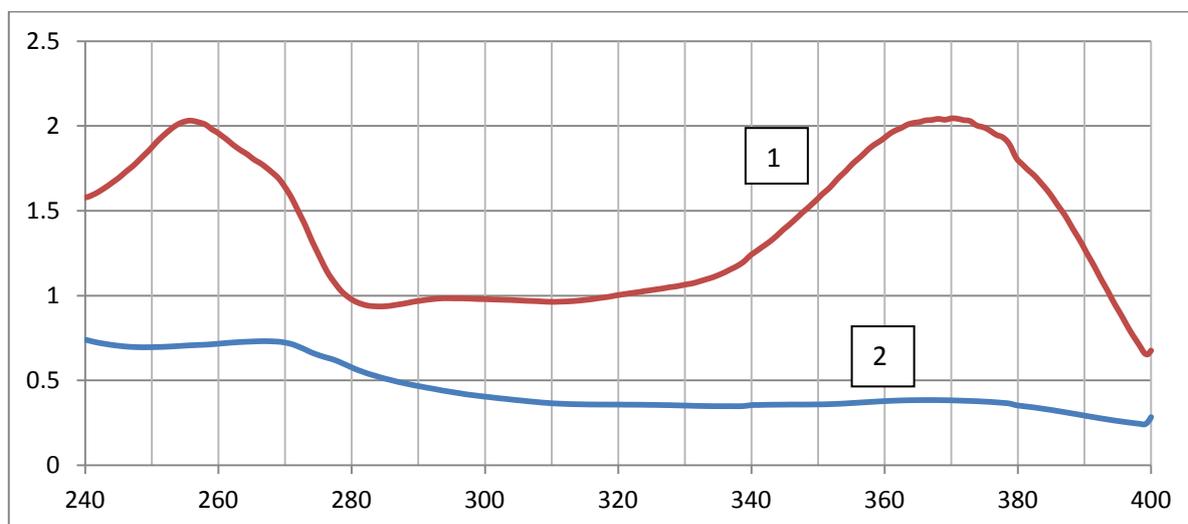


Fig 4.6.12a: UV spectrum of *T. jamnagarensis* :1 Quercetin (spectral max.254.,371.46),
2 Kaempferol (spectra max. 270, 369)

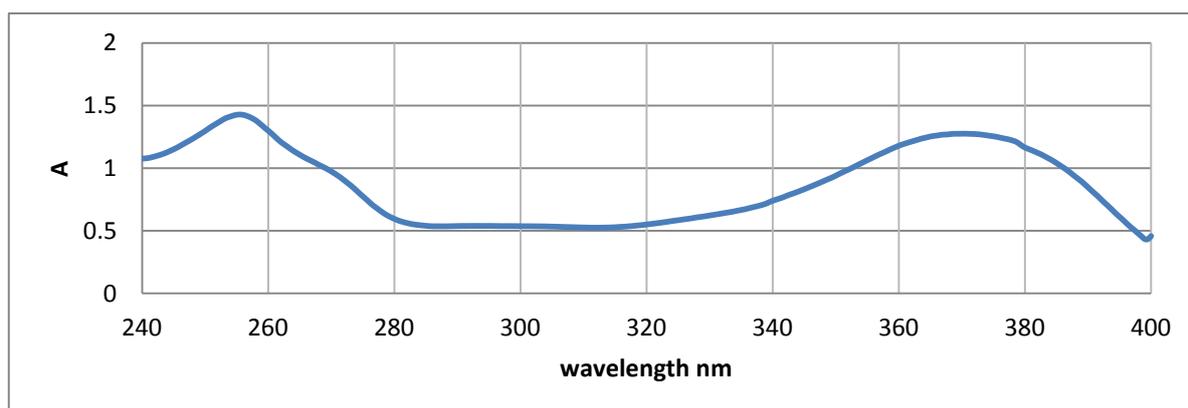


Fig 4.6.12b: UV spectrum of Spectra of *T. collina*: 1 Quercetin (spectral max. 254., 371.46)

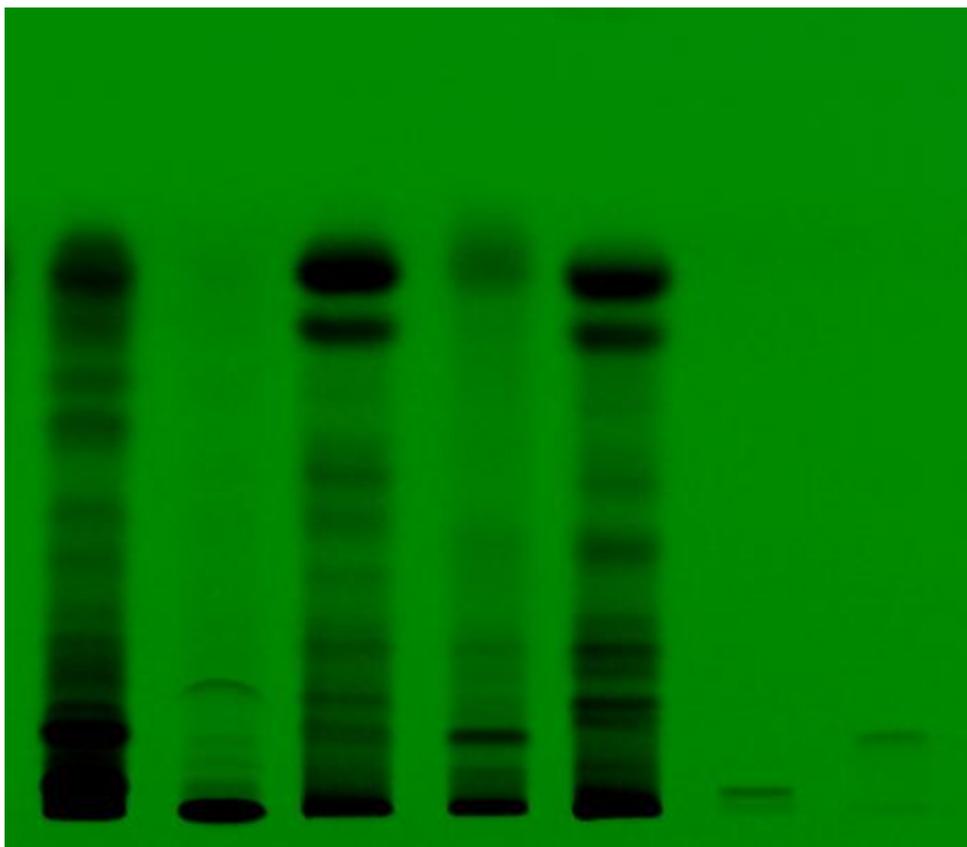


Fig 4.6.13 HPTLC of Flavonoids with samples

Track details: Track 1: TCL- 5 μ L, Track 2: TCS- 5 μ L, Track 3: TCR-5 μ L,Track 4: TJA- 5 μ L, Track 5: TJR- 5 μ L, Track 6: Quercetin (100 ppm)- 5 μ L, Track 7: Kaempferol (75 ppm)- 5 μ L

The HPTLC analysis for the flavonoids extract of different plant parts with standards at UV 254 showed that quercetin was present in all the samples while kaempferol was prominent in samples of TCL, TCR, TJA and TJR. The presence of both this Flavonoids Quercetin and Kaempferol is also recorded from other species *T. purpurea* and *T. villosa* (Chen *et al.*, 2014).

HPLC ANALYSIS OF FLAVONOIDS

HPLC analysis of flavonoids extract of different plant parts samples with standard of rutin, quercetin and naringenin was done. It was observed that rutin was present in *T. jamnagarensis* aerials extract (Fig 4.6.14a), *T. collina* stem and seed extracts (Fig 4.6.14.e & Fig 4.6.14.g) where as naringenin and quercetin were detected from *T. jamnagarensis* roots extract (Fig 4.6.14.b) and from *T. collina* leaves, root and seed extracts (Fig 4.6.14.d, 4.6.14.f & Fig 4.6.14.g). Naringenin was only detected in *T. jamnagarensis* seed extract (Fig 4.6.14.c).

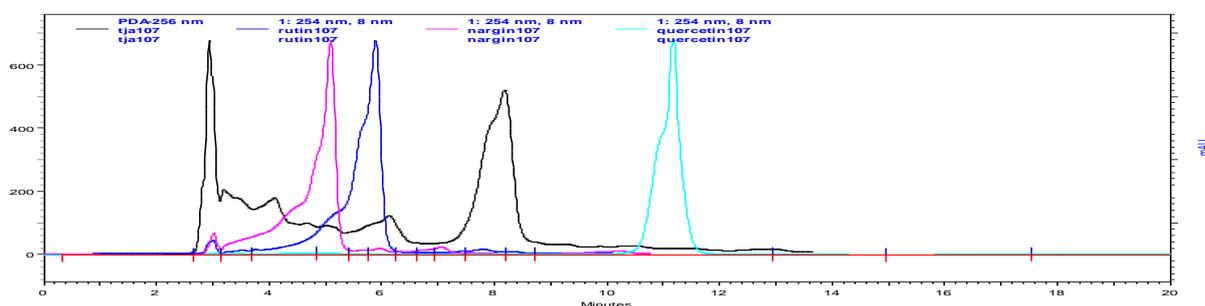


Fig 4.6.14a: HPLC profile of the TJA Extract; indicate presence of rutin

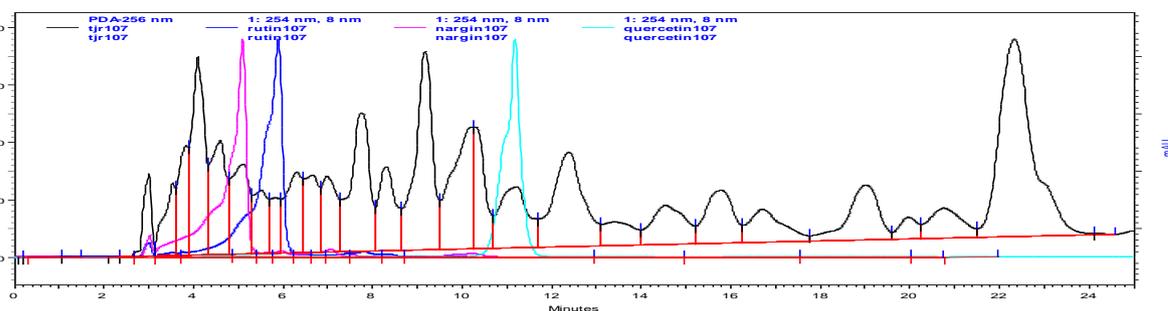


Fig 4.6.14.b: HPLC profile of the TJR Extract; indicate presence of Naringenin and quercetin

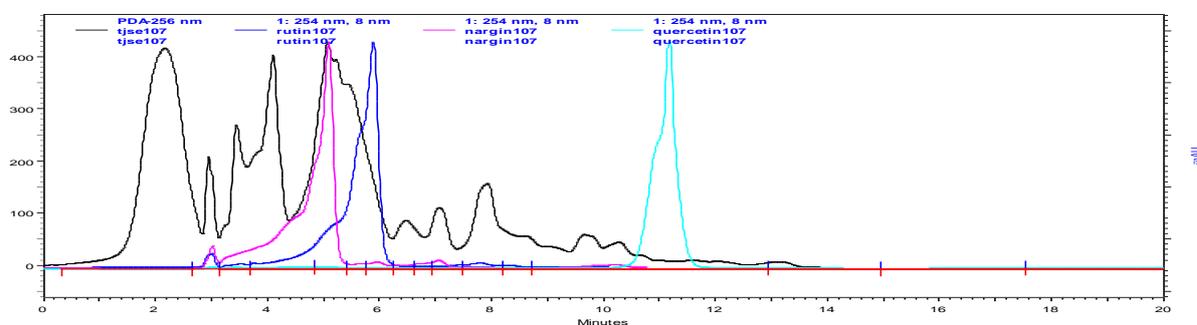


Fig 4.6.14.c: HPLC profile of the TJSE Extract; indicate presence of Naringenin

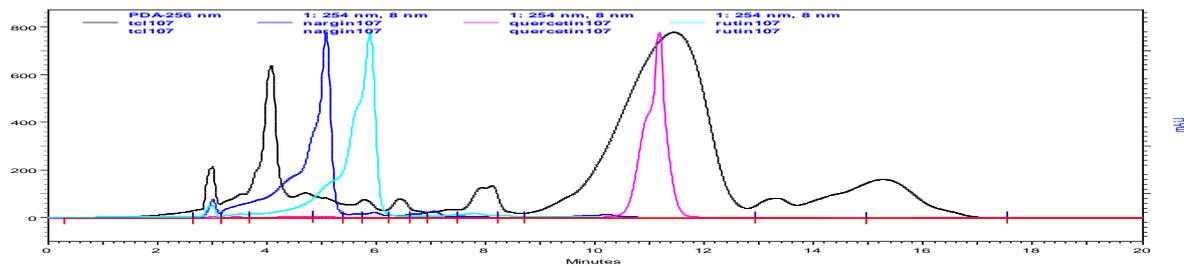


Fig 4.6.14.d: HPLC profile of the TCL Extract; indicate presence of naringenin and quercetin

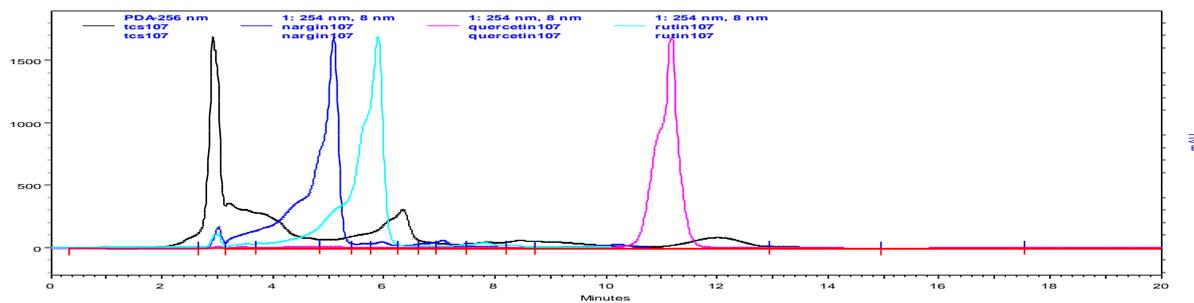


Fig 4.6.14.e: HPLC profile of the TCS Extract; indicate presence of rutin

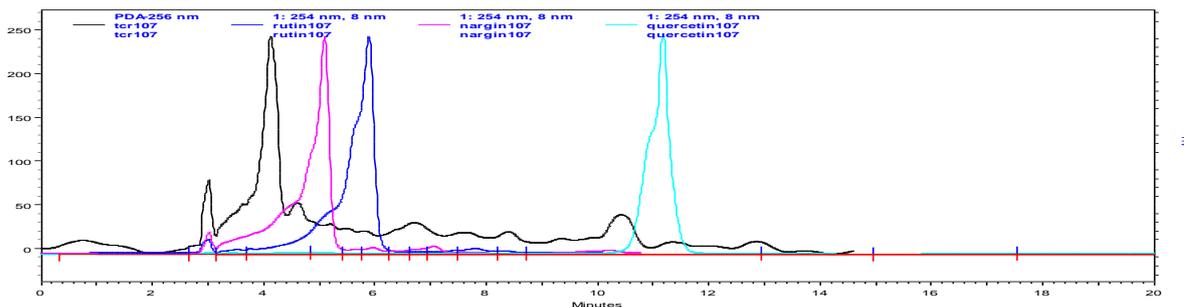


Fig 4.6.14.f: HPLC profile of the TCR Extract; indicate presence of naringenin and quercetin

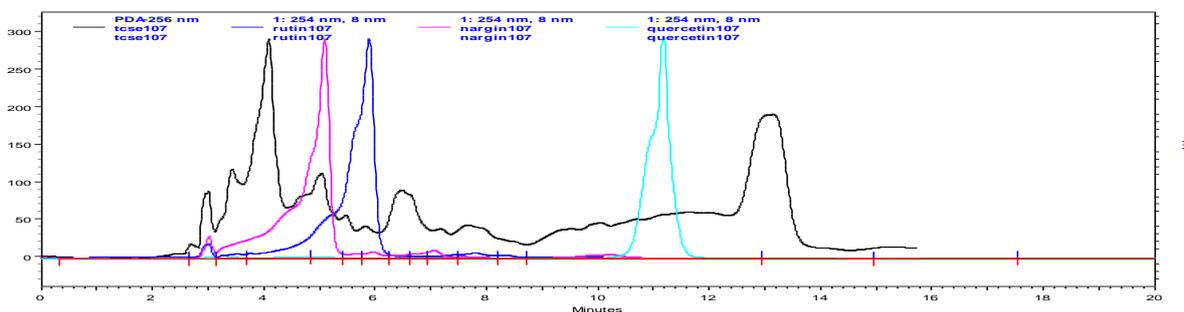


Fig 4.6.14.g: HPLC profile of the TCSE Extract; indicate presence of rutin, naringenin and quercetin

ROTENOIDS ANALYSIS OF TJ AND TC

Rotenoid-containing botanicals are important insecticides and fish poisons. This Phytoconstituents are reported to have anticancer activity in rats and mice (Fang and Casida, 1998). In genus *Tephrosia* species like *T. purpurea*, *T. villosa*, *T. tinctoria*, *T. candida*, *T. elata*, *T. pumila*, *T. uniflora* and *T. vogelii* had presence of different types of rotenoids. Among them major one are tephrosin, deguelin and rotenone (Chen *et al.*, 2014). The HPLC analysis of different plant parts of *T. jamnagarensis* and *T. collina* showed presence of rotenone only in the seeds of *T. jamnagarensis* (Fig 4.6.15a) and in roots of *T. collina* (Fig 4.6.15b).

