

# Conclusion

## RESEARCH HIGHLIGHT

This chapter summarize the data generated from the entire study. The aim of the study was to recognizing the phytochemical and pharmlological potentials of the endemic *Tephrosia* species namely *T. jamnagarensis* and *T. collina*. The finding can be highlight as follows.

- Plant samples were collect from the lastly cited site and authenticated from The Botanical survey of India, Jodhpur. There isotype was submitted in BSI Jodhpur.
- Macrophenological features analysis showed negligible deviation from the earlier stated details of these species. *T. jamnagarensis* seed showed the present of the endocarp which was not document earlier. Moreover it was found that *T. jamnagarensis* is more close to *T. strigosa* while *T. collina* to *T. villosa*.
- The organoleptic analysis showed that mucilaginous taste in the aerial parts of *T. jamnagarensis* while no specific taste in its roots. While *T. collina* roots had pungent taste while green tea odour in its aerial parts.
- The *matK* gene sequence of *T. jamnagarensis*, *T. collina*, *T. purpurea*, *T. strigosa* and *T. villosa* were generated and submitted to Genbank NCBI.
- 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
- Ecologically, during present 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.
- Population density of both this endemic species is affect by irregular rainfall and environmental threats due to overgrazing and invasive species.
- Seed germination of both these endemic species increase to 90% after per seed treatment with concentrated sulphuric acid.
- Phenological study of both these plant showed that *T. collina* had long vegetative phase in comparison to *T. jamnagarensis*. It was also found that *T. jamnagarensis* can

shows some of morphological aberrations like trifoliated leaf where as in *T. collina* leaves with higher leaflets pair shows aberration in last pair of leaflet.

- The distinct anatomical marker in *T. jamnagarensis* is presence of paedeomorphic rays and in *T. collina* secondary growth tissues in root and stem. The other anatomical markers are calcium oxalate prism shaped crystal in xylem fibers, anisocytic stomata and unicellular uniseriate trichomes.
- Physicochemical analysis, heavy metal analysis were found to be in accordance within the limits prescribed as per WHO/FDA. Heavy metal like lead and cadmium were not present and mercury in under permissible limit where as Arsenic contents was high.
- Preliminary phytochemicals analysis showed presence of phytochemicals like carbohydrates, protein, terpenoids, steroids, lignans, anthocyanin, flavonoids, quinines and phenolic acids in various plant parts of both these endemic plant.
- Details of phytochemical markers are summarise as follow:

The phytochemicals analysis showed the phytocomponents common with other *Tephrosia* species. In *T. jamnagarensis* showed presence of 41 compounds of which 20 compound are recorded from other *Tephrosia* species. On the contrary in *T. collina* 56 compounds were recorded of which 25 compounds are found in other *Tephrosia* species (Table 5.1)

**Table 5.1 Phytocomponents detected in TJ and TC**

Sr. No.	Phytocomponents	<i>T. jamnagarensis</i>	<i>T. collina</i>
<b>SUGAR ALCOHOL</b>			
1.	D -mannitol	-	+
<b>CARDIAC GLYCOSIDE</b>			
2.	Strophantidin	-	+
<b>AMINO ACID ESTER</b>			
3.	3,5-pyrimedicarboxylic acid	-	+
<b>FATTY ACID</b>			
4.	Palmitic acid (Hexadecanoic acid)	+	+
5.	Caprinic acid (Decanoic acid)	+	-
	Stearic acid (Octadecanoic acid)	+	-
6.	Sebacic acid (Decanedioic acid)	-	+

