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HRLC-MS analysis of *Tephrosia purpurea* (L.) Pers

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Abstract
The genus *Tephrosia*, is widely distributed in many tropical and subtropical countries of the world and have been used in folk medicine for the treatment of large number of diseases. Present investigation was undertaken in order to analyse bioactive constituents present in *Tephrosia purpurea* by using High Resolution- Liquid Chromatography-Mass Spectrometry (HR-LCMS).
The study revealed the presence of secondary metabolites like alkaloid (23-Acetoxysoladulcidine), Flavonoids (5,3'-Dihydroxy-2,4- dimethoxyydalbergiquinol), retinoids (16-Hydroxy-4-carboxyretinoic acid) Coumarin (1-Oxo1H2benzopyran3carboxaldehyde), cyanoguanidine (Pinacidil), stilbenoid, (Demethylbatatasin IV) Sesquiterpenoid, (13Hydroxy5'Omethylmelledonal), germacranolide (Molephantinin) and Phenolic compounds in the selected medicinal plants.

Keywords: *Tephrosia*, alkaloid, retinoids, coumarin, flavonoids, HR-LCMS

Introduction
The genus *Tephrosia Persoon* is currently recognized with ca. 350 species mostly inhabiting in the tropical and subtropical regions of the world with highest concentration in Africa (Mabberley, 2017) [6]. The genus *Tephrosia* is represented by 27 species and one variety in India (Sanjappa 1992) [5], of which 13 species are found in Maharashtra state, while 8 species in Marathwada region. *Tephrosia purpurea* (L.) Pers. is a perennial herb, characterised by compound stipulated Leaflets 13–19, oblanceolate, apex obtuse, retuse or mucronate, base cuneate. Flowers bright rosy–purple or violet. Pods linear, slightly curved, mucronate. In the Ayurveda system, is referred as *Sarwa wran vishapaha* which implies that it can heal any type of wound. It played an important role in the traditional medicine, (Akanksha *et al*., 2014) [1].

Material and Method
The root, stem and leaves of *Tephrosia purpurea* were collected from Dr. Babasaheb Ambedkar Marathwada University (B.A.M.U.), campus near Soneri Mahal Aurangabad, on 30/07/2021, and it was identified following Naik (1998) [7] and Singh *et al.* (2000) [10]. The Voucher specimen no. 010901 were deposited in BAMU Herbarium, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. (MS).
The root stem and leaves of selected medicinal plant were finely powdered. Bioactive compounds were extracted with Ethanol using a Soxhlet's extractor for 76 hrs. The extracts were concentrated to remove the solvents completely by using Rotary evaporator. Plant extract were sent for qualitative analysis of their chemical constituent at SAIF, IIT, Bombay by HRLC-MS technique. The instrument used is Agilent technology G6550A-ifunnel, Q-TOF, LC/MS. Column type is ZORBAX RRHDSBC18, with 100 mm length, 2.1 mm diameter and 1.8 pore size. It was carried out with mass spectrometry mainly for the classes of compound which are non-volatile like higher terpenoids, phenolic compounds, alkaloids, flavonoids, lipids, sugars, and amino acid etc.

Result and Discussion
The HRLC-MS analysis of *Tephrosia purpurea root* showed presence of 18 compounds as shown in table 1. Methyl-ophiopogonin-A is homo-Isoflavonoid from the roots of *Ophiopogon japonicus* and explored by their effects on the release of the inflammatory chemokine eotaxin, stimulated by IL-4 and the combination of IL-4 and TNF-α cells. (Hung *et al.*, 2010) [5]. Compound 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone, as the cytotoxic active metabolites, their most potent cytotoxic activity found against the K562 (Erythroleukemia) cell line (GI50) 8.4 and 7.4 µg/mL, respectively (Aponte *et al*., 2008) [2].
Stem showed presence of 17 compounds as shown in table 2. Pinacidil is cyanoguanidine a new antihypertensive vasodilator, probably acting directly on vascular smooth muscle

(Arrigoni-Martelli *et al.*, 1980) [3]. Spirapril is dipeptide angiotensin-converting enzyme (ACE) inhibitor with a long duration of action, to determine whether duration of inhibition of serum ACE activity may affect regional blood flow (RBF), compared spirapril with captopril, an ACE inhibitor with a short duration of action. (Van den *et al*., 1995) [11].
Leaves showed presence of 19 compounds as shown in table 3.