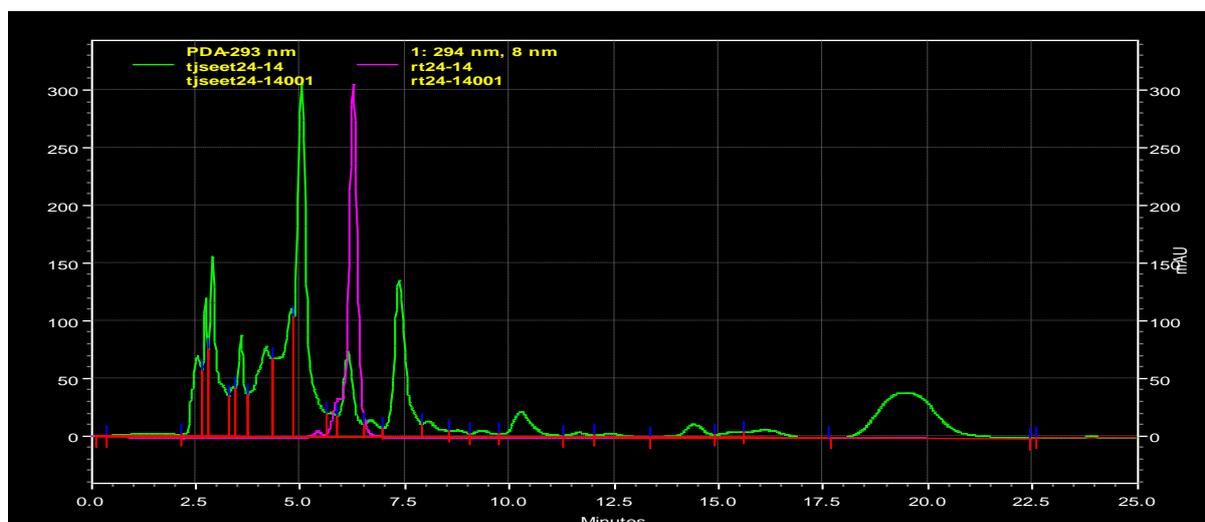


Fig 4.6.15.a: HPLC profile of the TJSE Extract

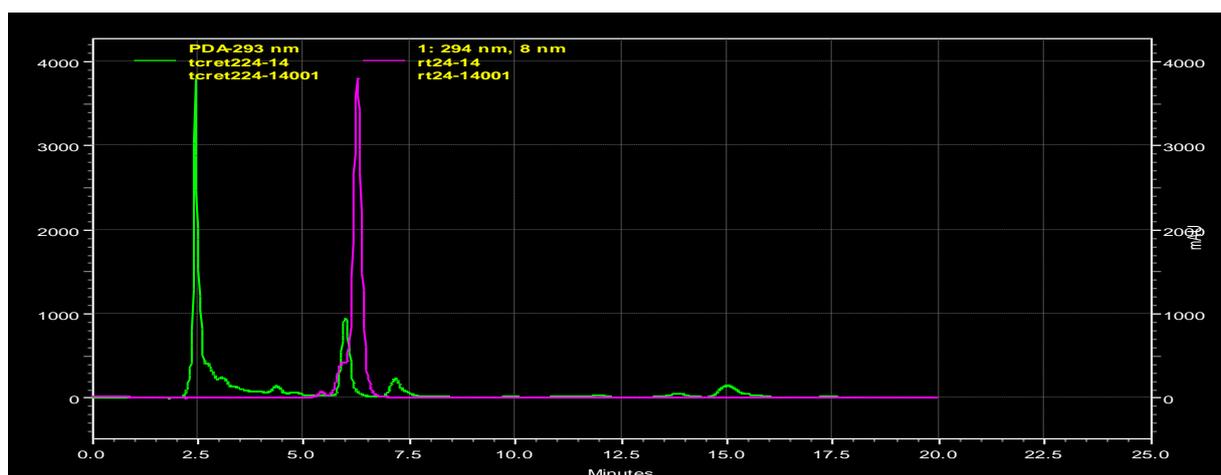
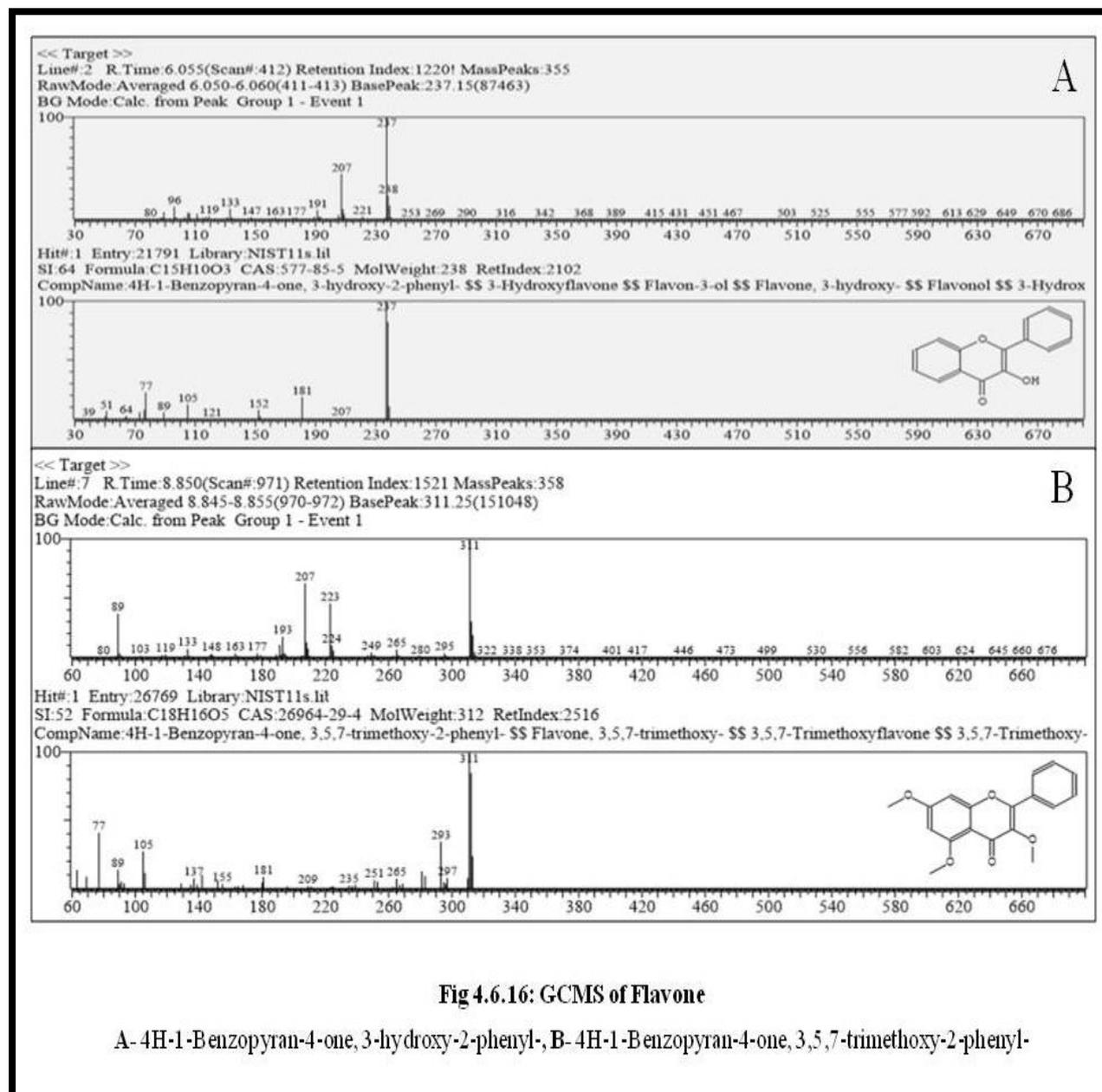


Fig 4.6.15b: HPLC profile of the TCR Extract

GCMS ANALYSIS

The GCMS analysis of aerial parts of *T. jamnagarensis* showed the presence of the flavones like 4H-1-Benzopyran-4-one, 3-hydroxy-2-phenyl-(Fig 4.6.16A) and 4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl-(4.6.16B), which was also reported from *T. purpurea*. These phytoconstituents were earlier recorded from *Kaempferia parviflora* and *Andrographis paniculata* (Jan, 2007).



LCMS ANALYSIS

The LCMS analysis showed the presence of various flavanoid glycoside and anthocyanins in TJ and TC (Table 4.6.3.4 and Table 4.6.3.5).

The mass spectra of the identified components were given from Fig 4.6.17. 1-14.

Table 4.6.3.4 List of phytochemical detected in LC-MS analysis *T. jamnagarensis*

m/z	Compound name	Groups	Aerial	Root	Seed
579	Daidzein 4',7-diglucoside	Flavonoids glycosides	-	+	-
609	Luteolin hexoside hexoside		+	-	+
565	Luteolin-7-oglucuronide		+	+	-
595	Kaempferol-3- o-(p-coumarolglycoside)		+	-	-
611	Quercetin -3-o-rutinoside		+	-	+
625	Quercetin-hexoside-hexoside		+	-	+
711	Quercetin-7-o-hexoside-3o-(malonyl) hexoside		+	-	-
593	Luteolin 7-o rutinoside		+	-	-
769	6- methoxy kaempferol		+	-	-
739	kaempferol	Flavones	-	-	+
727	Galloyl-A-type procyanidin dimer	Anthocyanin	+	+	-
649	p-Lariciresinola-9- sterate	Other flavonoids	+	+	+
548	Dereticulatin triacetate		-	+	+

Table 4.6.3.5 List of phytochemical detected in LC-MS analysis *T. collina*

m/z	Compound identified	Group	Aerial	Root	Seed
609	Luteolin hexoside hexoside	Flavonoids glycosides	+	+	+
565	Luteolin-7-oglucuronide		+	+	+
595	Kaempferol-3- o-(p-coumarolglycoside)		+	-	+
623	Isorhamnetin-3-Orutinoside		+	-	-
625	Quercetin-hexoside-hexoside		+	-	+
593	Luteolin 7-o rutinoside		+	+	+
769	6- methoxy kaempferol		+	-	-
579	Diadzin 4',7 diglucoside		+	-	-
727	Galloyl-A-type procyanidin dimer	Anthocyanin	+	+	-
649	Lariciresinola sterate	Other flavonoids	-	+	+

+ = Present, - = Absent.

Mass spectra of identified compounds

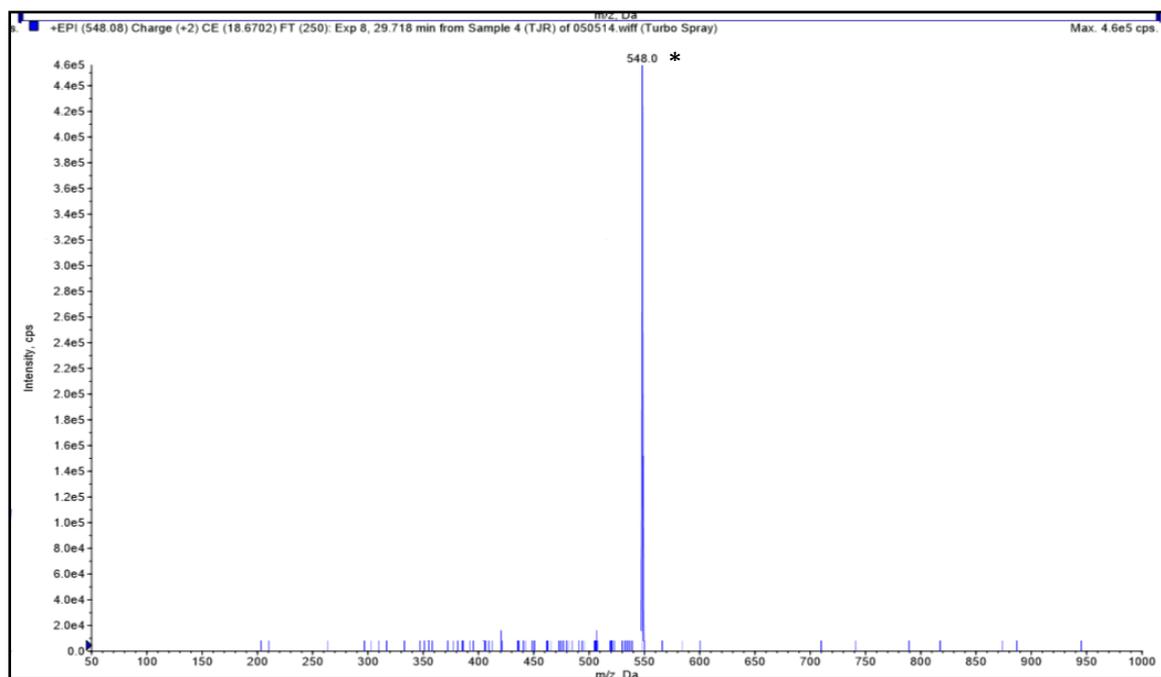


Fig 4.6.17.1: m/z 548-Dereticulatin triacetates (*)

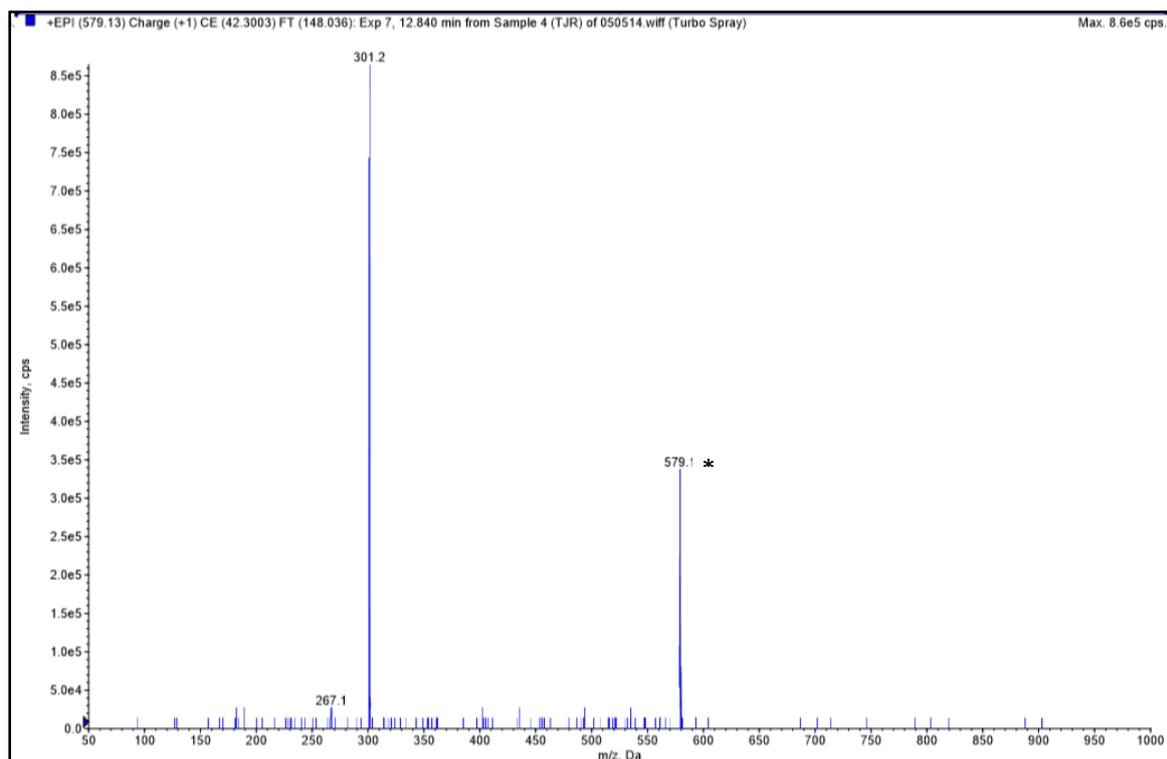


Fig 4.6.17.2: m/z 579- Daidzein 4',7-diglucoside (*)

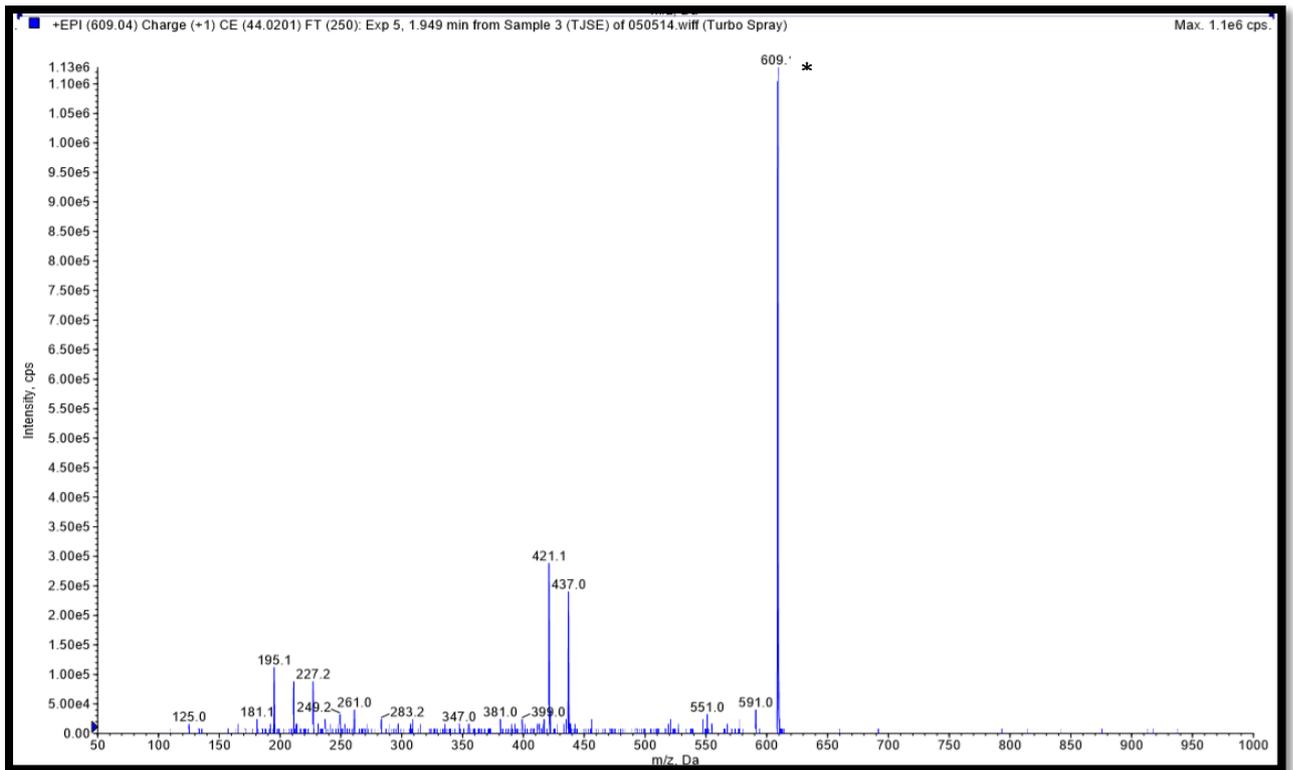


Fig 4.6.17.3:m/z 609-Luteolin hexoside hexoside (*)

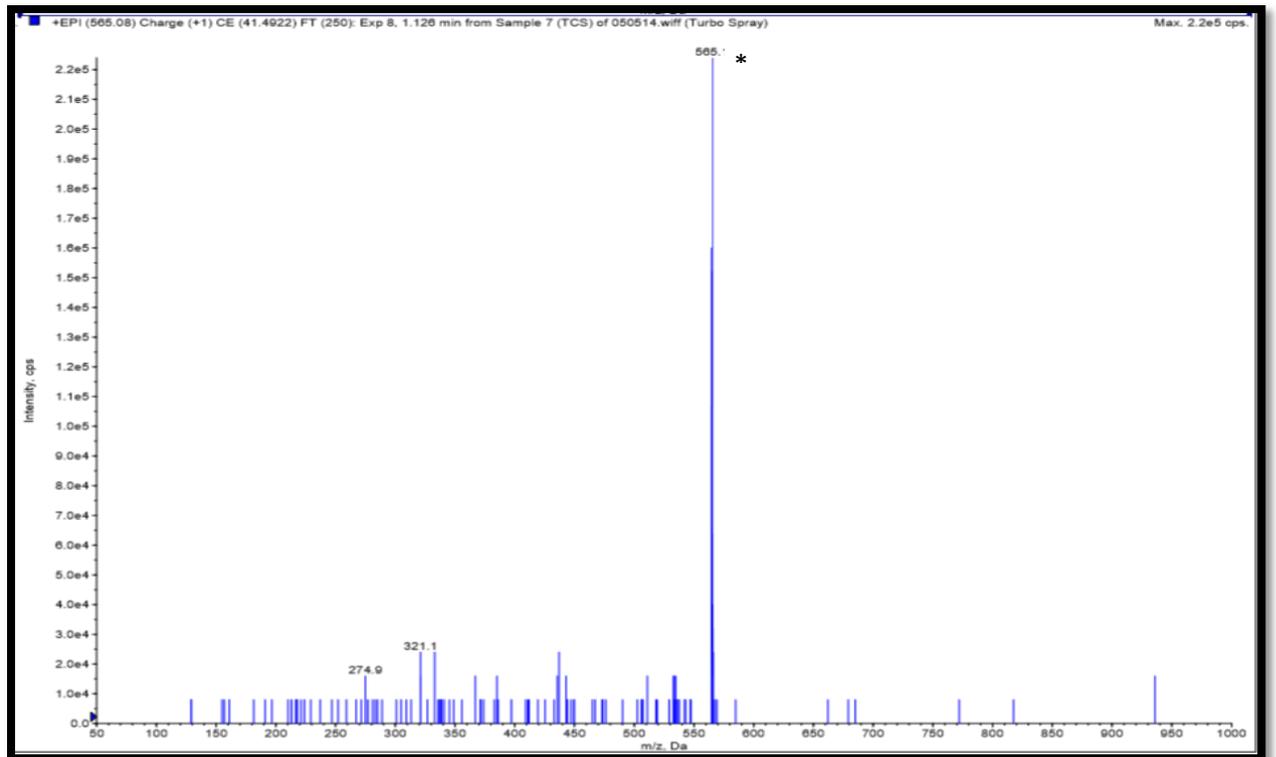


Fig 4.6.17.4 :m/z 565- luteolin 7-oglucurnide (*)

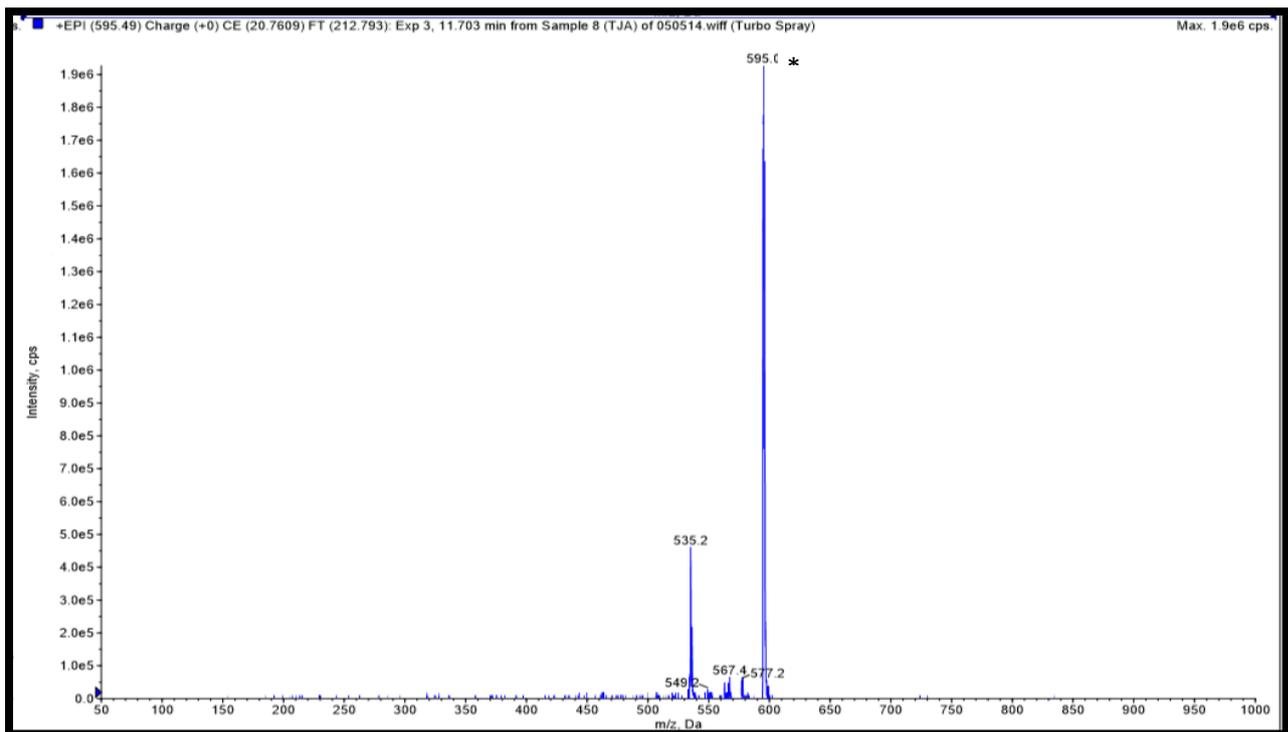


Fig 4.6.17.5: m/z 595- Kaempferol 3-O-(p-coumaroylglucoside) (*)

