Metabolomics profiling of *Tephrosia Purpurea*, *Cynodon dactylon* in dry form and *Cynodon dactylon* in wet form using Gas Chromatography-Mass Spectrometry and cytotoxicity study by using known antibiotics and plants phytochemicals

Raj Pratap Singh Chauhan[1], Abhijeet Garg[2], Zareen Baksh [3], Lakmikant Pandey[4], S. S. Sandhu[*5]

[1] Department of Biotechnology, St. Aloysius College, Sadar, Jabalpur (M.P)
[2] Biological Science Department Rani Durgavati University Jabalpur (M.P)
[3] Department of Botany and Microbiology, St. Aloysius College, Sadar, Jabalpur (M.P)
[4] Department of Biotechnology, St. Aloysius College, Jabalpur (M.P)
[*5] Bio Design Innovation Center Rani Durgavati University Jabalpur (M.P)
*Corresponding Author: S.S.Sandhu

Abstract: This study focuses on using Gas Chromatography-Mass Spectrometry (GC-MS)to do a thorough metabolic profiling of Cynodon dactylon. Additionally, it assesses the cytotoxicity of the plant in the presence of many plant phytochemicals and known antibiotics. Its varied biochemical characteristics and medicinal potential make Cynodon dactylon, a commonly used grass species, promising. A complex blend of fatty acids, steroids, terpenoids, and flavonoids was revealed by using GC-MS to identify and quantify several metabolites inside the plant. Bioactive substances that may be responsible for the therapeutic effects of the substance are indicated by the metabolic profile. Once the metabolic study was completed, we evaluated the cytotoxic effects of *Cynodon dactylon* extracts when combined with various antibiotics and phytochemicals. Using common cell viability tests against different cancer cell lines, cytotoxicity was quantified. major cytotoxic activity was demonstrated by the results.

Key words: Metabolic, potential, spectroscopy, biochemical, therapeutic, antibiotics, bioactive, cytotoxicity, pharmaceutical

I. Introduction

In this Study, we aim to investigate the metabolomic profiles of selected plants using Gas Chromatography-Mass spectrometry (GC-MS) and assess their cytotoxicity using the MTT assay. Metabolomics is a powerful technique that allows for the comprehensive study focuses on two significant medicinal plants: Dhurva Grass (*Cynodon dactylon*) and Sarpunkha (*Tephrosia purpurea*), which are used in traditional medicine for various therapeutic applications. Metabolomics is an emerging area that offers insights into the biochemical composition of biological systems. By studying small molecules (metabolites), it allows in information the metabolic pathways and their correlation with physiological states. Among the diverse tools used for metabolomic research, Gas Chromatography-Mass Spectrometry (GC-MS) is an exceptionally touchy and

reliable technique that lets in for the identification and quantification of numerous metabolites. In this look at, the metabolomic profiles of Tephrosia purpurea and Cynodon dactylon have been analyzed in special forms: the dry shape of Tephrosia purpurea and both dry and moist sorts of Cynodon dactylon. This vegetation is known for his or her wealthy phytochemical content material and therapeutic capacity. While Tephrosia purpurea is diagnosed for its traditional medicinal packages, specifically in liver problems, Cynodon dactylon is widely utilized in Ayurvedic medicinal drug for its antimicrobial and anti-inflammatory houses.

Plants Used in the Study

1. **Dhurva Grass (*Cynodon dactylon*)Classification**:

Kingdom: PlantaeFamily: Poaceae Genus: Cynodon
Species: *C. Dactylon*

Botanical Description: Dhurva grass, also known as Bermuda grass, is a perennial plant with a creeping growth habit. It has fine-textured leaves and stoloniferous stems, allowing it to form dense mats. The grass has small, spike- like inflorescences and typically grows in tropical and subtropical regions.

Medicinal Uses: Dhurva grass is highly valued in Ayurvedic medicine for its cooling, anti-inflammatory, and diuretic properties. It is commonly used for wound healing, treating skin diseases, and managing conditions such as diabetesand infections. It is also known to have a calming effect on the nervous system.

2. **Sarpunkha (*Tephrosia purpurea)*:Classification**:

Kingdom: Plantae Family: Fabaceae Genus: Tephrosia Species: T. Purpurea

Botanical Description: Sarpunkha is a small perennial herb or shrub that growsup to 1-1.5 meters in height. It has pinnately compound leaves and small, purplish-pink flowers arranged in axillary racemes. The plant produces flat, thinpods that contain several seeds. It thrives in dry, sandy soil and is commonly found in India.