Compound 23-Acetoxysoladulcidine is an alkaloid isolated from natural herbs exhibit antiproliferation, antibacterial, antiviral, insecticidal, and antimetastatic effects on various types of cancers both *in vitro* and *in vivo*, (Shi *et al.*, 2014) [9]. Batatasin IV is stilbenoid shows inhibitory effects against α-glucosidase regarding their antidiabetic activitieties, (Hashimoto, & Tajima, (1978) [4].

Table 1: Phytochemical compounds present in the root of *Tephrosia purpurea*

Sr. No.	Compound name	Formula	Mass	R.T.	D.B. Diff. (PPM)
1.	Epicatechin pentaacetate	$C_{25}H_{24}O_{11}$	500.1313	8.323	1.08
2.	O-Demethylfonsecin	$C_{14}H_{12}O_6$	276.0637	8.385	-1.28
3.	Aquayamycin	$C_{25}H_{26}O_{10}$	486.1519	8.577	1.33
4	Methylophiopogonone A	$C_{19}H_{16}O_6$	340.0954	10.235	-1.98
5	2',4'-Dihydroxy-4,6'- dimethoxydihydrochalcone	$C_{17}H_{18}O_5$	302.1158	11.6	-1.14
6	Isosativan	$C_{17}H_{18}O_4$	286.1208	11.791	-1.08
7	Hemiariensin	$C_{22}H_{24}O_7$	400.1522	11.184	0.11
8	Hydroxyisonobilin	$C_{20}H_{26}O_6$	362.1727	11.228	0.59
9	5,3'-Dihydroxy-2,4- dimethoxydalbergiquinol	$C_{17}H_{18}O_4$	286.1207	12.396	-0.53
10	Molephantinin	$C_{20}H_{24}O_6$	360.1578	12.522	-1.5
11	Montanin A	$C_{19}H_{20}O_4$	312.1362	12.904	-0.02
12	Grandiflorone	$C_{19}H_{22}O_4$	314.1517	13.061	0.39
13	16-Hydroxy-4-carboxyretinoic acid	$C_{20}H_{24}O_5$	344.1625	13.141	-0.34
14	Angoletin	$C_{18}H_{20}O_4$	300.1369	12.97	-2.58
15	Eudesmin	$C_{22}H_{26}O_6$	386.1721	13.361	2.09
16	2',4',6'-Trihydroxy-3'- prenyldihydrochalcone	$C_{20}H_{22}O4$	326.1517	13.377	0.47
17	Isoeugenol phenylacetate	$C_{18}H_{18}O_3$	282.1257	13.578	-0.49
18	4'-Hydroxy-5,7-dimethoxy-8- methylflavan	$C_{18}H_{20}O_4$	300.1331	13.648	0.18