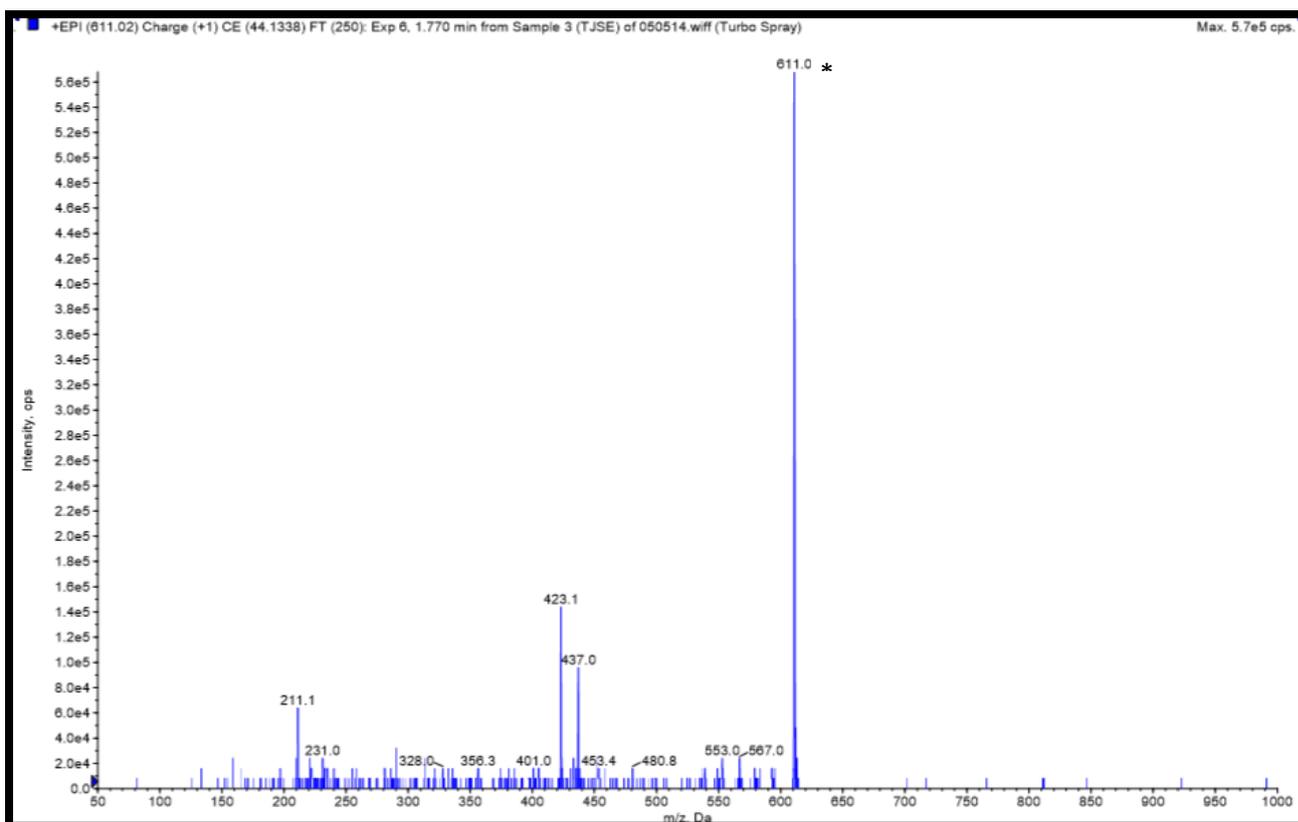


Fig 4.6.17.6: m/z 611-Quercetin-3-O-rutinoside (*)

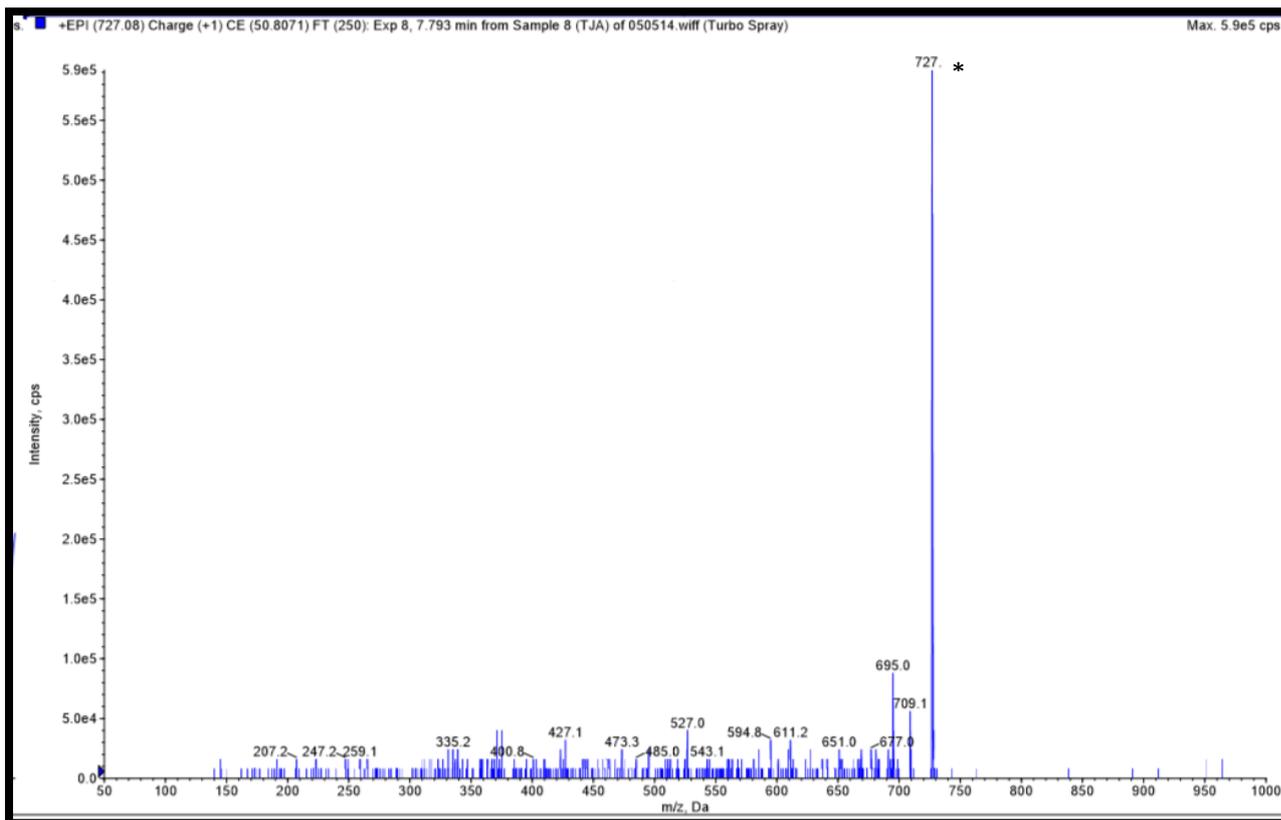


Fig 4.6.17.7: m/z 727 -Galloyl-A-type procyanidin dimer (*)

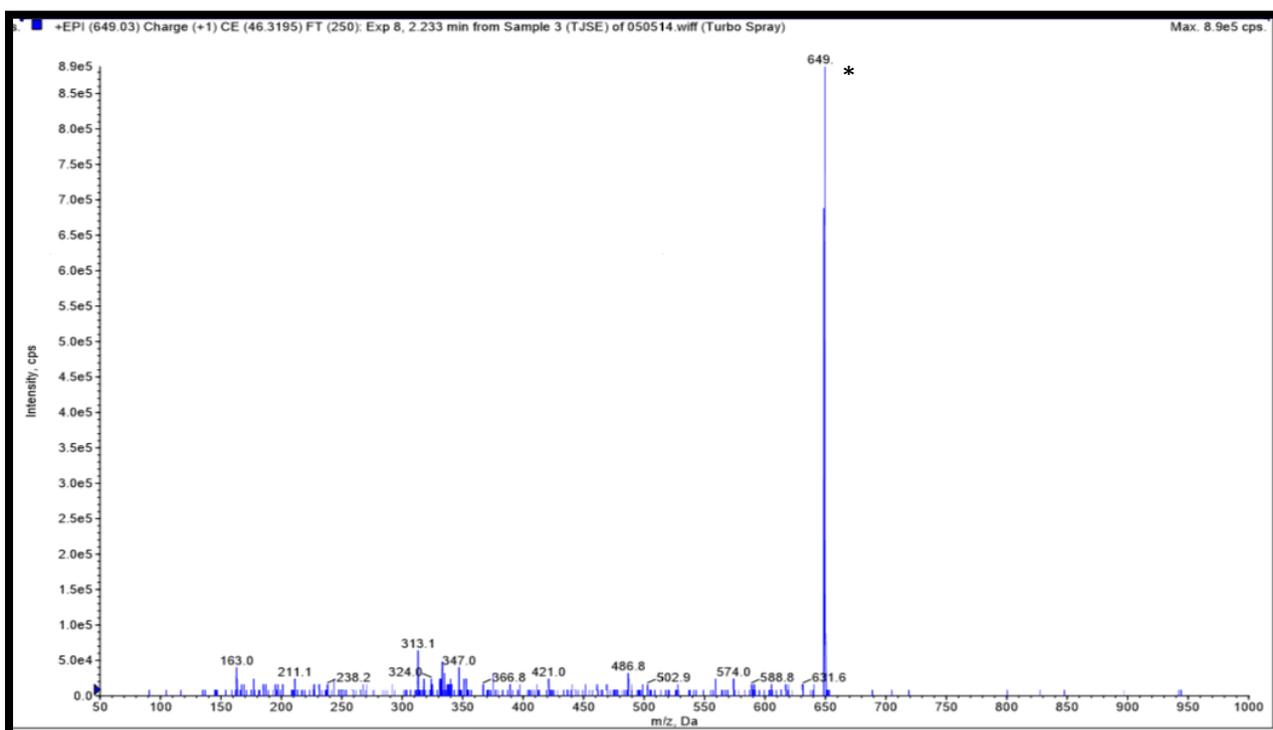


Fig 4.6.17.8: m/z 649- (p)-Lariciresinol 9'-Stearate (*)

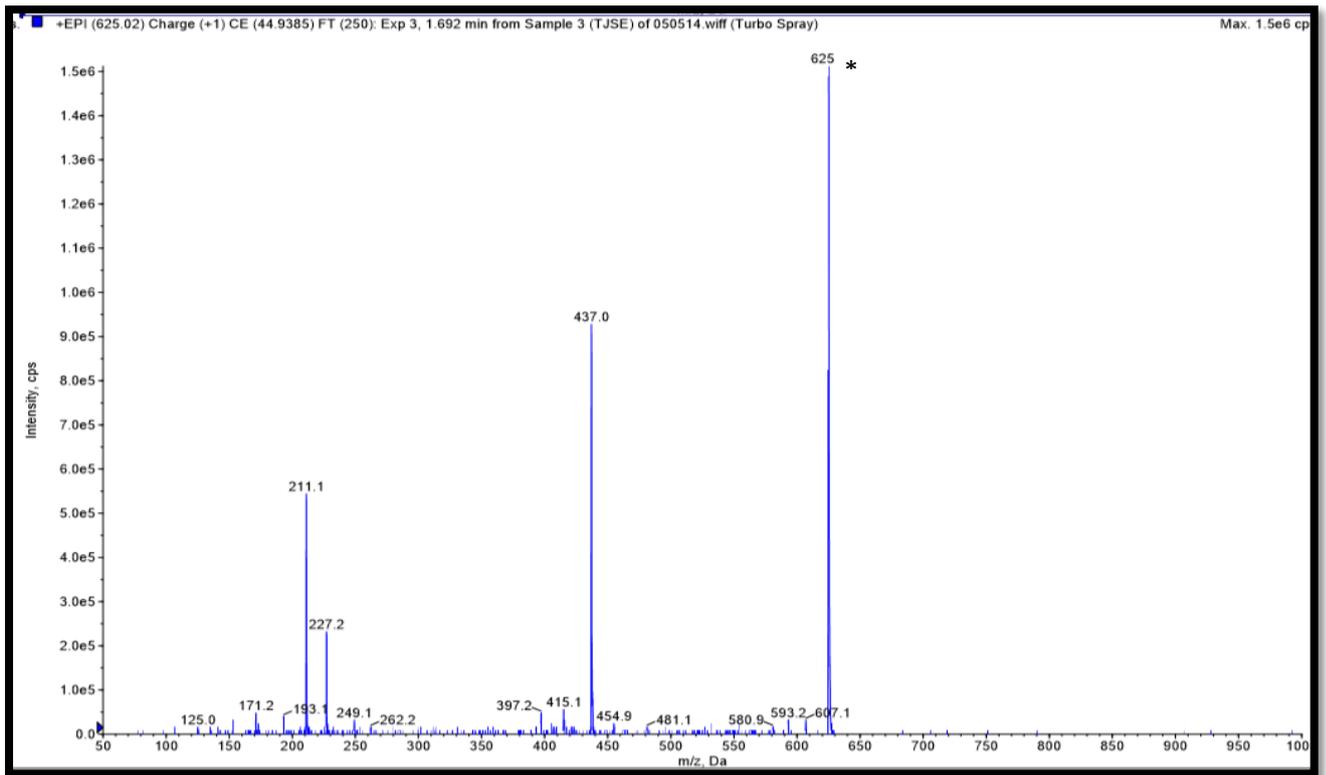


Fig 4.6.17.9: m/z 625 - Quercetin-hexoside-hexoside (*)

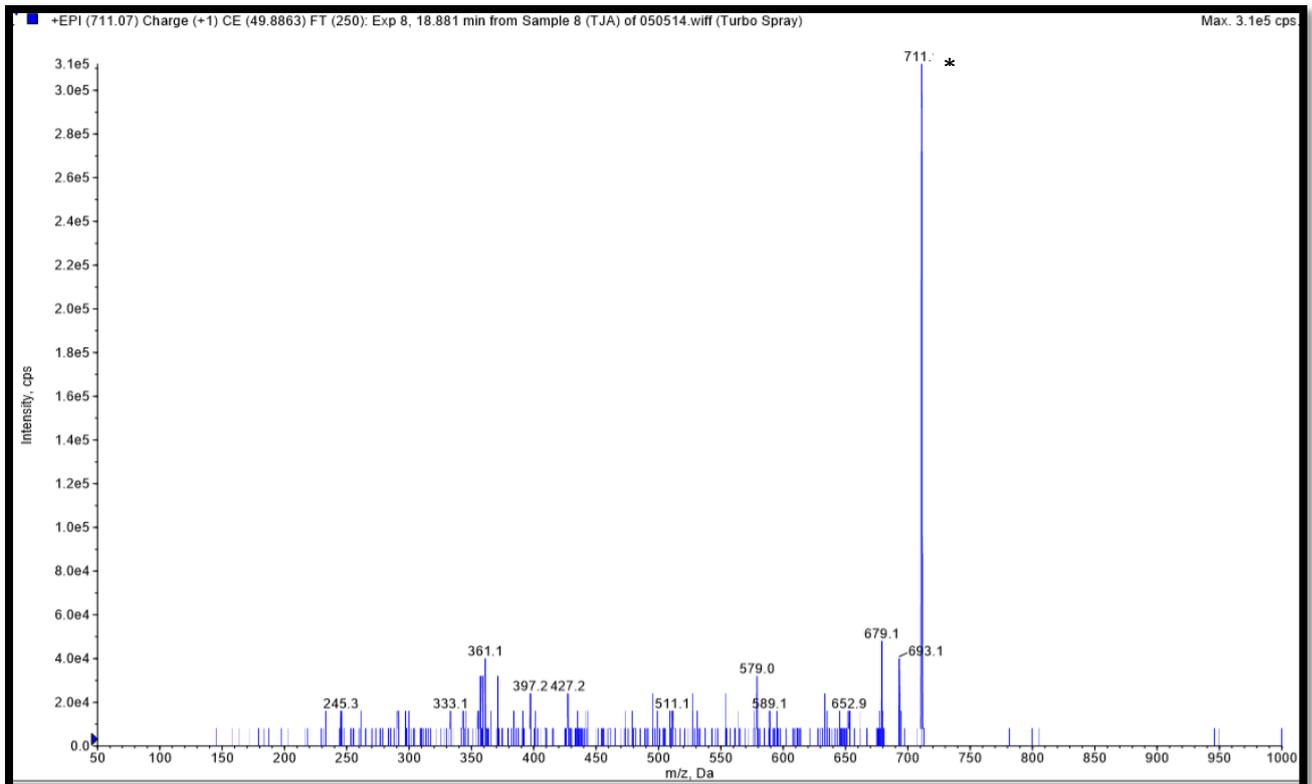


Fig 4.6.17.10: m/z 711-Quercetin-7-O-hexoside-3-O- (malonyl) hexoside (*)

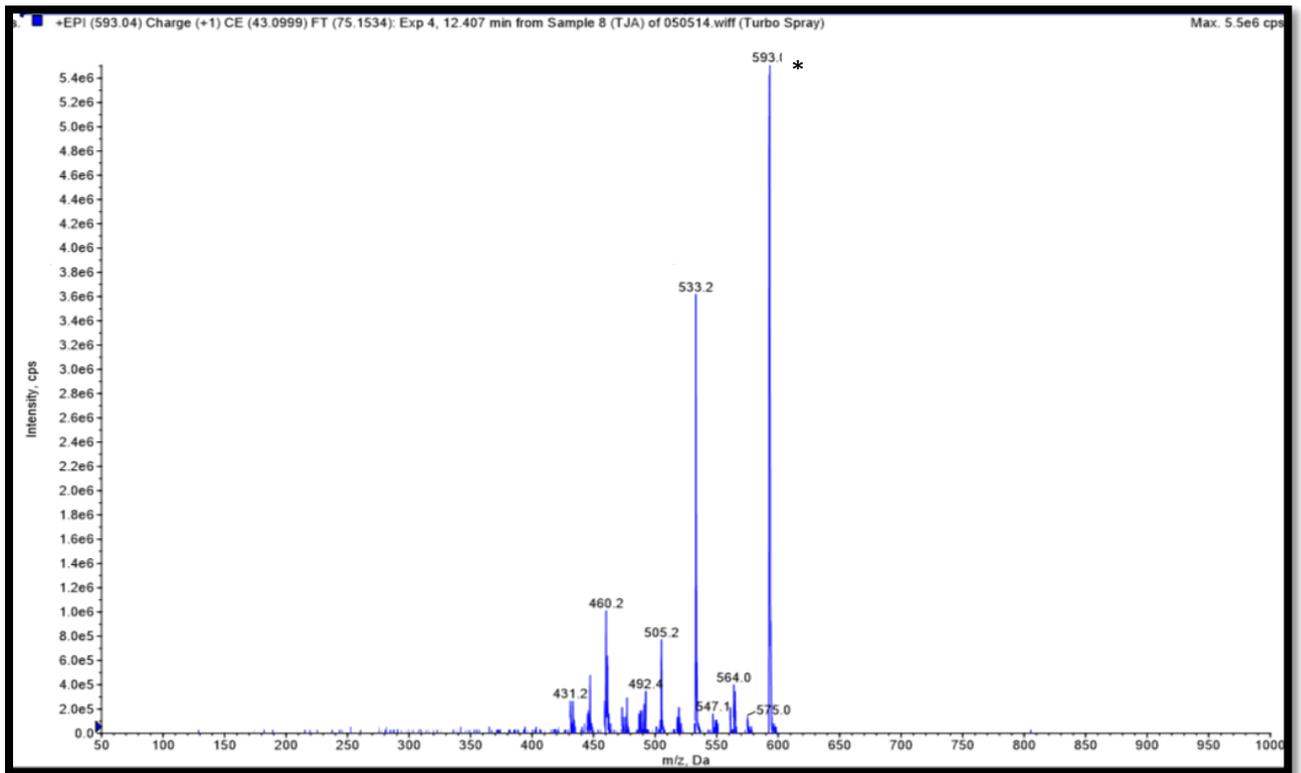


Fig 4.6.17.11: m/z 593- luteolin7-o-rutinoside (*)