Medicinal Uses: Sarpunkha is widely known for its hepatoprotective properties and is used in traditional medicine to treat liver disorders. It also has anti- inflammatory and antimicrobial effects, making it useful in treating various infections and skin conditions. In addition, it is believed to support the immune system and improve respiratory health Both of these plants have been chosen for their rich phytochemical profiles and potential therapeutic properties. This research will utilize GC-MS to identify thechemical constituents of these plants.

This study aimed to:

- Characterize the metabolite profiles of *T. purpurea* and both dry and wet forms of *C. dactylon* using GC-MS.
- Evaluate the cytotoxic and antibacterial potential of these plants through an MTT assay, comparing their effects with known antibiotics.

2. Materials and Methods

2.1 Plant Material Collection and Identification

- *Tephrosia purpurea* and *Cynodon dactylon* (dry and fresh) were collected from local regions of Jabalpur and authenticated by a botanist.

Instruments

- Gas Chromatography-Mass Spectrometry (Shimadzu GC-MS):

- Soxhlet Apparatus
- ELISA Reader (MTT Assay)
- Centrifuge
- 96 vial micro tittle plate

Cytotoxicity Assessment (MTT Assay)

Bacterial Strain

- *Escherichia coli* was isolated and cultured on nutrient agar plates.

Antibiotic Preparation

- Chloramphenicol was diluted to final concentrations of 5%–50%.

Chemicals required

- MTT reagent
- Ethanol
- Methanol
- Antibiotic (Chloramphenicol)
- Distilled water