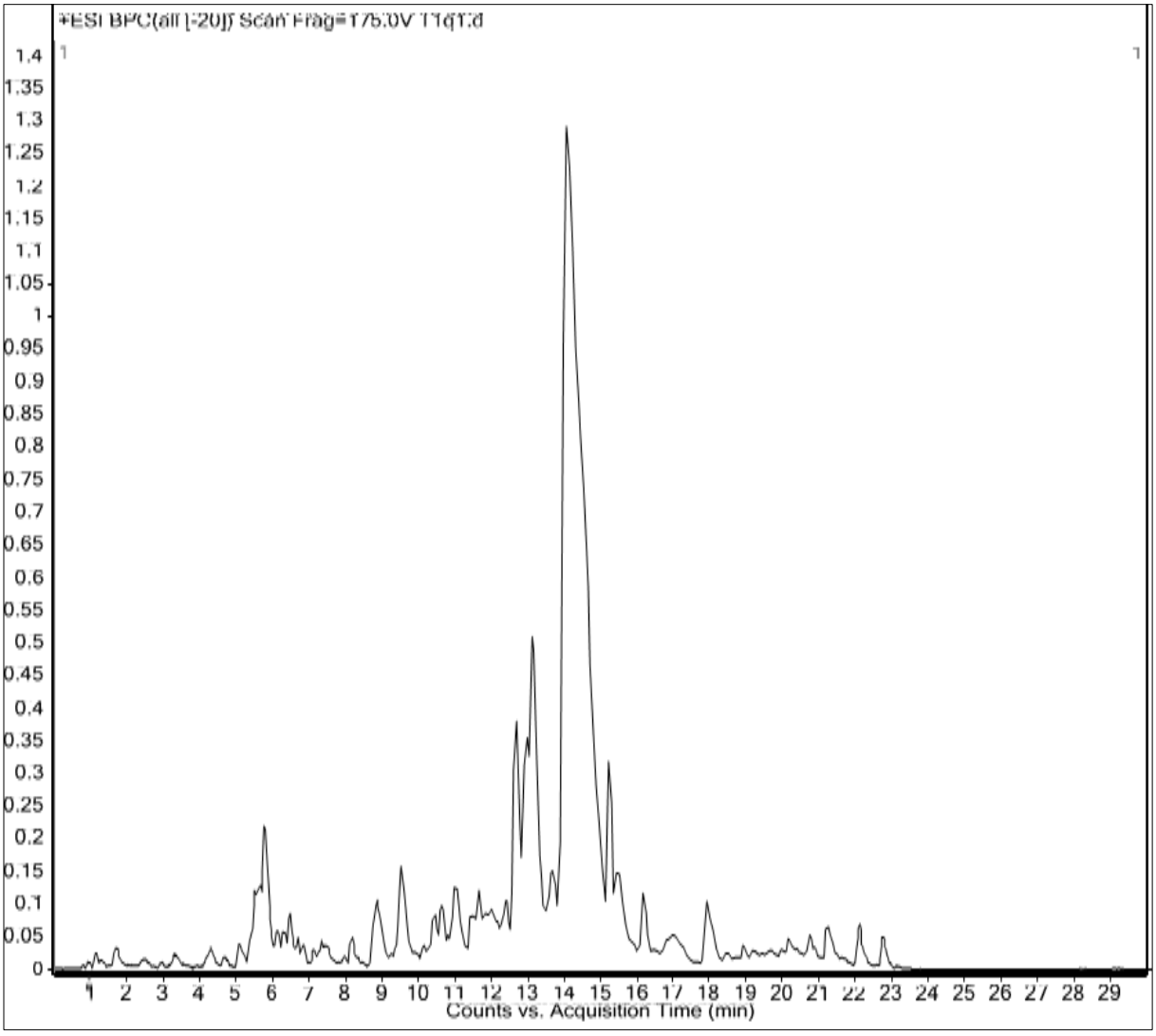

Fig 1: (HR)-LCMS Spectrogram of *Tephrosia purpurea root*

Table 2: Phytochemical compounds present in the stem of *Tephrosia purpurea*

Sr. No.	Compound name	Formula	Mass	R.T.	D.B. Diff. PPM
1	Pinacidil	$C_{13}H_{19}N_5$	245.1622	3.624	7.48
2	N-2-[4-(3,3- Dimethyl allyloxy) phenyl]ethylcinnamide	$C_{22}H_{25}NO_2$	335.196	4.202	-6.06
3	Hydrocortamate	$C_{27}H_{41}NO_6$	475.2945	5.149	-2.31
4	Villinol	$C_{24}H_{22}O_8$	438.13	8.801	3.43
5	Dihydro-deoxy streptomycin	$C_{21}H_{41}N_7O_{11}$	567.2853	8.944	1.98
6	Dihydro-obliquin	$C_{14}H_{14}O_4$	246.0897	9.405	-2.11
7	13-Hydroxy-5'-O-methylmelledonal	$C_{24}H_{30}O_9$	462.1883	10.06	1.5
8	Isodonal	$C_{22}H_{28}O_7$	404.1828	10.151	1.62
10	Ethyl 4-methylphenoxyacetate	$C_{11}H_{14}O_3$	194.095	11.175	-3.76
11	1-Oxo-1H-2-benzopyran-3- carboxaldehyde	$C_{10}H_6O_3$	174.0304	11.282	7.68
12	Spirapril	$C_{22}H_{30}N_2O_5S_2$	466.1617	11.47	-4.42
13	Furano [2",3":6,7] aurone	$C_{17}H_{10}O_3$	262.0617	11.659	4.81
14	Glyinflanin H	$C_{19}H_{16}O_4$	308.1041	11.736	2.52
15	Nb-trans-Feruloylserotonin glucoside	$C_{26}H_{30}N_2O_9$	514.1962	12.123	-2.06
16	Magnosalicin	$C_{24}H_{32}O_7$	432.2144	13.349	1.03
17	Isoeugenol phenylacetate	$C_{18}H_{18}O_3$	282.126	13.559	-1.29