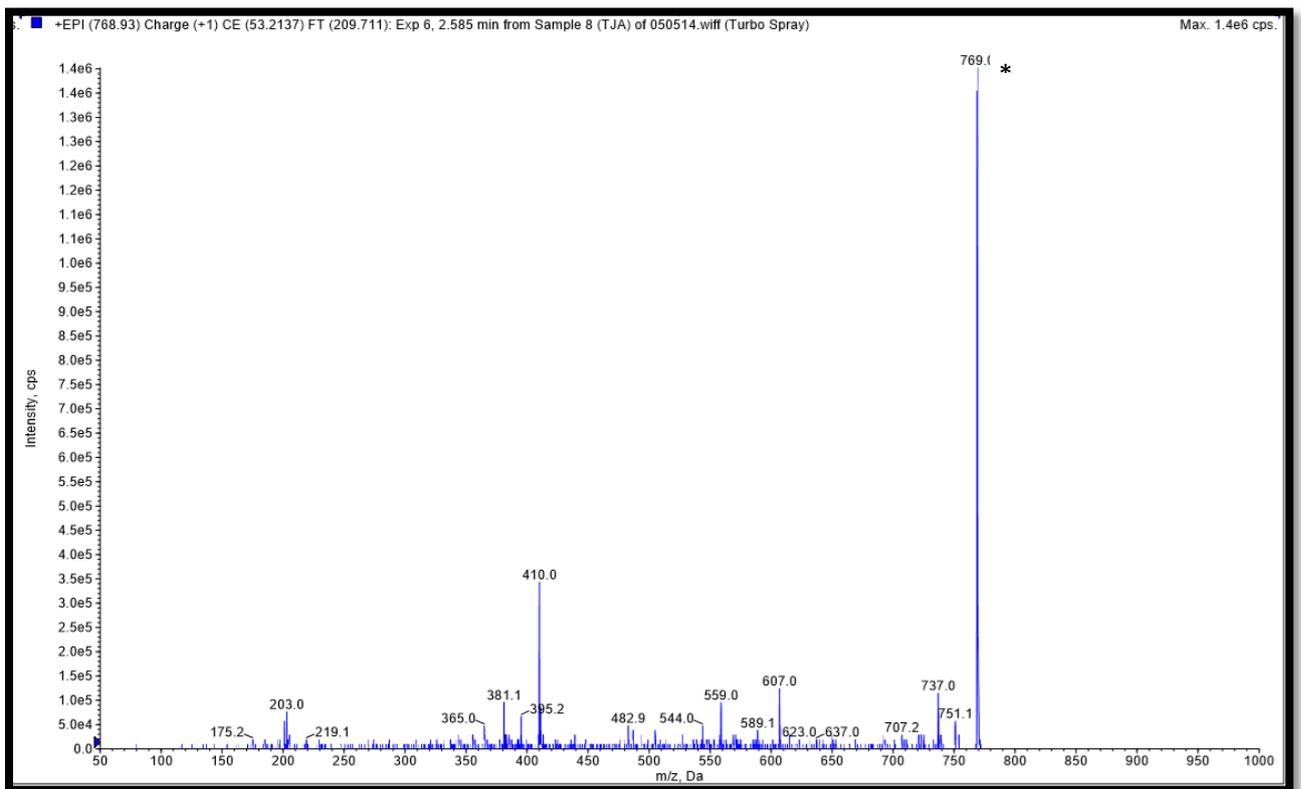


Fig 4.6.17.12: m/z 769-6-methoxykaempferol (*)

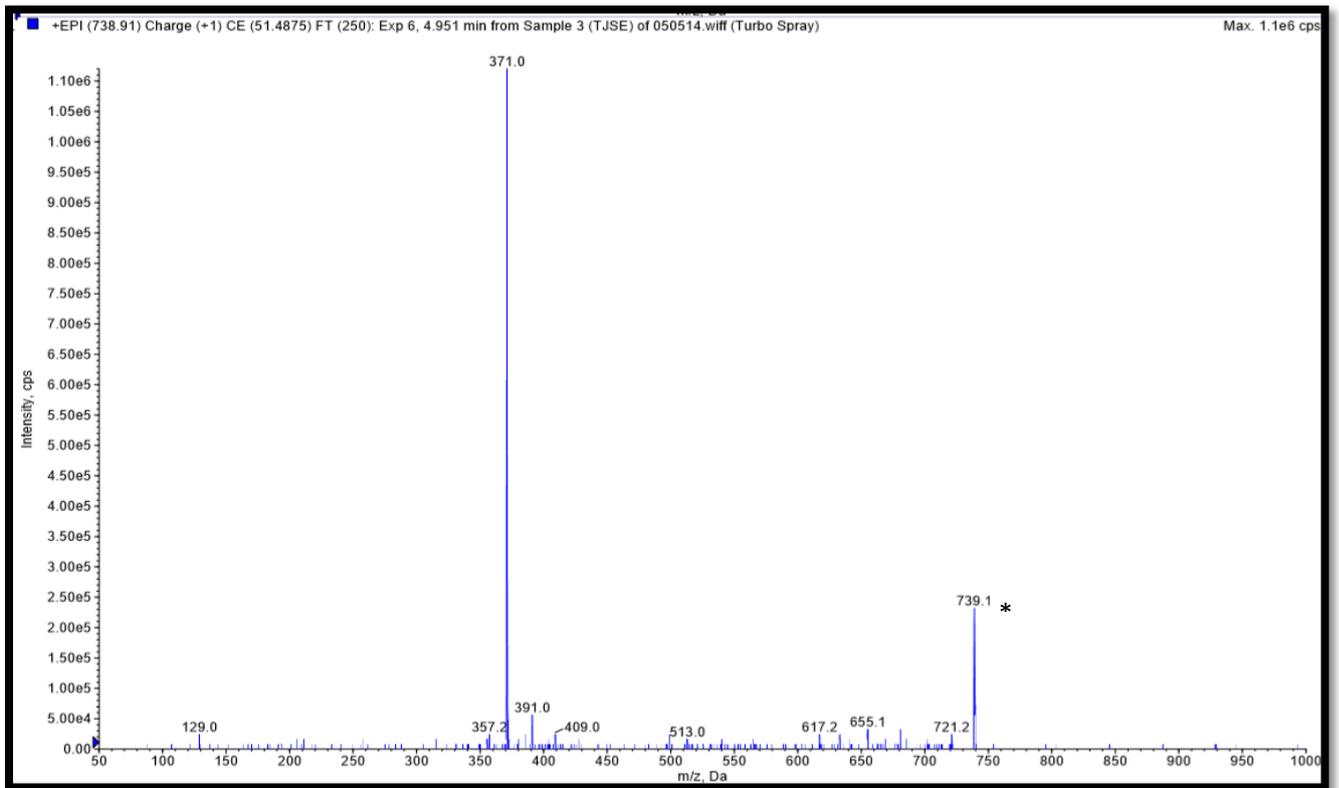


Fig 4.6.17.13: m/z 739-Kaempferol (*)

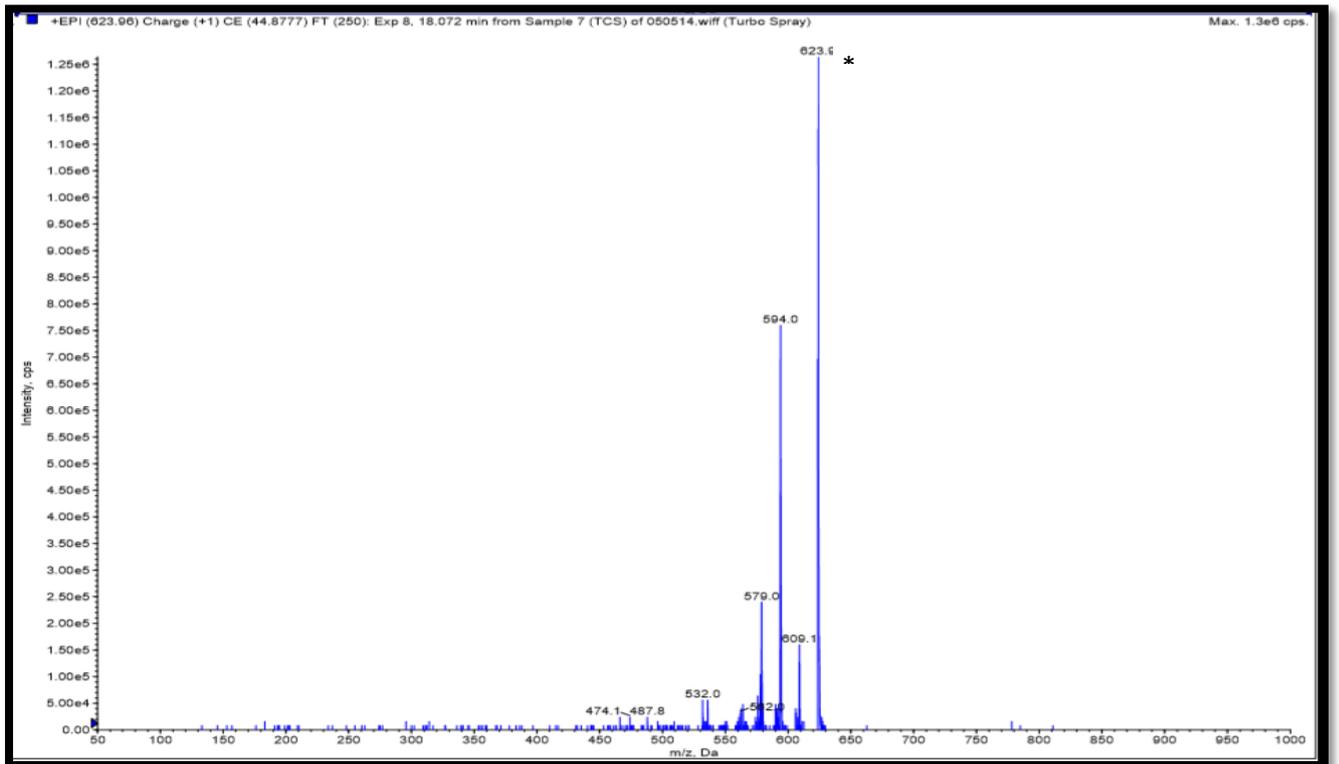


Fig 4.6.17.14: m/z 623- Isorhamnetin-3-O-rutinoside (*)

Flavonoids were the most main constituents of the genus *Tephrosia*, even of the Leguminosae family. From the year of 1971, 161 flavonoids isolated from the genus *Tephrosia* are divided into several categories depending on their skeletons (*c.f.* chapter 2 section 2.4.). In present study the flavonoids analysis observations are summarised as below:

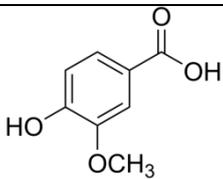
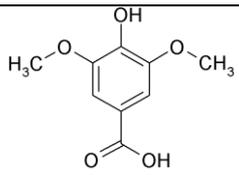
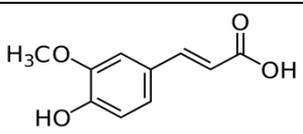
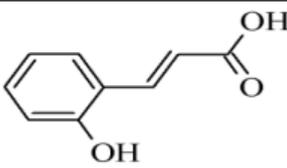
- In both species aerial parts showed presence of more flavonoids compare to roots and seed. Both species presence of flavonol, flavones, flavonol glycosides, anthocyanin, isoflavonoids and other flavonoids. The analysis showed presence of 19 flavonoids in *T. jamnagarensis* and 14 flavonoids in *T. collina*.
- In *T. jamnagarensis* four flavonols i.e., quercetin, rutin, kaempferol and methoxykaempferol were detected where as in *T. collina* only three were detected.
- Only two flavones i.e., 4H-1-Benzopyran-4-one, 3-hydroxy-2-phenyl- and 4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl- were detected from *T. jamnagarensis*. *T. collina* showed absence of flavones.
- Both the species showed presence of eight flavonol glycosides.
- Only single Isoflavonoid (rotenoids) rotenone was detected from seed of *T. jamnagarensis* and root of *T. collina*.
- Galloyl-A-type procyanidin dimer an anthocyanin was detected from both the species.
- Other flavonoids i.e., p lariciresinola-9-sterate was detected from both species where as dereticulatin triacetate was detected from *T. jamnagarensis* only.

PHENOLIC ACID

Phenolic compounds are all aromatic so that they all show intense absorption in the UV region of the spectrum. In addition, phenolic compound characteristically exhibit bathochromic shift in their spectra in the presence of alkali. Spectral method are therefore, especially important for the identification and quantitative analysis of phenols. With this understanding the phenolic components were analysed by two dimensional paper chromatography. **It showed the presence of**

- **Vanillic and syringic acid in all the plant parts of both the *Tephrosia* species.**
- ***cis* and *trans* ferulic acid in *T. jamnagarensis* aerial parts and roots.**
- ***o*-coumaric acid was detected in the aerial part and roots of *T. jamnagarensis* as well as in roots of *T. collina* (Table 4.6.3.6 and Fig 4.6.18).**
- **LCMS analysis showed the presence of phenolic compounds like 3,5-di-ocaafferyl quinic acid (Fig 4.6.19A) as well as quinicquinic caffeic acid ester (Fig 4.6.19B) in all the parts of both the species.**

Table 4.6.3.6 Phenolic acids and its distribution in TJ and TC

Phenolic acid	Structure	Distribution
Vanillic acid (dihydroxybenzoic acid)		<i>T. jamnagarensis</i> aerial parts and roots, <i>T. collina</i> all plant parts
Syringic acid (trihydroxybenzoic acid)		<i>T. jamnagarensis</i> aerial parts and roots, <i>T. collina</i> all plant parts
ferulic acid (hydroxycinnamic acid)		<i>T. jamnagarensis</i> aerial parts and roots.
O-coumaric acid (hydroxycinnamic acid)		<i>T. jamnagarensis</i> aerial parts and roots, <i>T. collina</i> in roots

Based on paper chromatographic studies following phenolic acid were identified

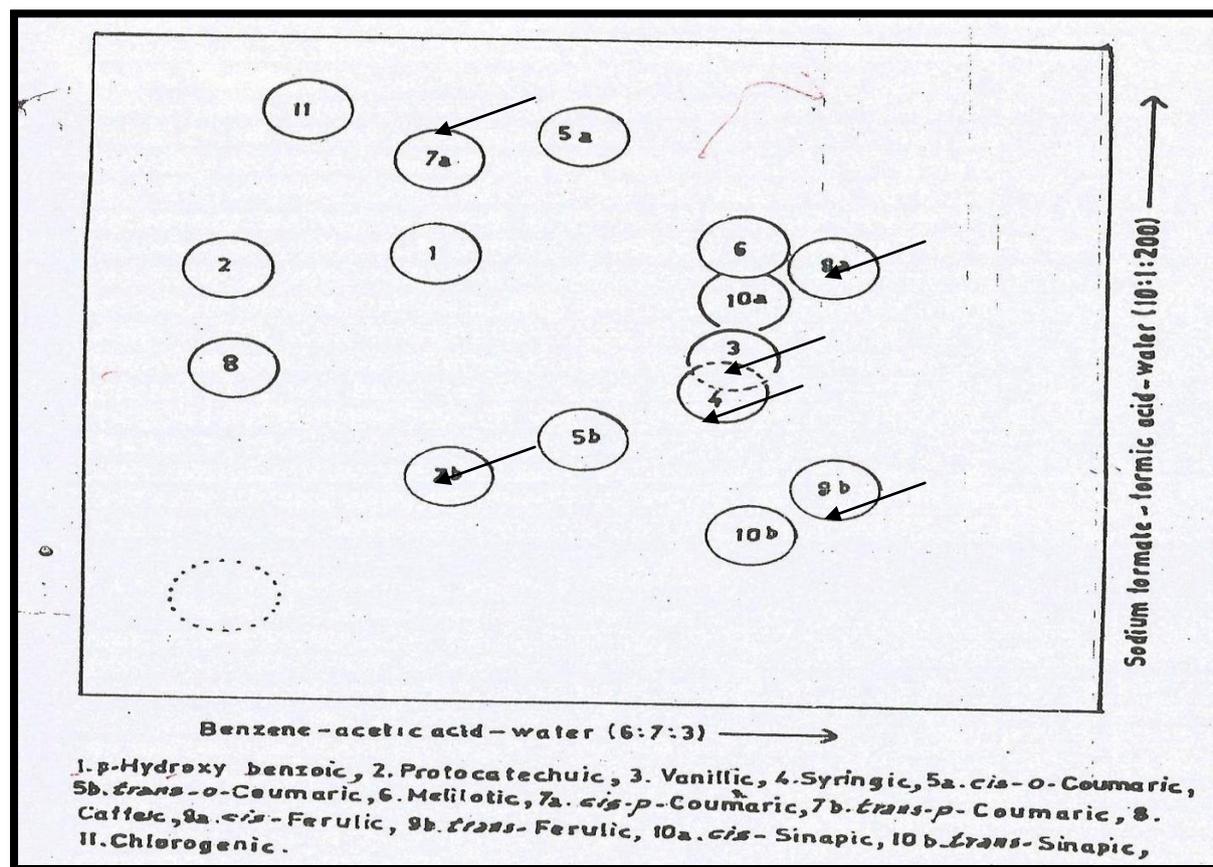


Fig 4.6.18: 2-D chromatogram showing the position of the phenolic acid (Daniel, 1991)

Table 4.6.3.7 The colour reaction of phenolic acid (Daniel, 1991)

Sr no.	Phenolicacids	Colors		
		UV	Diaxotized p-nitraniline	Diazotised Sulphanilic acid
1	Vanillic acid		Purple	Orange
2	Syringic acid		Blue	Red
3	Ferulic acid	Blue	Bluish green	Purple
4	Cis-o-coumaric acid	Bluish yellow	Purple	Orange
5	Trans-o-coumaric acid	Bluish yellow	Purple	Orange

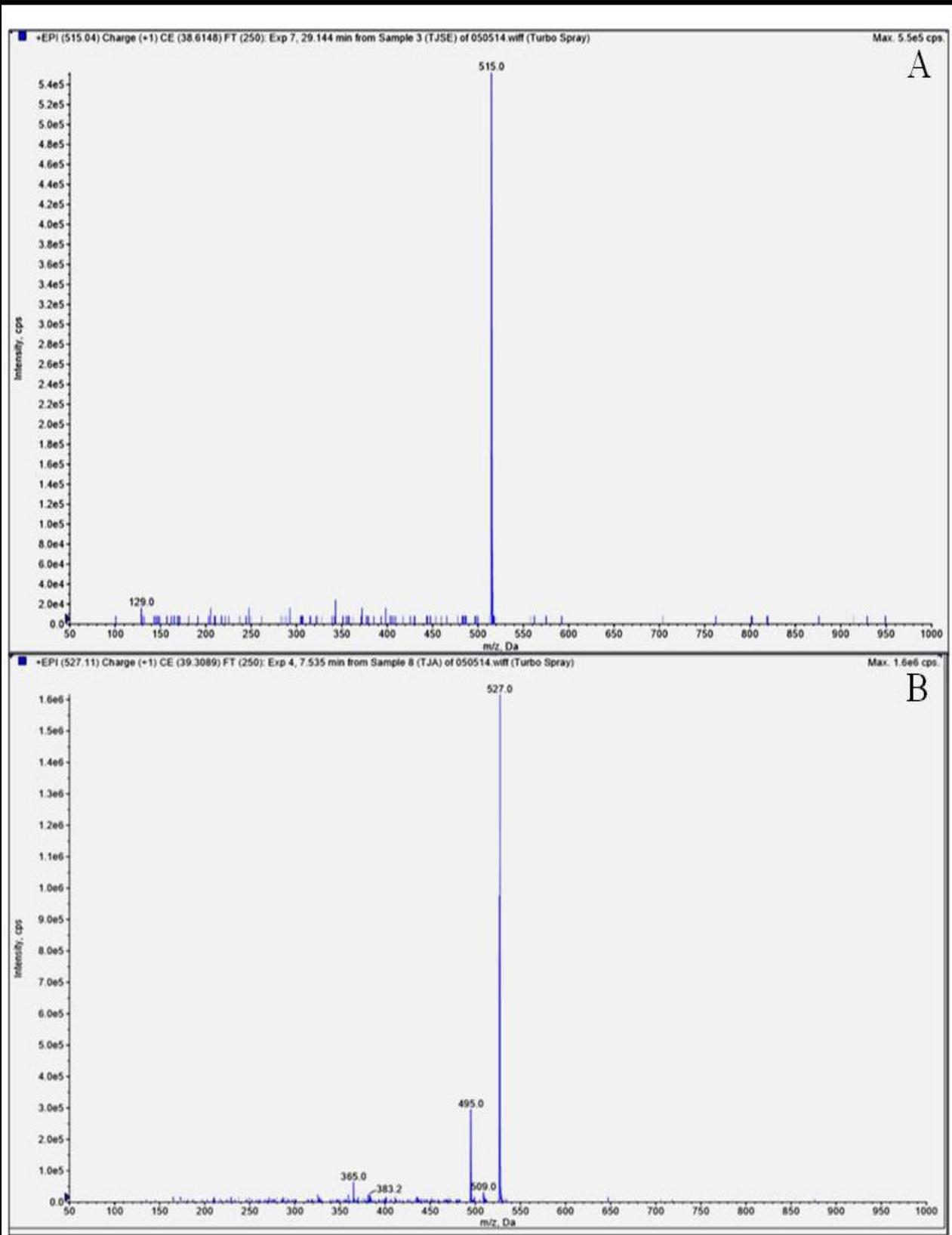


Fig 4.6.19: LCMS of Phenolic acid

A- 3, 5-Di-O-caffeoylquinic acid (m/z 515), B- quinic quinic caffeic acid esters (m/z 527)

STEROLS AND TERPENOIDS

Phytosterols, which encompass plant sterols and stanols are steroid compounds similar to cholesterol which occurs in animals. Stanols are saturated sterols. Phytosterols enrich foods and dietary supplements have been marketed for decades (Weingartner *et al.*, 2008). Like sterols, terpenoids are also most widespread and chemically diverse groups of natural products and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms.