<b>Sr. No.</b>	<b>Phytocomponents</b>	<b><i>T. jamnagarensis</i></b>	<b><i>T. collina</i></b>
7.	methyl 14 methyl pentadeconate (Pentadecanoic acid, 14 methyl)	+	+
8.	Cetene (1 hexadecene)	+	-
9.	Trans-2-undecenoic acid	+	-
10.	Vaccenic acid (9, 11- Octadecadienoic acid).	+	+
11.	Oleic acid (9, Octadecadienoic acid Z-)	+	+
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 )	-	+
<b>FATTY ACID ALCOHOL</b>			
16.	Behenic alcohol	-	+
<b>ALKALOIDS</b>			
17.	Norephedrine	+	-
18.	Diethyl 2,6-dimethyl-4-phenyl-3,5- pyridinedicarboxylate	+	-
19.	(-)-Quebrachidin	-	+
<b>CHALONES</b>			
20.	Piperonylic acid	+	-
21.	2 propen-1-one, 3-(4-nitrophenyl)-1- phenyl	+	-
<b>FLAVONOIDS</b>			
22.	Quercetin	+	+
23.	Rutin	+	+
24.	4H-1-benzopyran-4-one(Flavone-3- hydroxy)	+	-

<b>Sr. No.</b>	<b>Phytocomponents</b>	<b><i>T. jamnagarensis</i></b>	<b><i>T. collina</i></b>
25.	4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl-	+	-
26.	Dereticulatin triacetates	+	-
27.	Daidzein <sup>4'</sup> ,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	-	+
<b>PHENOLIC ACIDS</b>			
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	+	+
<b>STERIODS</b>			
48.	Cholestan 3-ol	+	+
49.	β-sitasterol	+	+

<b>Sr. No.</b>	<b>Phytocomponents</b>	<b><i>T. jamnagarensis</i></b>	<b><i>T. collina</i></b>
50.	Lanosterol		+
51.	Stigmasterol		+
52.	Stigmast-5,22 dien-34,21 diol-34,21hexadecanote	+	+
<b>TERPENOIDS</b>			
53.	Anethole	-	+
54.	Phytol	-	+
55.	$\beta$ -Eudesmol	-	+
56.	Lanost-8-en-3ol,	-	+
57.	Alpha santol	-	+
58.	Caryophyllene	-	+
59.	Alpha amyrin	-	+
60.	Lup20(29)en-3-one	-	+
61.	Friedelan-3-one	-	+
62.	Beta amyrin	-	+
63.	Lupeol	+	+
64.	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	+	+
<b>ESSENTIAL OILS</b>			
65.	2-allyl-4-methylphenol	-	+
66.	1H-Cycloprop[e]azulen-4-ol,dehydro-1,1	-	+
67.	p-cymen-7-ol	-	+
<b>BENZOQUINONE</b>			
68.	2,5-di-tert-Butyl-1,4-benzoquinone	-	+
<b>SAPONIN</b>			
69.	2H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	-	+

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

- 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*.
- Strophanthidin 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 ( $\alpha$ -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 (c.f. Table 5.1) .
- 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 & 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 in *T. collina*.
- Some toxic components detected are *T. jamnagarensis* aerial part alkaloid (-)-Norephedrine, and in *T. collina* aerial part showed saponin 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl.
- Aqueous extract of *T. collina and T. jamnagarensis* aerial part was significant ( $p < 0.05$ ) in *Invitro* Bioassay against HepG2 (human Liver carcinoma cells) at 100  $\mu\text{g/ml}$  and 150  $\mu\text{g/ml}$
- Aqueous extract of *T. collina and T. jamnagarensis* aerial part was not significant ( $p < 0.05$ ) in *invitro* bioassay against lymphocytes MTT assays while it was significant in Trypan blue test at 100  $\mu\text{g/ml}$  concentrations
- *In vivo* bioassay of methanolic and aqueous extract of *T. collina* aerial part showed that methanolic extract had significant hepatoprotective property correlation the standard Silymarin.
- The detection of the insecticides in GCMS analysis mainly Phthalates, Fenvalerate and Pyrenes indicate that the surrounding agricultural field are regularly sprayed with insecticides and herbicides.
- **Recommendation:**

The presence of the phytocomponents like flavonoids, steroids, terpenes and chalcones in aerial and seed of *T. jamnagarensis* and *T. collina* prove that these plants possess antioxidant property and other biological activities which are yet to be

explored. Hence further there need for checking their medicinal potential. In addition, the conservation measures for both these plants are must for their sustainability in the grassland ecosystem of the Saurashtra peninsula.