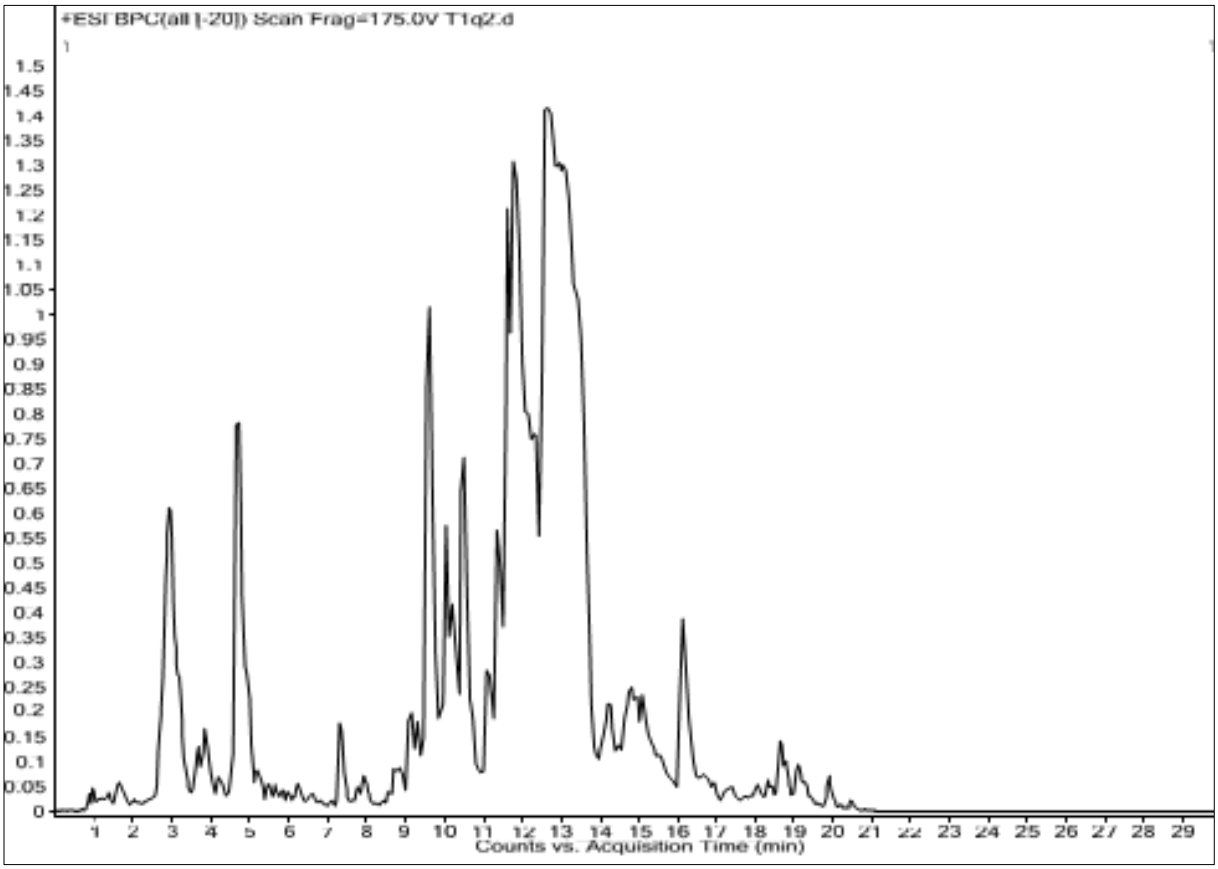

Fig 2 (HR)-LCMS Spectrogram of *Tephrosia purpurea stem*

Table 3: Phytochemical compounds present in the leaves of *Tephrosia purpurea*

Sr. No.	Compound name	Formula	Mass	R.T.	D.B. Diff. PPM
1.	Quercetin	$C_{15}H_{10}O_7$	302.0404	5.668	7.49
2	Rutin	$C_{27}H_{30}O_{16}$	610.1496	5.671	6.28
3	1-Oxo-1H-2-benzopyran-3- carboxaldehyde	$C_{10}H_6O_3$	174.0304	7.337	7.49
4	N-Isobutyl-2,4,8,10,12- tetra-decapentaenamide	$C_{18}H_{27}NO$	273.2098	7.724	-1.96
5	N-tetradecanoyl-L-Homoserine lactone	$C_{18}H_{33}NO_3$	311.2465	8.019	-1.47
6	AMP-Deoxy nojirimycin	$C_{22}H_{39}NO_5$	397.2796	8.145	8.02
7	Myxalamid C	$C_{24}H_{37}NO_3$	387.2778	8.878	-1.24
8	3-methyl-octanoic acid	$C_9H_{18}O_2$	158.1318	9.311	-7.06