The preliminary analysis of the extract of both this plant showed presence of sterols and terpenoids. For the detail analysis HPTLC, GCMS and LCMS analysis of both the endemic *Tephrosia* species was done. **The HPTLC analysis of both these endangered plant showed presence of triterpenoids lupeol and beta sitosterol in all the plant parts (Fig4.6.20).**

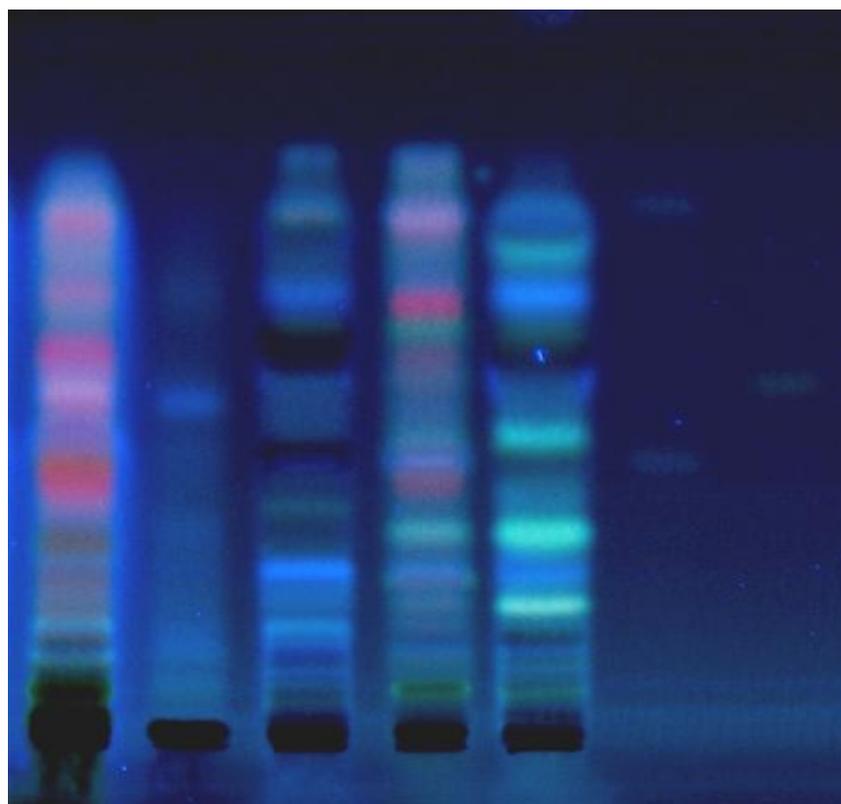


Fig 4.6.20: HPTLC of steroids and terpenoid with samples

Track details: Track 1: TCL- 5 µL, Track 2: TCS- 5 µL, Track 3: TCR- 5 µL, Track 4: TJA- 5 µL, Track 5: TJR- 5 µL, Track 6: Betasitosterol (10 ppm)- 10 µL, Track 7: Lupeol (40 ppm)- 5 µL.

In order to correlate with other species, HPTLC analysis with extracts of aerial parts of *T. purpurea*, *T. villosa*, *T. jamnagarensis* and *T. collina* with standard of lupeol and beta sitosterol was done. It showed that all species had lupeol and beta sitosterol in their extract (Fig 4.6.33).

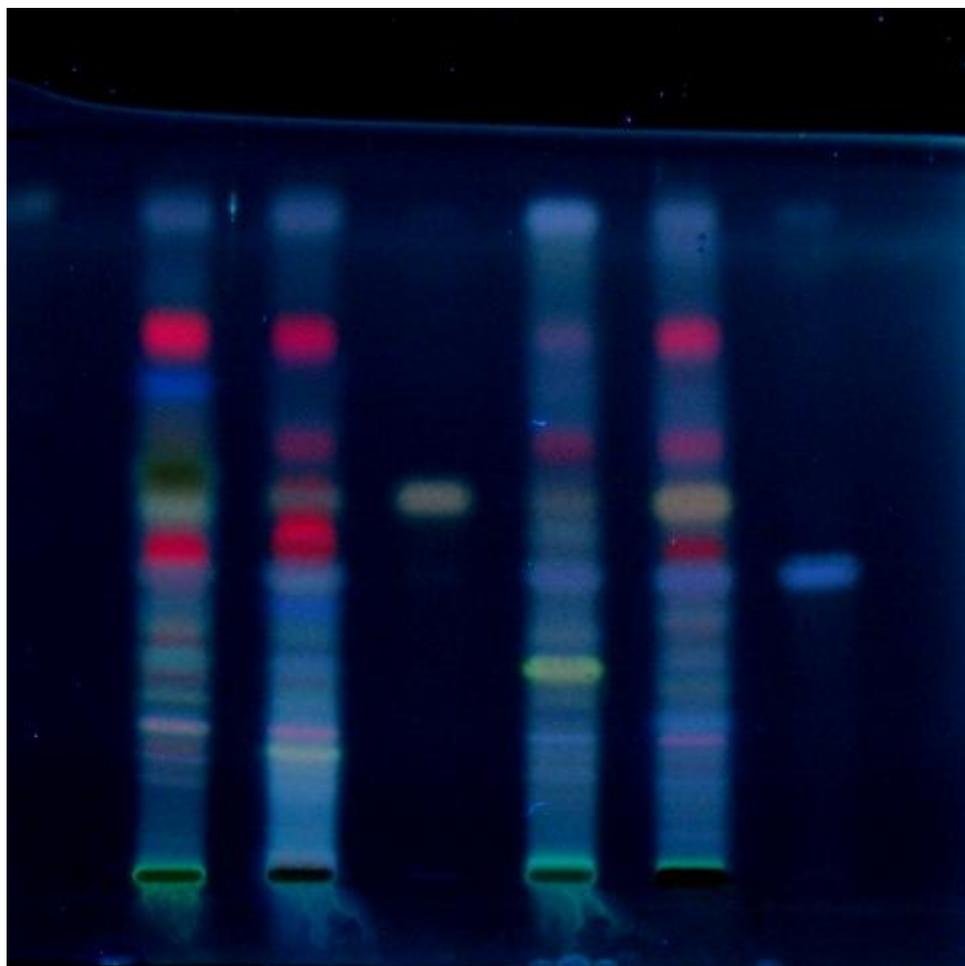


Fig 4.6.21: HPTLC fingerprint of *Tephrosia* species

Track details: -Track 1: *T. purpurea*, Track 2: *T. villosa*, Track 3: Lupeol (50 ppm), Track 4: *T. jamnagarensis*, Track 5: *T. collina*, Track 6: β -sitosterol (50 ppm).

GCMS analysis of petroleum ether extracts of both these endemic plants also showed presence of other sterols.

- TCA showed presence of sterol like Beta sitosterol (Fig 4.6.22A), stigmasterol (Fig 4.6.22B) were detected where as in its seed extracts Lanosterol (Fig 4.6.22C) was detected.
- TJA showed presence of Cholestan-3-ol (Fig 4.6.22D), which develops as a secondary metabolites (Hu, 2009).

- Phytosterols like Beta sitosterol and Stigmasterol have also been earlier recorded from *Tephrosia purpurea* (Chang *et al.*, 1997), *T. strigosa* (Sreenivasulu and Sharma, 1998), *T. uniflora* (Abreu and Luis, 1996) and *T. villosa* (Prashant and Krupadanam, 1993).
- **LCMS analysis showed presence of stigmast-5-22dien-34,21diol-34,21-dihexadecanoate (Fig 4.6.23) in both the plant all parts**, which was also detect in *T. purpurea* (Sharma *et al.*, 2008).

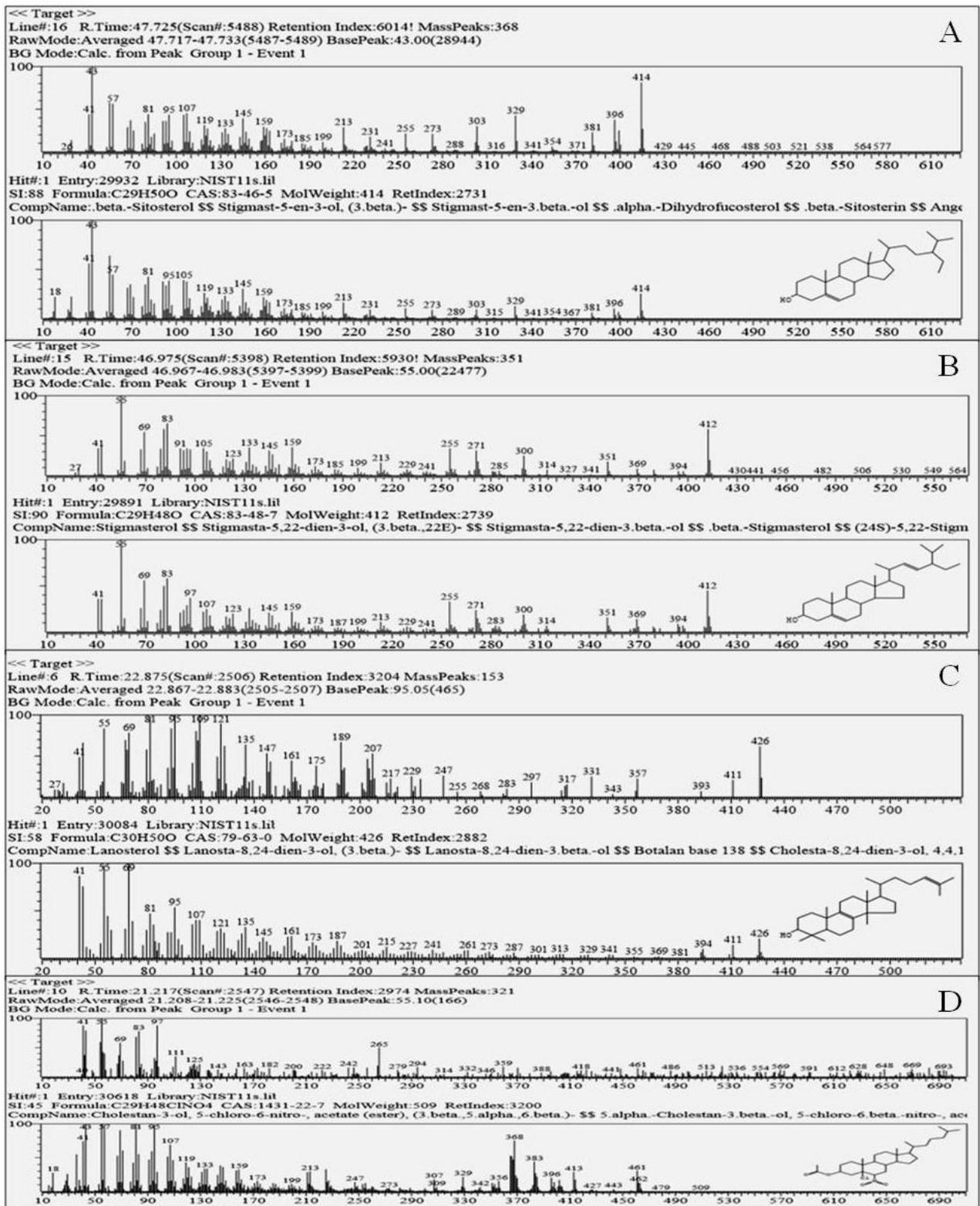


Fig 4.6.22: GCMS of Steroids

A- Beta-sitosterol, B- Stigmasterol, C- Lanosterol, D- Cholestan-3-ol

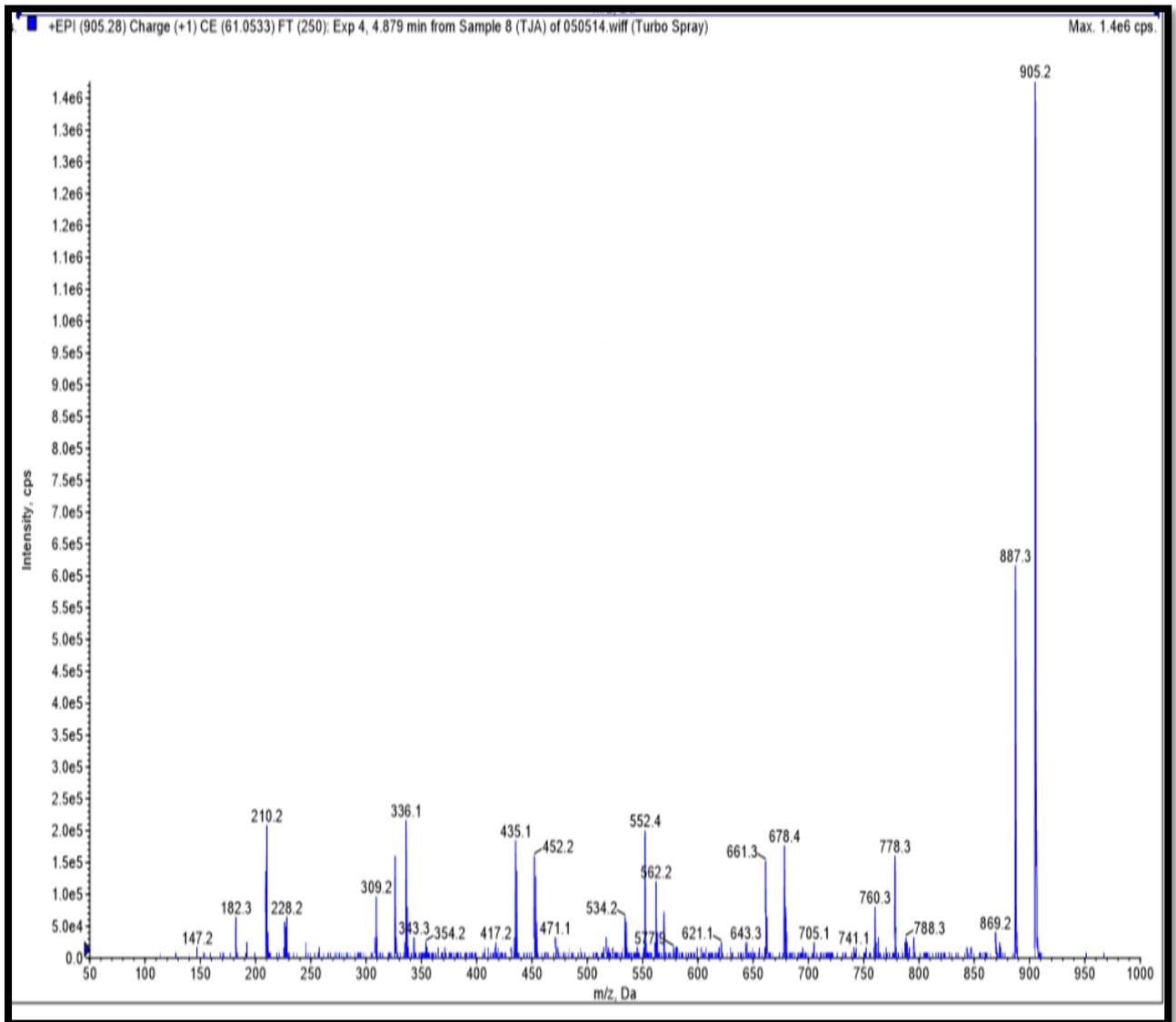


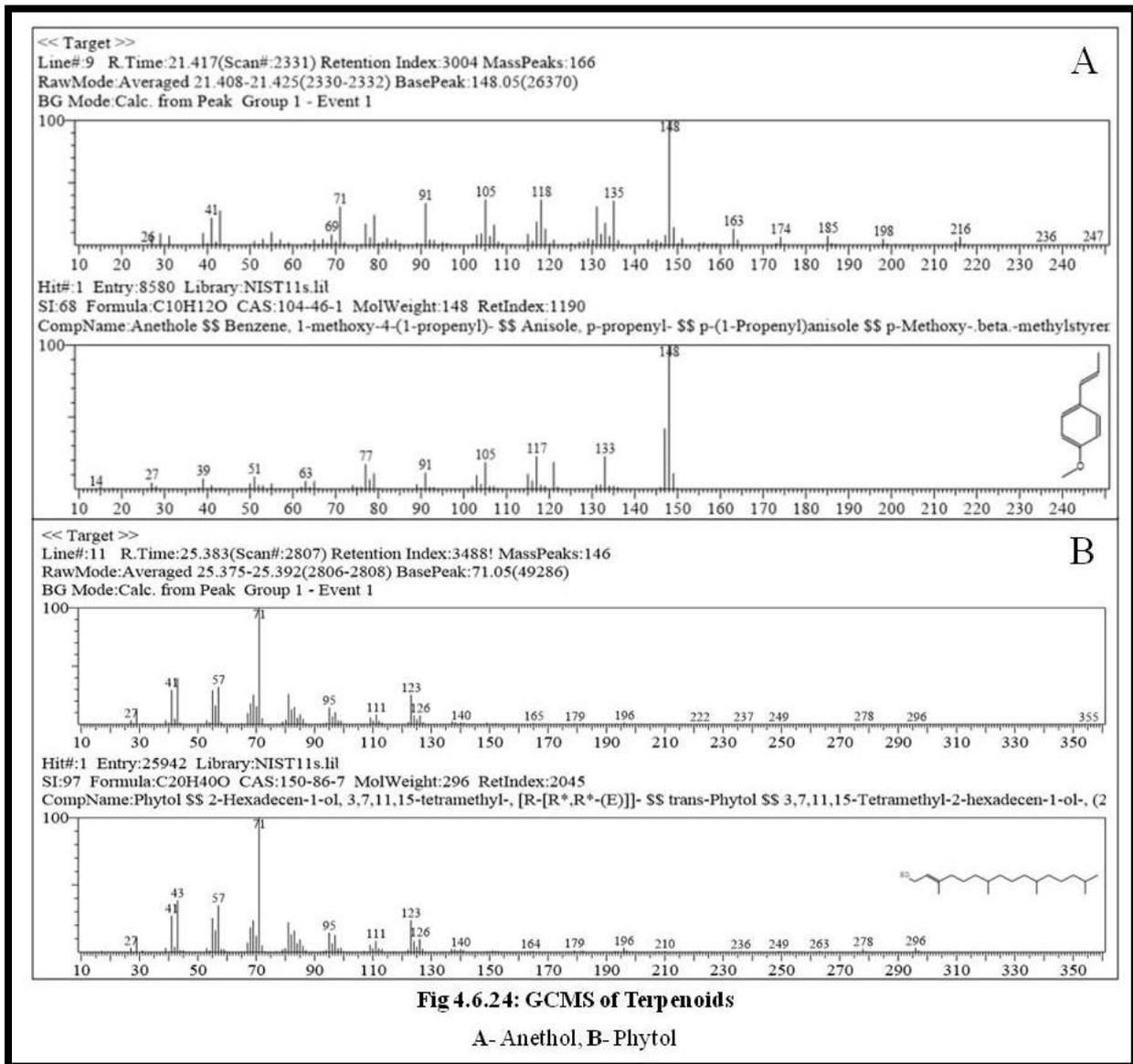
Fig 4.6.23: LC MS of m/z 905 stigmast-5-22-dien-34, 21-diol,34,21,hexadonate

TERPENOIDS

The GCMS extract of *T. jamnagarensis* did not showed presence of any terpenoids except lupeol. While the *T. collina* extracts of aerial and seed parts showed presence of various terpenoids (Fig 4.6.24 to Fig 4.6.26).

- Monoterpenes: Anethole.
- Diterpenes: Phytol from the aerial part extract.
- Sesquiterpenes: Lanost-8-en-3ol, Caryophellene, Beta Endesmol and Alpha santol.
- Further the LCMS showed presence of sesquiterpene in *T. jamnagarensis* and *T. collina* extracts was Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S(1.alpha.,7.alpha.,8a.beta)]-(Fig 4.6.26), which was earlier detected from one of the *Tephrosia* species i. e, *T. purpurea*. it is known to impact anti-inflammatory activity
- Triterpenoids: Alpha amyirin, beta amyirin, friedelan-3-one and Lup20(29)en3one .

Among detected terpenoids some of these were also recorded form other *Tephrosia* species. Caryophyllene was earlier recorded from *T. vogelii* and *T. densiflora*. Alpha and Beta Amyrin are triterpenes earlier recorded from *T. strigosa* (Sreenivasulu and Sarma, 1988) while Lupenone which caused a significant reduction in fasting blood glucose (FBG) levels in diabetic rats (Feng Xu *et al.*, 2014), is earlier recorded from *T. villosa* (Prashant and Krupadanam, 1993).



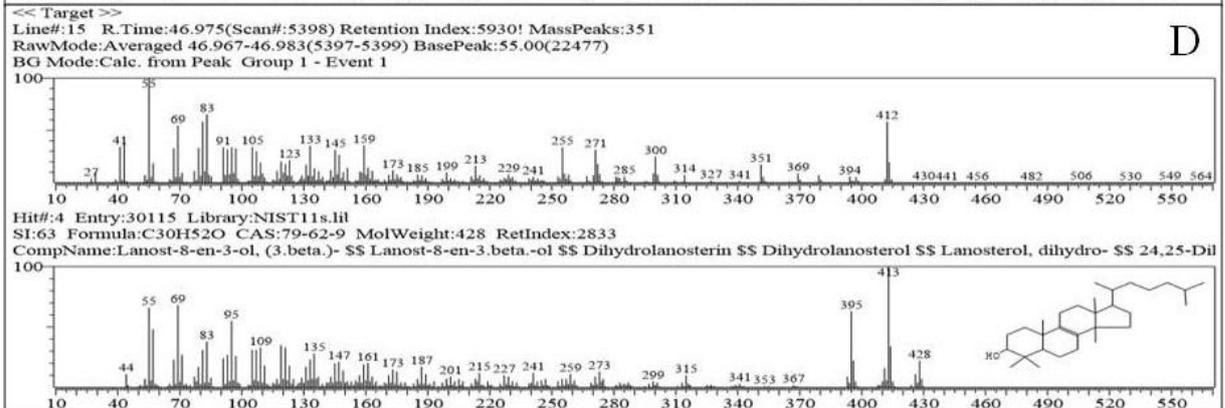
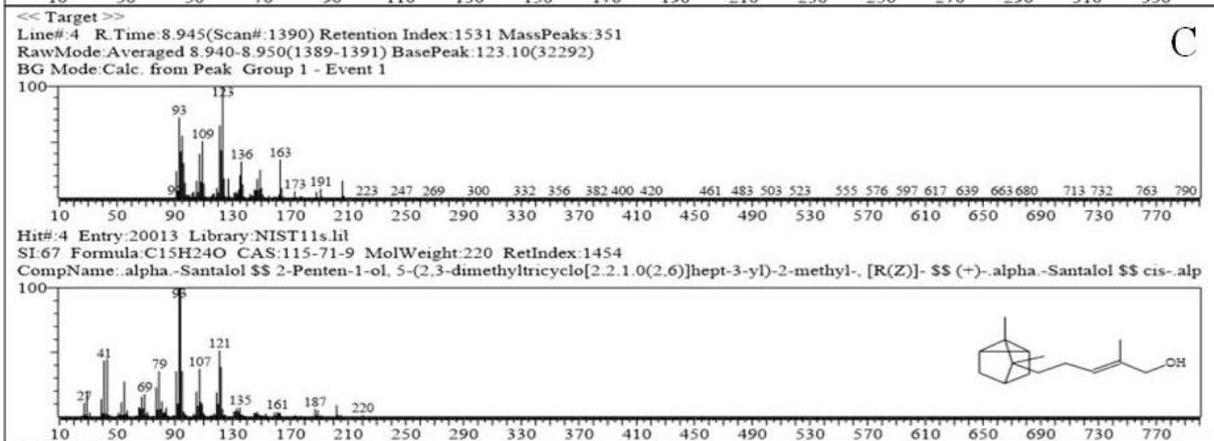
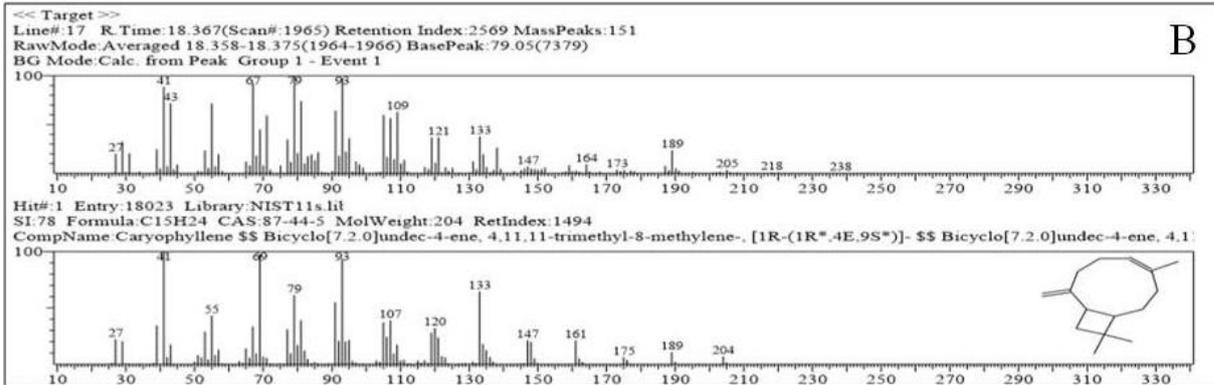
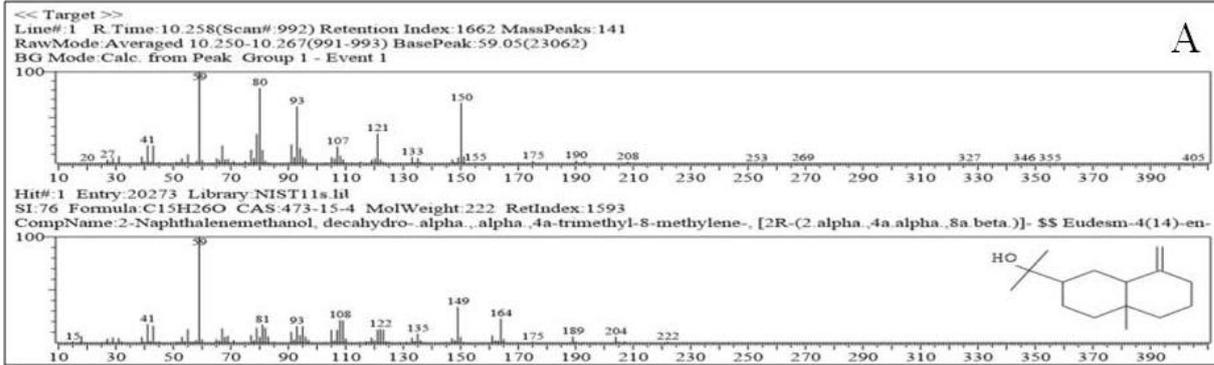


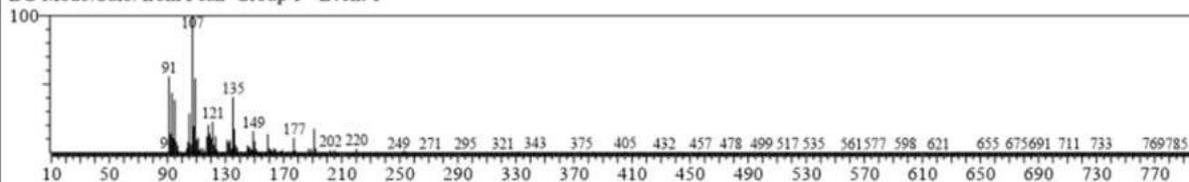
Fig 4.6.25: GCMS of Sesquiterpenes

A- β -Eudesmol, B- Caryophyllene, C- Alpha-santalol, D- Lanost-8-en-3-ol

<< Target >>

Line#:2 R. Time: 8.700(Scan#:1341) Retention Index:1506 MassPeaks:420
RawMode:Averaged 8.695-8.705(1340-1342) BasePeak:107.10(13105)
BG Mode Calc. from Peak Group 1 - Event 1

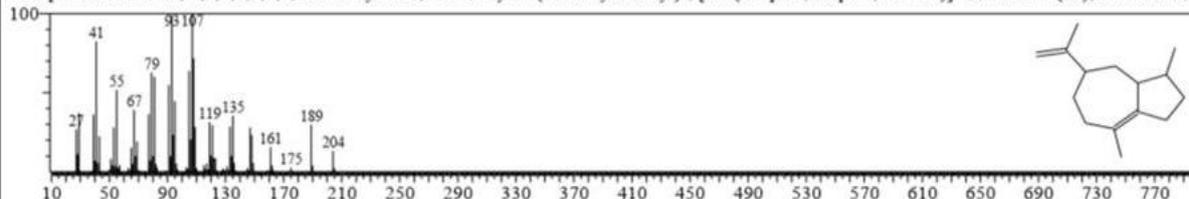
A



Hit#:1 Entry:18093 Library:NIST11s.lil

SI:74 Formula:C15H24 CAS:3691-11-0 MolWeight:204 RetIndex:1490

CompName:Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-



■ +EPI (531.03) Charge (+1) CE (39.5344) FT (250): Exp 8, 1.105 min from Sample 4 (TJR) of 050514.wiff (Turbo Spray)

Max. 3.8e5 cps.

B

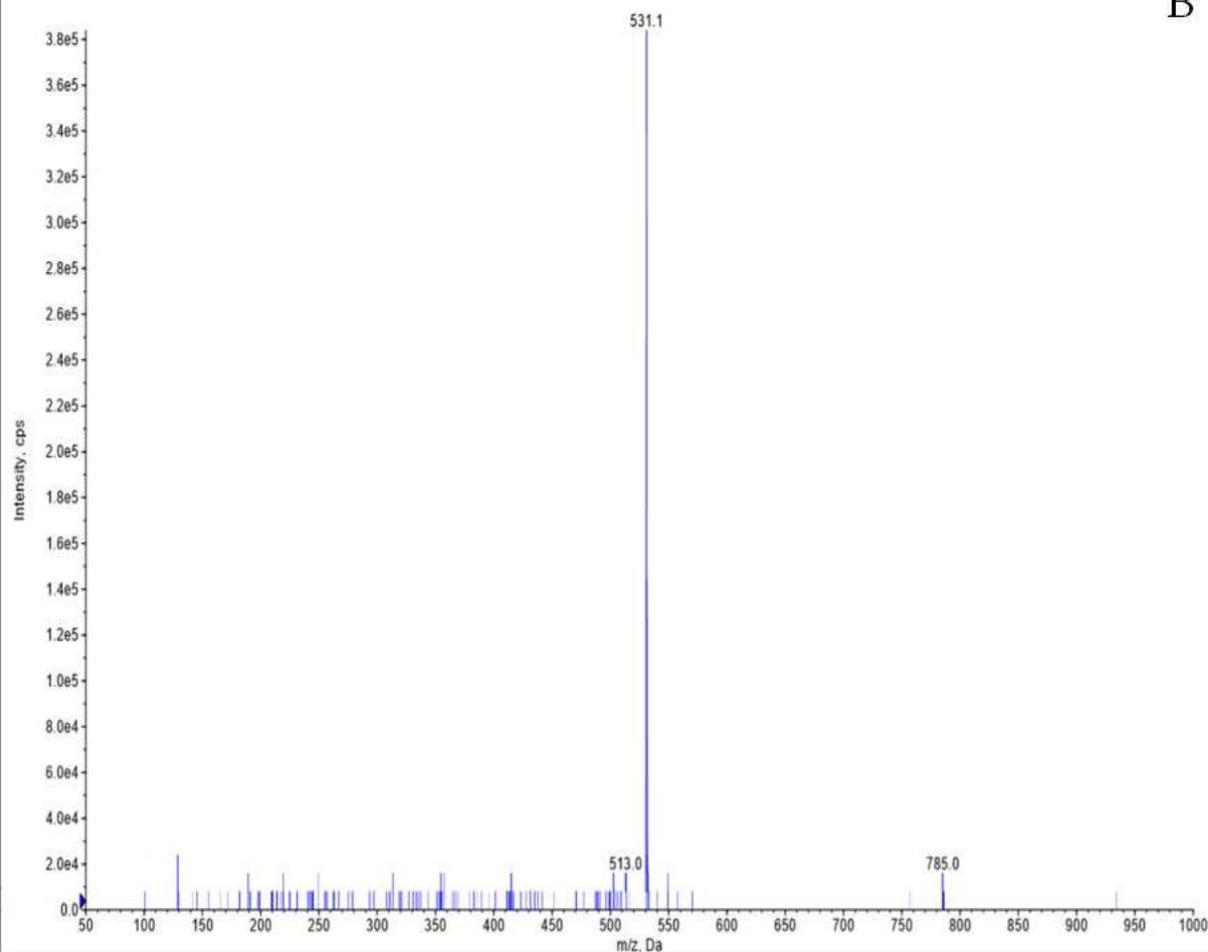


Fig 4.6.26: Sesquiterpene-Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-

A- GCMS, B- LCMS

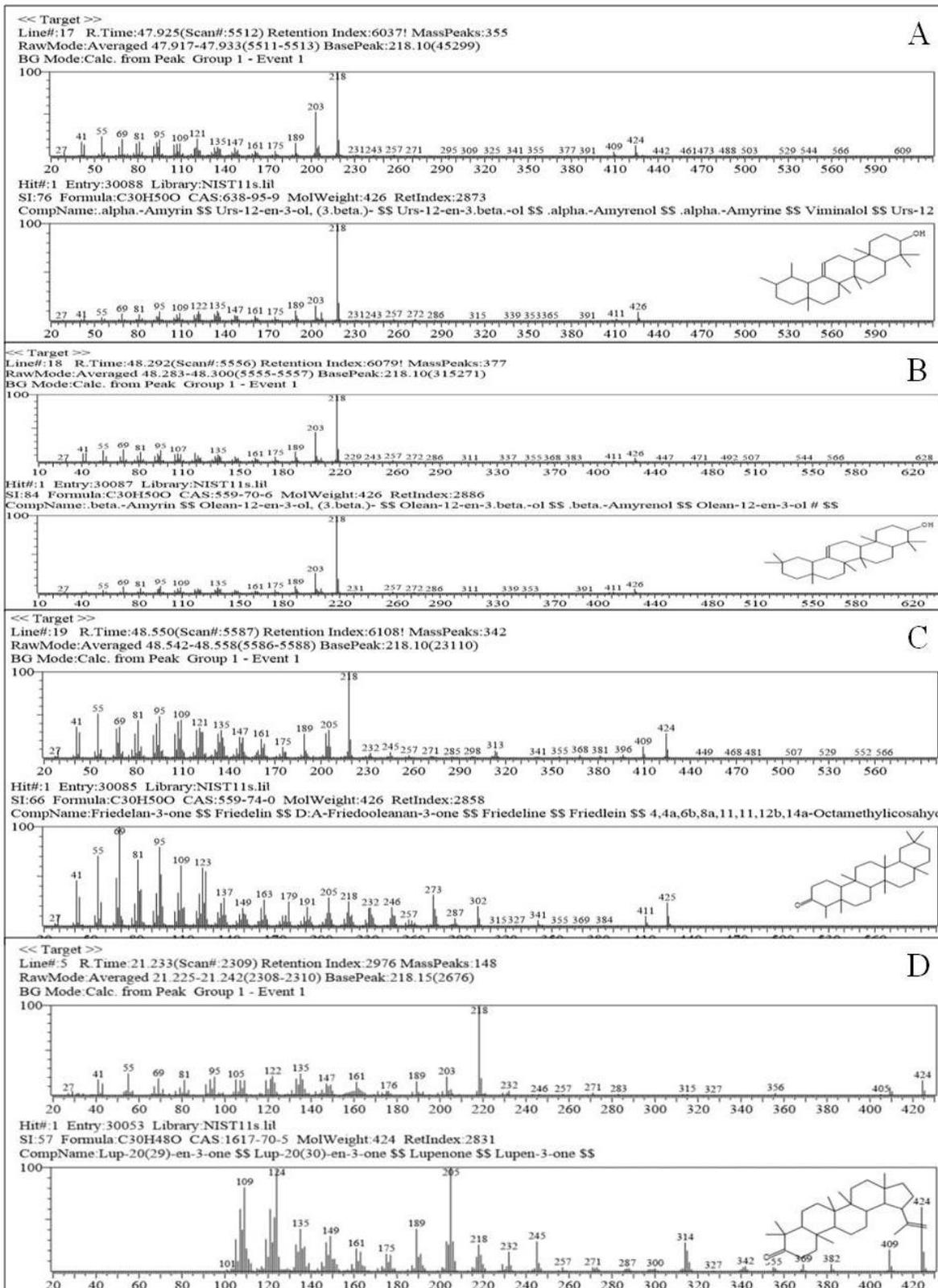


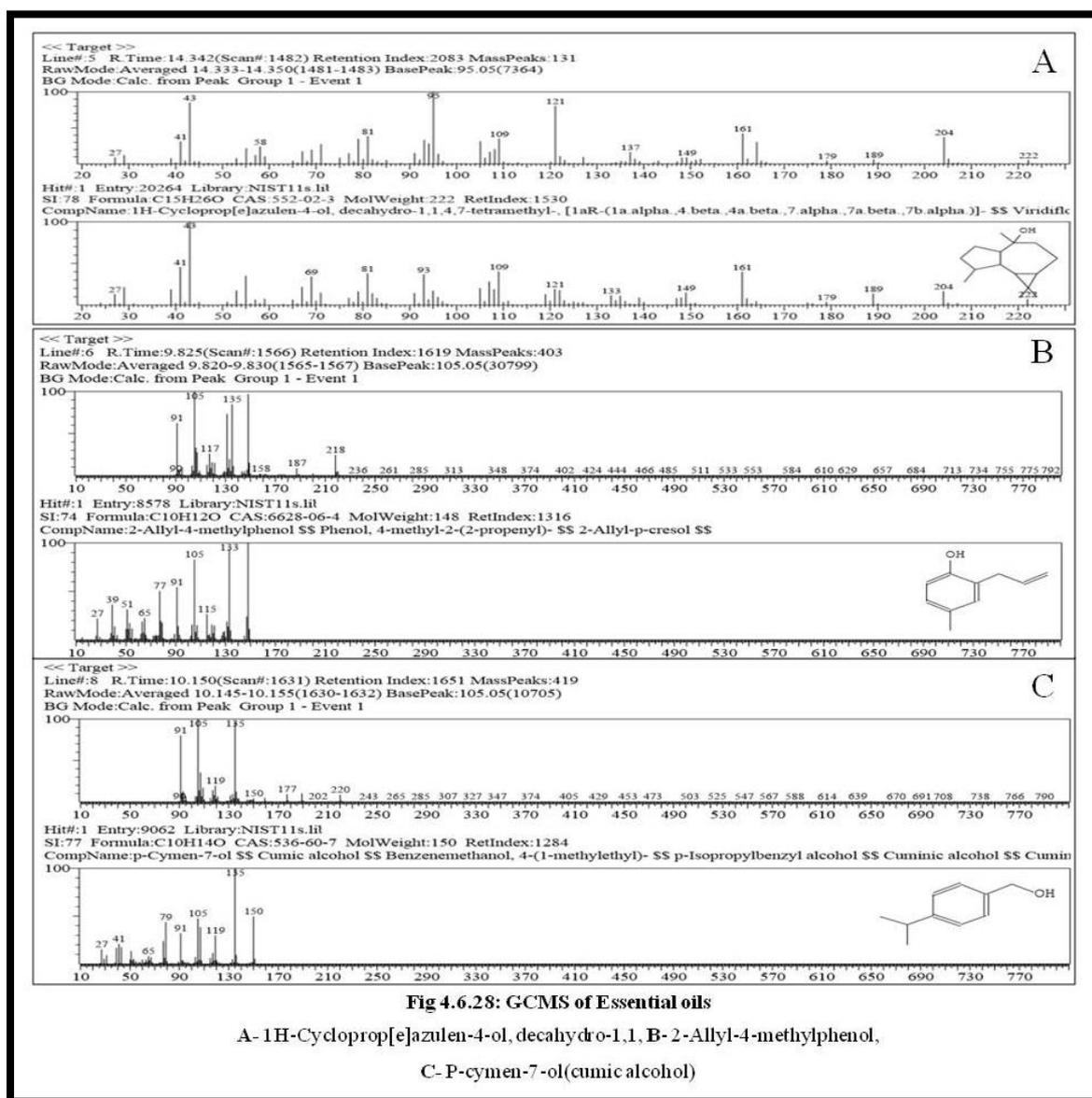
Fig 4.6.27: GCMS of Triterpenes

A- Alpha amyryn, B- Beta- amyryn , C- Friedelan-3-one, D- Lup20(29)en-3-one

ESSENTIAL OILS

The GCMS analysis of the *T. collina* aerial part and seed extract showed presence of various essential oils. The details of its are given below

- Similarly, 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1 (Fig 4.6.27B) was detected from *T. collina* aerial extracts. It was also recorded from other plant species *Aglaia lawii* leaves (Lavate *et al.*, 2014) as well as *Endostemon obtusifolius* leaves (Sadashiva *et al.*, 2013).
- 2-Allyl-4-methylphenol and P-cymen-7-ol (cumic alcohol) (Fig 4.6.27 C&D) both these volatile oils were also recorded from the *Mentha rotundifolia* L. which gives mentha oil property of antimicrobial and antioxidant properties (Riahi *et al.*, 2013).