9	23Acetoxysoladulcidine	$C_{29}H_{47}NO_4$	473.3476	10.397	6.18
10	Eupatocunin	$C_{22}H_{28}O_7$	404.1831	10.49	1.03
11	Demethylbatatasin IV	$C_{14}H_{14}O_3$	230.094	11.066	-2.38
12	1-Oxo-1H-2-benzopyran-3- carboxaldehyde	$C_{10}H_6O_3$	174.0304	11.308	7.45
13	Gmelinol	$C_{22}H_{26}O_7$	402.1685	11.39	-1.64
14	9-Hydroxy-3',4'-dimethoxy-3,4- methylenedioxy-9,9'- epoxylignan	$C_{21}H_{24}O_6$	372.158	12.549	-1.91
15	Neobavaisoflavone	$C_{20}H_{18}O_4$	322.1211	12.554	-1.96

16	4Z,15E-Bilirubin IXa	$C_{33}H_{36}N_4O_6$	584.2627	17.05	1.39
17	Pheophorbide a	$C_{35}H_{36}N_4O_5$	592.2668	17.559	3.07
18	(3Z)- Phycoerythrobilin	$C_{33}H_{38}N_4O_6$	586.2785	17.695	1.05
19	Euphornin	$C_{33}H_{44}O_9$	584.2991	17.984	-1.03

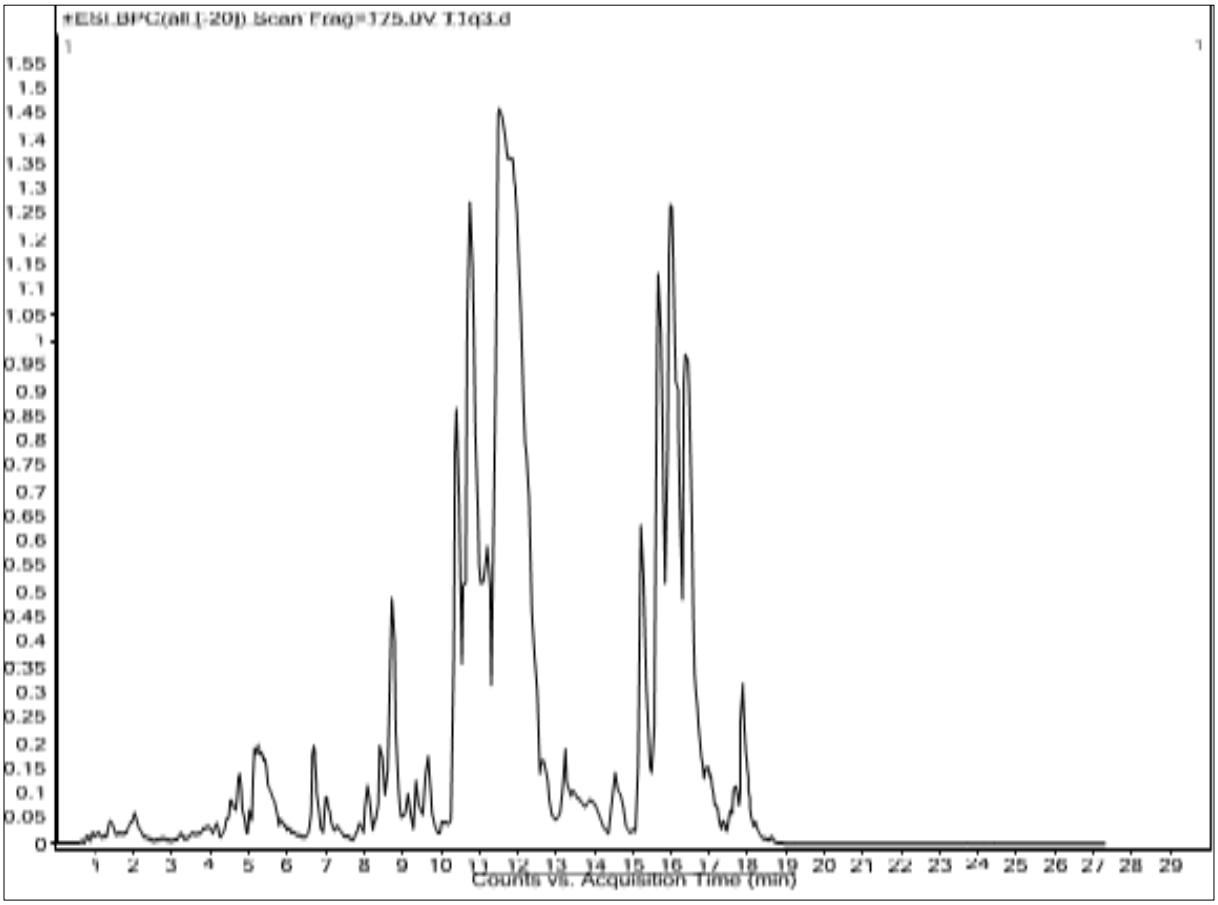

Fig. 3 (HR)-LCMS Spectrogram of *Tephrosia purpurea leaves*

Conclusion

The ethanolic extract of *Tephrosia purpurea* root, stem and leaves revealed the presence of important bioactive compounds like alkaloids, flavonoids, Phycoerythrobilin, flavones, stilbenoid, pheophorbide, glycoside, terpenoids, sesquiterpenoids, retinoids, Cyanoguanidine, germacranolide, coumarins, Chalcones and Steroid, using high-resolution liquid chromatography-mass spectrometry (HRLC-MS) analysis. These compounds are species specific and can be used to standardize the species.

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