BENZOQUINONE

The aerial part of *T. collina* showed presence of 2,5-di-tert-butyl-1,4-benzoquinone (Fig 4.6.29), which possesses antioxidant property. Similar compounds were detected from the other Leguminosae members like *Acacia melanoxylon* and *Caesalpinia pulcherima* (Bisky, 1994)

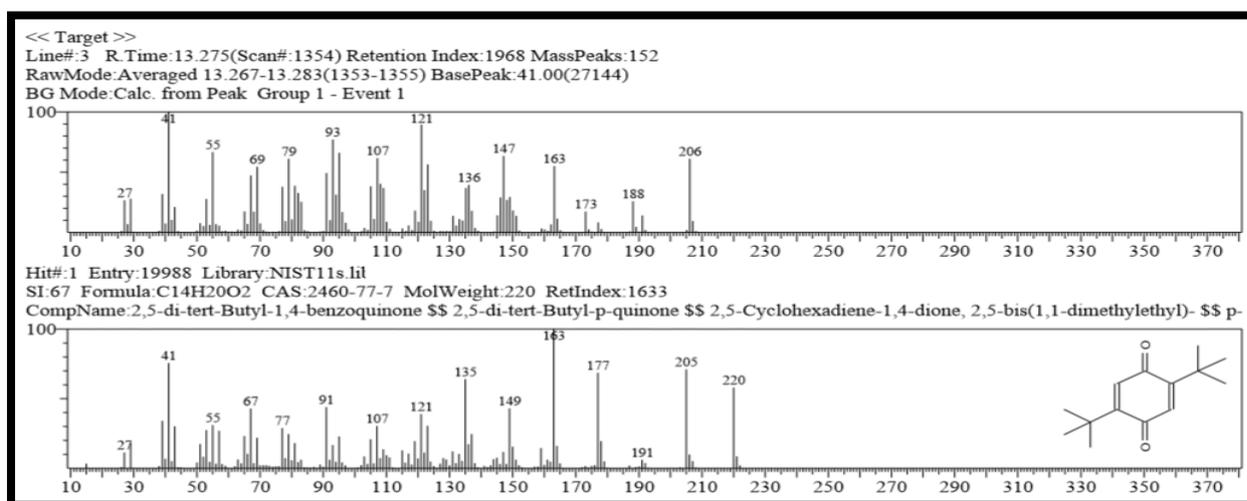


Fig 4.6.29: GCMS of 2,5-di-tert-butyl-1,4-benzoquinone

SAPONIN

GCMS analysis of *T. collina* showed presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (Fig 4.6.30) . It is a conjugated saponin named soyasaponin alpha. This had been also isolated from many Fabaceae members like *Phaseolus coccineus* (Yoshiki *et al.*, 1994). It is also foundns as a nonenzymic product in dehydrated and cooked foods.

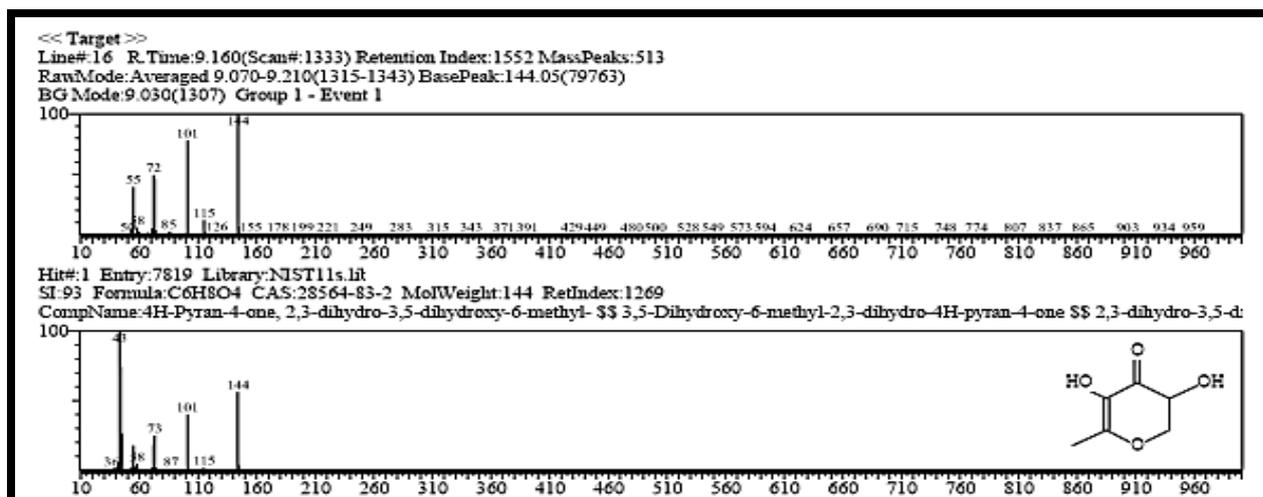


Fig 4.6.30: GCMS of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl

Similarity between other *Tephrosia* species with *T. jamnagarensis* and *T. collina*.

Phytochemical analysis of *T. collina* and *T. jamnagarensis* showed presence of some Phytoconstituents common with other *Tephrosia* species. *T. jamnagarensis* showed presence of 41 compounds of which 20 compound are recorded from other *Tephrosia* species (Table 4.6.3.8). On the contrary in *T. collina* 56 compounds were recorded of which 25 compounds are found in other *Tephrosia* species (Table 4.6.3.8).

Table 4.6.3.8: Phytochemical similarity of TJ and TC with other *Tephrosia* species.

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i> Detected compounds	<i>T. collina</i> Detected compounds	Other <i>Tephrosia</i> sp. (c.f. chapter 2; section 2.4)
SUGAR ALCOHOL				
1.	D -mannitol	-	+	-
CARDIAC GLYCOSIDE				
2.	Strophantidin	-	+	-
AMINO ACID ESTER				
3.	3,5-pyrimedicarboxylic acid	-	+	-
FATTY ACID				
4.	Palmitic acid (Hexadecanoic acid)	+	+	+
5.	Caprinic acid (Decanoic acid)	+	-	-
	Stearic acid (Octadecanoic acid)	+	-	+
6.	Sebacic acid (Decanedioic acid)	-	+	-
7.	methyl 14 methyl pentadecanoate (Pentadecanoic acid, 14 methyl)	+	+	-
8.	Cetene (1 hexadecene)	+	-	-
9.	Trans-2-undecenoic acid	+	-	
Unsaturated Fatty acid				
10.	Vaccenic acid (9, 11- Octadecadienoic acid).	+	+	+
11.	Oleic acid (9, Octadecadienoic acid Z-)	+	+	+

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i> Detected compounds	<i>T. collina</i> Detected compounds	Other <i>Tephrosia</i> sp. (c.f. chapter 2; section 2.4)
12.	Octadec11-enoic acid (11-Octadecenoic acid)	-	+	+
13.	linolenic acid (9,12,15-Octadecatrienoic acid, (Z,Z,Z)-)	-	+	+
14.	<i>cis</i> 9 hexedecenal (Z-9-hexadecenal)	+	-	-
15.	Octadecanedioic acid (Hexadecanedicarboxylic acid)	-	+	-
FATTY ACID ALCOHOL				
16.	Behenic alcohol	-	+	+
ALKALIOIDS				
17.	Norephedrine	+	-	-
18.	Diethyl 2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate	+	-	-
19.	(-)-Quebrachidin	-	+	-
CHALCONES				
20.	Piperonylic acid	+	-	-
21.	2 propen-1-one, 3-(4-nitrophenyl)-1-phenyl	+	-	-
FLAVONOIDS				
22.	Quercetin	+	+	+
23.	Rutin	+	+	+
24.	4H-1-benzopyran-4-one(Flavone-3-hydroxy)	+	-	-
25.	4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl-	+	-	+
26.	Dereticulatin triacetates	+	-	-

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i> Detected compounds	<i>T. collina</i> Detected compounds	Other <i>Tephrosia</i> sp. (c.f. chapter 2; section 2.4)
27.	Daidzein ^{4',7} -diglucoside	+	+	+
28.	Luteolin hexoside hexoside	+	+	-
29.	Luteolin-7-oglucuronide	+	+	-
30.	Kaempferol-3- o-(p-coumarolglycoside)	+	+	-
31.	Quercetin -3-o-rutinoside	+	+	-
32.	Galloyl-A-type procyanidin dimer	+	+	-
33.	p-Lariciresinola-9- sterate	+	+	+
34.	Quercetin-hexoside-hexoside	+	+	-
35.	Quercetin-7-o-hexoside-3o-(malonyl) hexoside	+	+	-
36.	Luteolin 7-o rutinoside	+	+	-
37.	6- methoxy kaempferol	+	+	-
38.	Kaempferol	+	+	+
39.	Naringenin	+	+	-
40.	Rotenone	+	+	+
41.	Isorhamnetin-3-Orutinoside	-	+	-
PHENOLIC ACIDS				
42.	Vanillic acid	+	+	+
43.	Syringic acid	+	+	+
44.	Ferulic acid	+	+	-
45.	Cis-o-coumaric acid	+	+	-
46.	Quinicquinic caffeic acid ester	+	+	+
47.	3,5-di-O-cafferyl quinicacid	+	+	+
STEROIDS				
48.	Cholestan 3-ol	+	+	-
49.	β-sitasterol	+	+	+

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i> Detected compounds	<i>T. collina</i> Detected compounds	Other <i>Tephrosia</i> sp. (c.f. chapter 2; section 2.4)
50.	Lanosterol		+	-
51.	Stigmasterol		+	+
52.	Stigmast-5,22 dien-34,21 diol-34,21hexadecanote	+	+	+
TERPENOIDS				
MONOTERPENE				
53.	Anethole	-	+	-
DITERPENE				
54.	Phytol	-	+	+
SESQUITERPENES				
55.	β -Eudesmol	-	+	-
56.	Lanost-8-en-3ol,	-	+	-
57.	Alpha santol	-	+	-
58.	Caryophyllene	-	+	+
59.	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	+	+	+
TRITERPENES				
60.	Alpha amyirin	-	+	+
61.	Lup20(29)en-3-one	-	+	+
62.	Friedelan-3-one	-	+	+
63.	Beta amyirin	-	+	+
64.	Lupeol	+	+	+
ESSENTIAL OILS				
65.	2-allyl-4-methylphenol	-	+	-
66.	1H-Cycloprop[e]azulen-4-	-	+	-

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i> Detected compounds	<i>T. collina</i> Detected compounds	Other <i>Tephrosia</i> sp. (c.f. chapter 2; section 2.4)
	ol,dehydro-1,1			
67.	p-cymen-7-ol	-	+	-
BENZOQUINONE				
68.	2,5-di-tert-Butyl-1,4-benzoquinone	-	+	-
SAPONIN				
69.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	-	+	-

*+ sign indicate presence of components, - sign indicate absence of it

PHYTOCHEMICALS FOUND IN TJ AND TC IN BRIEF

- Primary phytochemical metabolites analysis showed the presence of **carbohydrates** like Glucose, mannose, maltose, fructose, ribose, xylose and sucrose in both the plants.
- D-mannitol a sugar alcohol recorded from seed extracts of *T. collina*.
- Strophantidin a cardiac glycoside was first time recorded from Fabaceae family in aerial plant part of *T. collina*.
- The aerial and roots extracts of both plant showed absence of one essential **amino acid** Histidine and one non essential amino acid is Asparagine.
- *T. jamnagarensis* showed presence of ten **fatty acid** of which two fatty acid Pentadecanoic acid, 14 methyl and methyl stearate were present in aerial part while eight fatty acid i.e, n-Hexadecanoic acid, Decanoic acid, 9,11-Octadecadienoic acid (linoleic acid), 9-Octadecadienoic acid (oleic acid), Oleic acid, 1-hexadecene, octadecanedioic acid and Cis-9-hexadecenal in seed.
- In *T. collina* eight fatty acid were detected of which octadecanoic acid was found in both aerial and seed parts while fatty acid like 1-Nonadecene and 9,12,15 octadecatrienoic acid (α -Linolenic acid) were present in aerial parts. The fatty acids of seed of *T. collina* were 9-Octadecanoic acid, 9,12-Octadecadienoic acid,

hexadecanoic acid, 11-octadecenoic acid methyl ester and pentadecanoic acid 14methyl.

- Secondary metabolite phytochemicals analysis in *T. jamnagarensis* showed presence of polyphenols like flavonoids, chalcones, phenolic acids, steroids, and alkaloids where as in *T. collina* it includes alkaloids, steroids, flavonoids, phenolic acid, terpenoids, saponin, benzoquinone and essential oils.
- Two **alkaloids** namely (2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate and (-)-norephedrine, were recorded from aerial plant of *T. jamnagarensis* where as *T. collina* showed presence of one alkaloids (-)- Quebrachidin in its seeds.
- **Chalcones** were only detected in *Tephrosia jamnagarensis* aerial part namely piperonylic acid and 2propen-1-one,3-(4-nitrophenyl)-1-phenyl.
- **Flavonoids** like Quercetin, Kaempferol, Rutin and Naringenin were detected in aerial and root extracts of both the plant. Along with that, it also showed the presence of Flavonls glycoside and anathocyanin (cf. Table 4.6.3.8).
- Rotenoids were only detected in the extracts of *T. jamnagarensis* seeds and *T. collina* roots.
- Both the endemic plant showed presence of vanillic, syringic, 3,5-di-o-cafferyl quinic, quinic ester and *cis and trans o*-coumaric acid where as ferulic acid.
- The phytosterols like Beta sitosterol and stigmasterol which are powerful anti-inflammatory agents were present in aerial extract of TC and TJ.
- Lupeol was only terpenoid found in both the plant aerial parts. In *T. collina* other terpenoids were also recorded. The details are as follows
Monoterpenes - Anethole
Diterpenes - Phytol
Sesquiterpenes - Lanost-8-en-3ol, caryophellene, Beta Endesmol and Alpha santol, Azulene
Triterpenoids - Alpha amyirin, beta amyirin, friedelan-3-one and Lup20(29)en3one.
- The other phytochemicals like essential oils-1H-Cycloprop[e]azulen-4-ol, decahydro-1,1; 2-Allyl-4-methylphenol, P-cymen-7-ol (cumic alcohol) and benzoquinone were also detected *T. collina*.

- TJA showed presence of toxic components like alkaloid (-)- Norephedrine, and in *T. collina* aerial part showed saponin 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl .

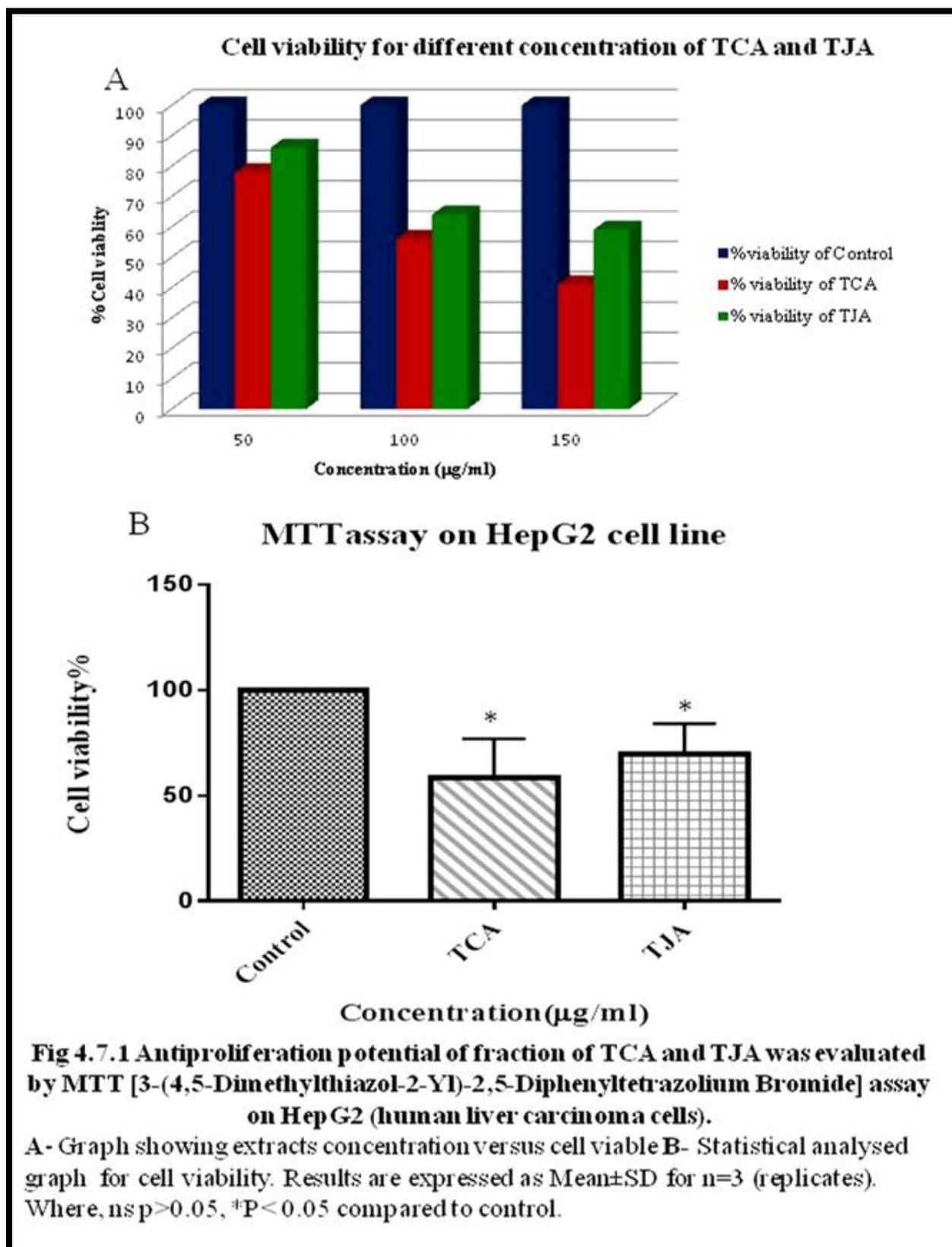
4.7 PHARMACOLOGY

4.7.1 *In-vitro*

Tephrosia purpurea had been worked out for its biological activity especially related to liver disorder, spleen and jaundices (*c.f.* chapter 2; section 2.5). The hepatoprotective activity in *T. purpurea* is due to presence of principle phytochemicals Tephrosin and Quercetin (Kashaw *et al.*, 2011). In present phytochemical analysis of *T. jamnagarensis* and *T. collina* presence of Quercetin along with other components were detected (*c.f.* Chapter 4; section 4.4.6). However till today there are no report on biological activity related to TJ and TC. Hence the aqueous extract of the two endemic species aerial parts [*T. jamnagarensis* aerial part (TJA) and *T. collina* aerial part (TCA)] were taken to study the hepatoprotective property.

In-vitro evaluation of hepatoprotective property was studied by antiproliferation potential by MTT [3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide] assay on HepG2 cell line and Lymphocyte. Tryphan blue assay on Lymphocyte cell was also done.

Aqueous extracts of *T. jamnagarensis* and *T. collina* aerial parts were added at different concentrations (50, 100 and 150 µg/ml) to cultured the HepG2 cells, and incubated for 48 hrs. After incubation, it was observed that, *T. collina* and *T. jamnagarensis* aerial part extracts were cytotoxic to the liver cancer cell line at all the tested concentrations (Fig 4.7.4). results revealed that TCA and TJA accounted for significant antiproliferative effect (< 50% viability) at 100 and 150 µg/ml against HepG2 cells. The IC₅₀ value for these were found to be <150 µg/ml in *T. collina* where as its >150 µg/ml in *T. jamnagarensis* (Fig 4.7.1). Cell count and percentage viability of HepG2 cells treated with *T. collina* and *T. jamnagarensis* aerial clearly indicate that these plant extracts inhibit the proliferation of HepG2 cells at 50, 100 and 150 µg/ml concentrations (percentage viability decreased as the concentration of extracts increased) when compared to that of the controls (Fig 4.7.4).



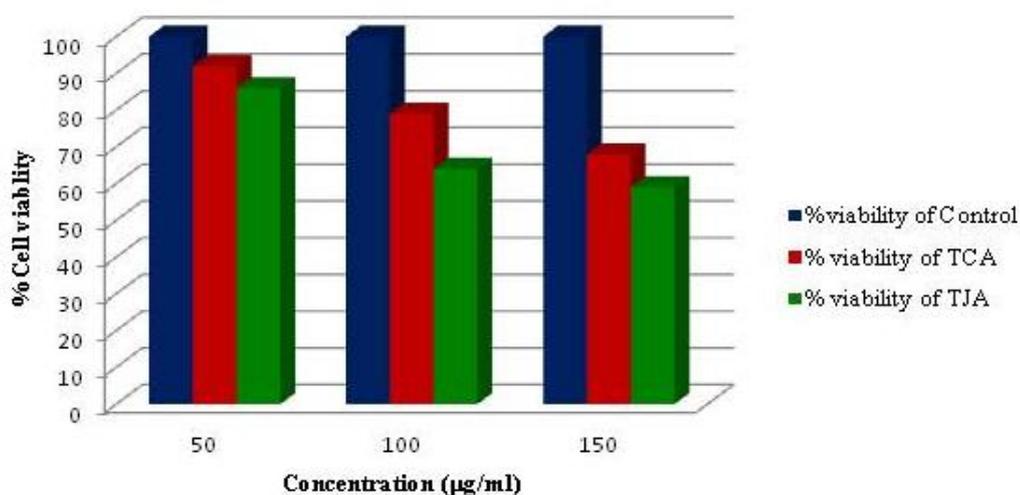
In lymphocytes MTT assay analysis, it was observed that the percentage viability was nearing to 70 % almost in all the concentration of both the plant aerial extracts. (Figure 4.7.2 and Fig 4.7.4) and the differences were not statistically significant ($p>0.05$). *T. collina*

and *T. jamnagarensis* extracts treatments have reduced the percentage viability of lymphocytes to 80%-75% at 50 and 100µg/ml concentrations. This indicates that aqueous extract of *T. collina* and *T. jamnagarensis* is not effective against lymphocytic cell line.

These results indicate that the *T. collina* and *T. jamnagarensis* aerial part (TCA and TJA) extracts which were inhibitory to the proliferation of HepG2 cell line were less cytotoxic to lymphocytes and appear to be quite safe for humans. The analysis trypan blue dye exclusion method on *T. collina* and *T. jamnagarensis* extracts treatments have reduced the percentage viability of lymphocytes to 90% at 50 and 100µg/ml concentrations (Fig 4.7.3).

The phytochemicals like 7,4'-dihydroxy-3',5'-dimethoxyisoflavone and (+)-tephropurpurin were obtained from *T. purpurea*, were effective bioassay against based cell line cultured Hepa 1c1c7 mouse hepatoma cells (Chang *et. al.*,1997). In present study, 4H-1-benzopyran-4-one(Flavone-3-hydroxy) and 4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl- were detected from the *T. jamnagarensis* aerial parts, and its extract was significantly effective for bioassay based on cell line cultured HepG2 human hepatoma cells. In *T. collina* the presences of Flavonols like Quercetin and Rutin, along with terpenoids were considered to effective for the bioassay based on cell line cultured HepG2 human hepatoma cells and lymphocytes cells.

A Cell viability for different concentration of TCA and TJA



B MTT Assay on Lymphocyte cell line

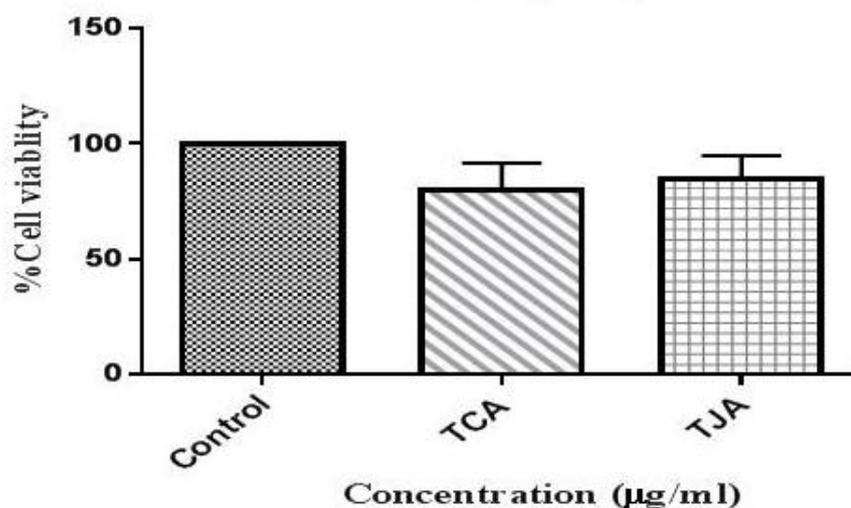
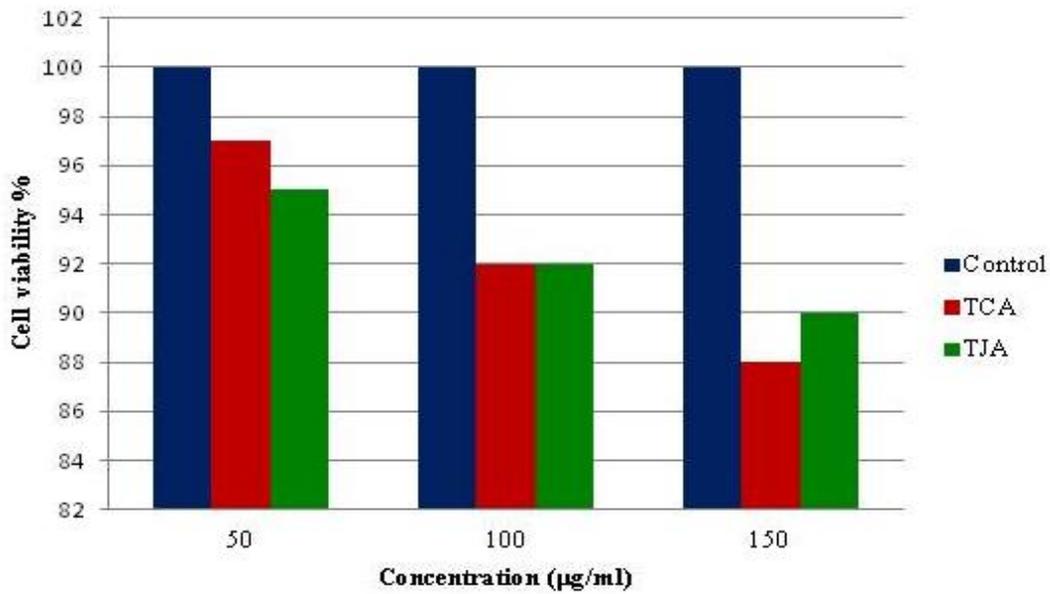


Fig 4.7.2 Antiproliferation potential of fraction of TCA and TJA was evaluated by MTT [3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide] assay on lymphocyte cells.

A- Graph showing extracts concentration versus cell viable B- Statistical analysed graph for cell viability. Results are expressed as Mean±SD for n=3 (replicates). Where, non significant $p > 0.05$, compared to control. Here result are non significant

A

Cell viability for different concentration of TCA and TJA



B

Tryphan blue Assay on Lymphocyte cell

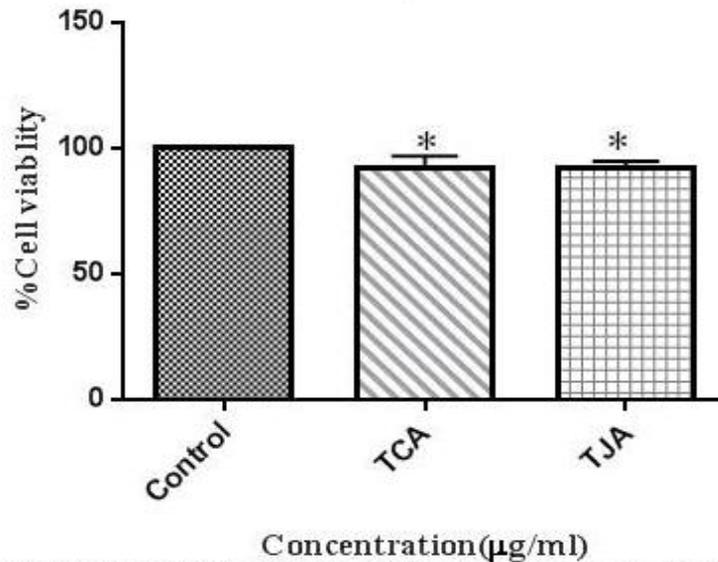
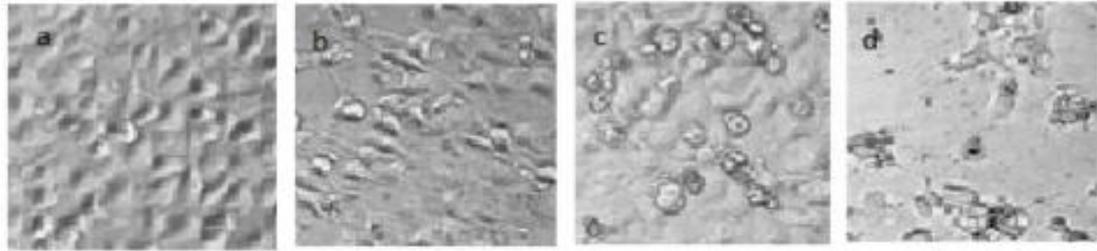
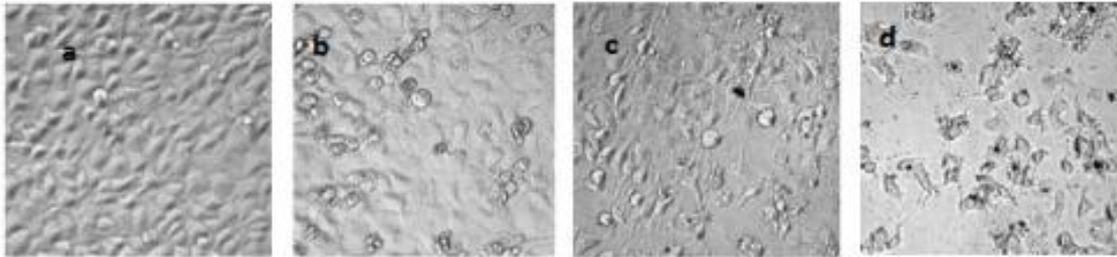


Fig 4.7.3 Antiproliferation potential of fraction of TCA and TJA was evaluated by Tryphan blue assay on Lymphocyte cells.

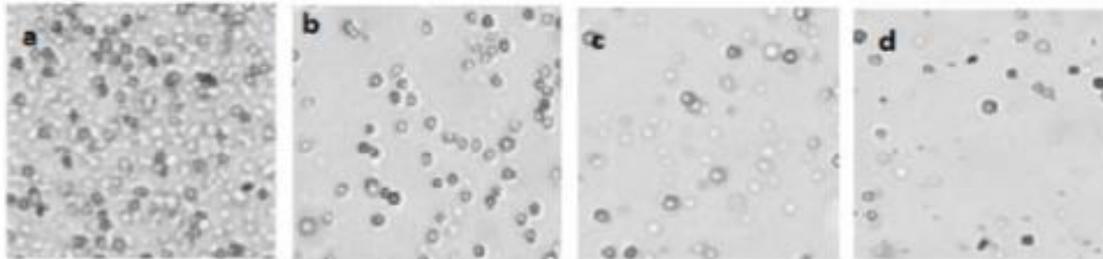
A- Graph showing extracts concentration versus cell viable B- Statistical analysed graph for cell viability. Results are expressed as Mean±SD for n=3 (replicates). Where, ns p>0.05, *p < 0.05 compared to control.



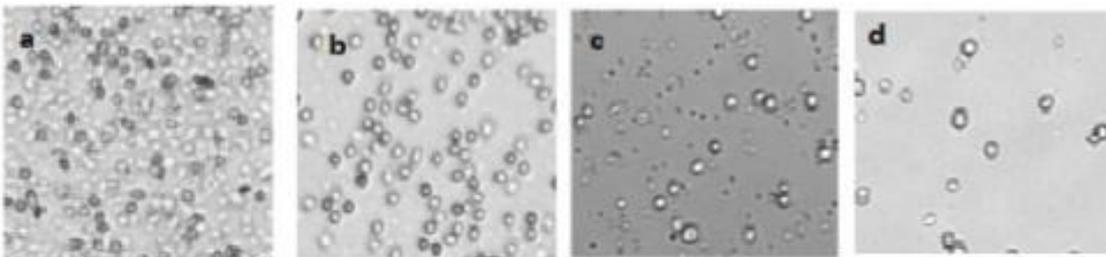
HpeG2- TCA: a) Control b) 50 µg/ml c) 100 µg/ml d) 150 µg/ml



HpeG2- TJA: a) Control b) 50 µg/ml c) 100 µg/ml d) 150 µg/ml



Lymphocute- TCA: a) Control b) 50 µg/ml c) 100 µg/ml d) 150 µg/ml



Lymphocute- TJA: a) Control b) 50 µg/ml c) 100 µg/ml d) 150 µg/ml

Fig 4.7.4 : Cell count photograph of MTT assay of TCA and TJA extracts

4.7.2 *In-vivo* analysis

The methanol extract and aqueous extract of TCA were subjected to acute toxicity determination as per Organisation for Economic Co-operation and Development (OECD) guidelines. None of these showed mortality even at the dose level of 2000 mg/kg and therefore considered to be safe.

All the extracts were investigated for the hepatoprotective activity in rat using different models.

1. Hepatoprotective activity:

The extracts and fractions of the selected plant were subjected to hepatoprotective activities *In-vivo*.

2. Hepatoprotective activity *in-vivo*:

All the extracts were administered at dose levels of 100, 200, 300 mg/kg, where as their fraction were administer at dose levels of 50, 150 mg/kg dose level. Silymarin was used as positive control and was administered at dose level of 100 mg/kg.

3. Hepatoprotective activity of the extracts:

Methanol extract (ME), and aqueous extract (AE) of aerial parts of *T. collina* were subjected to preliminary evaluation of hepatoprotective activity *In-vivo*, against CCL₄- induced toxicity by assessing them through biochemical parameters and histopathological observations. The selected extracts were tested at fixed dose level of 200 mg/kg p.o. while the silymarin at dose level of 100 mg/kg p.o. was used as positive control.

Effect of TCA extracts against CCL₄-induced hepatotoxicity:

CCL₄ intoxication in normal rats elevated the serum levels of GOT (141.83±29.46 to 345.33 ± 34.36), GPT (117.30 ±14.44 to 249.02 ±37.36), ALKP (332.50 ± 21.59 to 455.0 ±19.66), TBL (1.23 ±0.19 to 2.37 ±1.16) and ALB (3.90 ± 0.26 to 1.98 ± 0.17) was significantly (p< 0.05) when compared to control indicating actue hepato cellular damage and biliary obstruction leading to necrosis. The rats treated with ME and silymarin extract, showed a significant decreased in all the elevated GOT, GPT, ALKP, TBL, and CHL levels and significant increase in TPTN and ALB levels. The rats treated with AE have shown significant decrease in the levels of GOT and CHL and increase in the levels of ALB. The results are depicted in Table 4.7.1.

Table: 4.7.1 Effect of ME and AE of *Tephrosia collina* against CCL₄-induced hepatotoxicity in rats.

GROUP	GOT(IU/L)*	GPT(IU/L)	ALKP(IU/L)	TBL(mg/dl)	ALB (g/dl)
Control	141.83±29.46	117.3±14.44	332.50±21.59	1.23±0.19	3.90±0.26
CCL ₄	345.33±34.36	249.02±37.36	455.00±19.66	2.37±1.16	1.98±0.17
Silymarin	140.33±28.03	115.50±19.98	352.50±24.95	1.07±0.16	3.26±0.18
AE	173.83±30.56	217.16±31.47	396.17±27.36	1.53±0.20	2.33±0.21
ME	169.67±29.73	127.00±17.54	358.33±19.90	1.16±0.30	3.40±0.33
F Calculated	7.89	6.07	4.50	2.99	10.99

* Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), Alkaline phosphatase (ALKP), total bilirubin (TBL), Albumin (ALB). Values are mean ± SEM of six animals, F theoretical = 2.76(p <0.05). AE: Aqueous extract; ME: methanol extract.

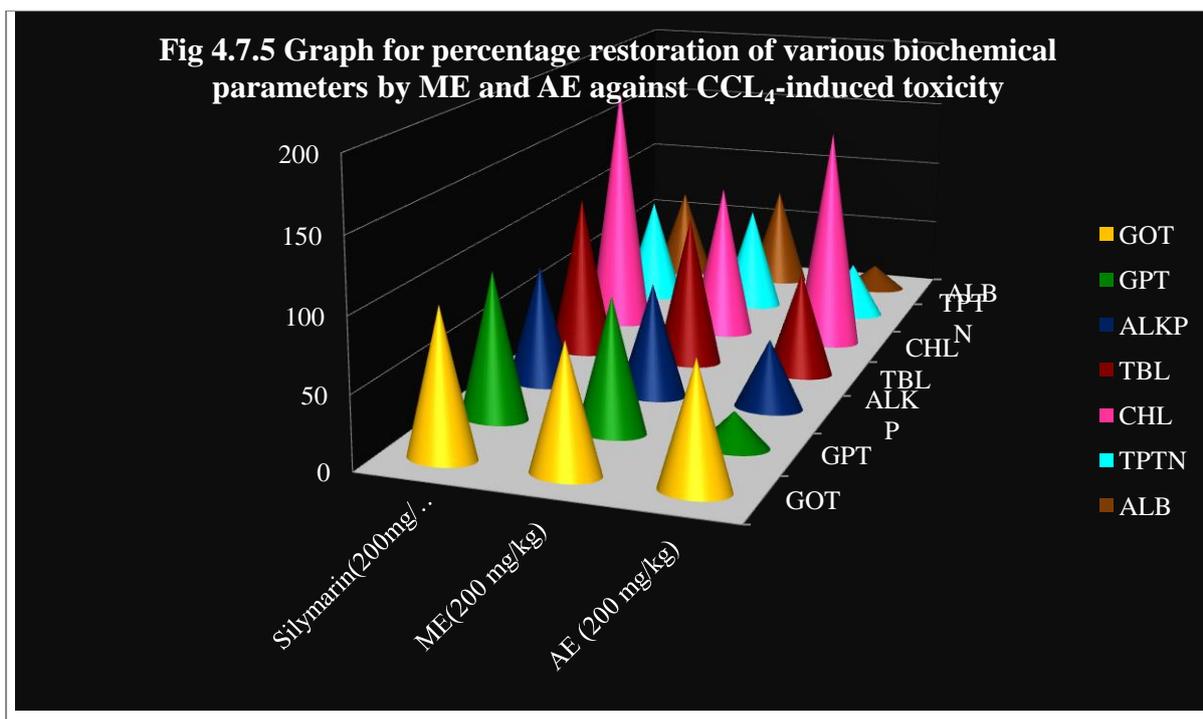
Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. Disarrangement of hepatic cells with intense centrilobular necrosis and vacuolization were observed in CCL₄-intoxicated liver. The sections of the rat's liver treated with ME and intoxicated with CCL₄, were less vacuole without necrosis similar to those in case of silymarin, suggesting the protective effect of the extract. The visible changes were observed in section of rat liver treated with AE and intoxicated with CCL₄, were on the lower side compared to those observed in ME treated rat liver sections.

The percentage restoration of various biochemical parameters in case of silymarin, ME, and AE against CCL₄ was done (Table 4.7.2. and Fig 4.7.5). ME at selected dose offered maximum protection.

Table 4.7.2. Percentage restoration of various biochemical parameters by ME and AE against CCL₄-induced toxicity.

GROUP	GOP	GPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin(200mg/kg)	100.45	101.36	83.64	114.02	180.42	76.28	66.66
ME(200 mg/kg)	86.07	92.01	78.88	106.12	111.24	75.54	73.95
AE (200 mg/kg)	84.03	24.18	48.01	73.67	158.83	39.25	18.23

* Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), Alkaline phosphatase (ALKP), total bilirubin (TBL), Albumin (ALB), Chorophyllin (CHL), total parenteral nutrition (TPTN), AE: Aqueous extract; ME: Methanol Extracts



The present investigation of TCA ME consistently had shown better hepatoprotective property than its AE. The possible reason for these observations may be the fractionation of active polar constituents. The polar compounds are more easily extracted in methanol as compare to water. This fact, in turn may be considered responsible for the methanolic extract (ME) exhibiting better hepatoprotective property than the aqueous extract (AE). This indicates that the methanolic fraction can be further fractionated and individual active compound can be exploited for the treatments of liver and spleen disorders. Similarly in *T. purpurea* flavonoids fraction extracted in ethanolic showed a significant hepatoprotective activity against CCL₄ induced liver damage in rats (Jain *et al.*, 2006).