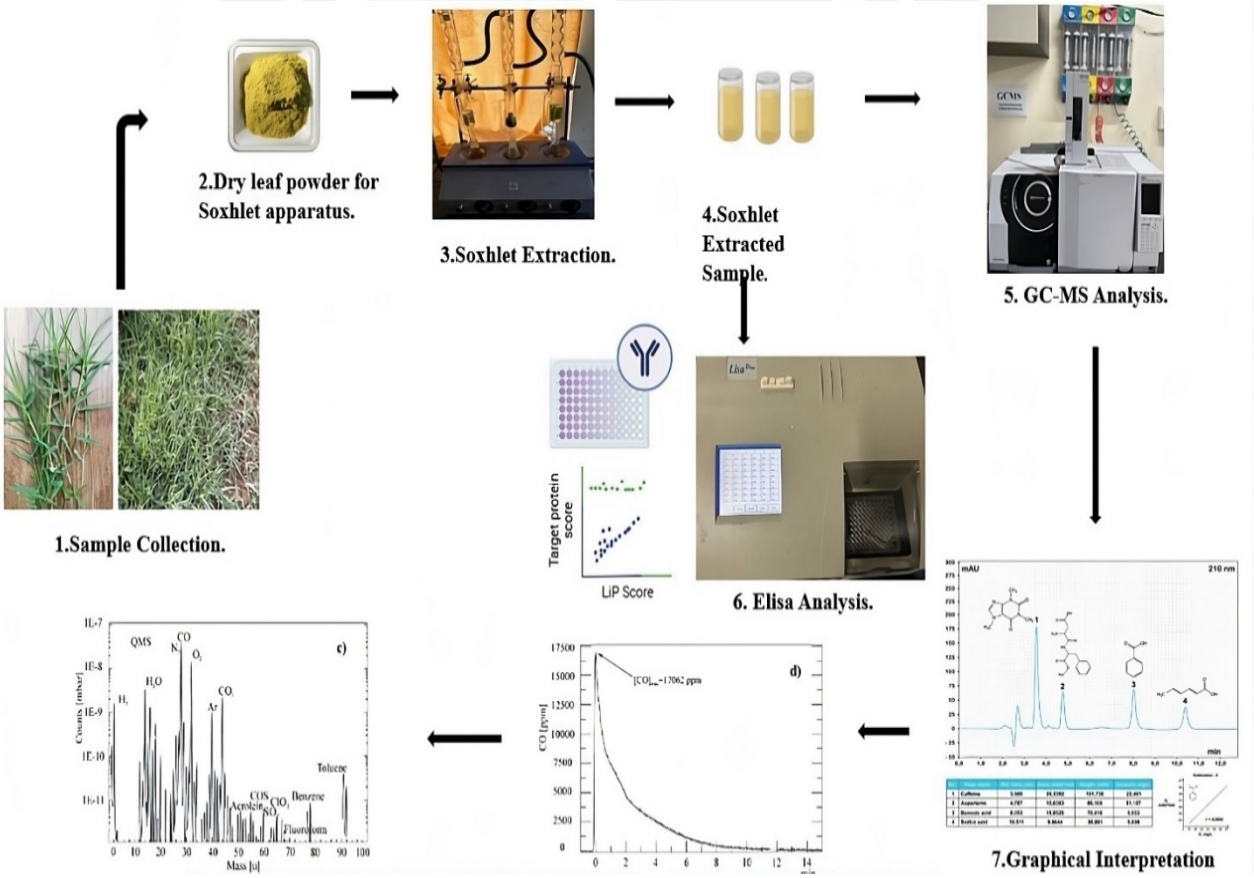


Fig: 1 **Overall procedure of Metabolic Profiling of *Mimosa pudica***

Methodology

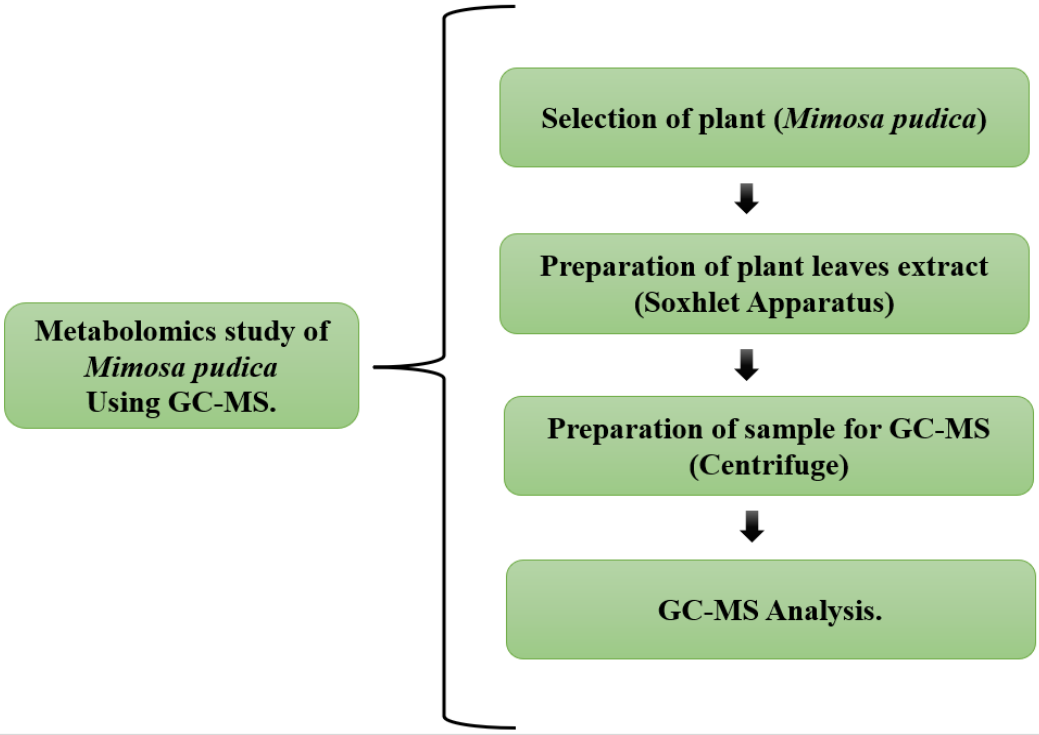


Fig: 2 **Metabolomic Study of Various Plants Using GC-MS and Cytotoxity Study with MTT Assay (ELISA)**

Preparation of sample for Gas Chromatography-Mass Spectrometry(GC-MS)

In this step, three different plant samples were prepared for GC-MS analysis. Thesamples were:

Sample 1: Dry Dhurva grass (*Cynodon dactylon*)

Sample 2: Wet Dhurva grass (*Cynodon dactylon*)

Sample 3: Sarpunkha (*Tephrosia purpurea*)

Sample Preparation for GC-MS

We took 2 ml of each plant extract from the prepared solution and put it into a centrifuge tube. The tubes were then spin in a centrifuge at 4000 rpm for 5 minutes at 4°C. After spinning, the liquid part (supernatant) was carefully collected, and the solid residue at the bottom (pellet) was discarded. The collectedliquid from each sample was then placed in separate vials for analysis using GasChromatography-Mass Spectrometry (GC-MS).

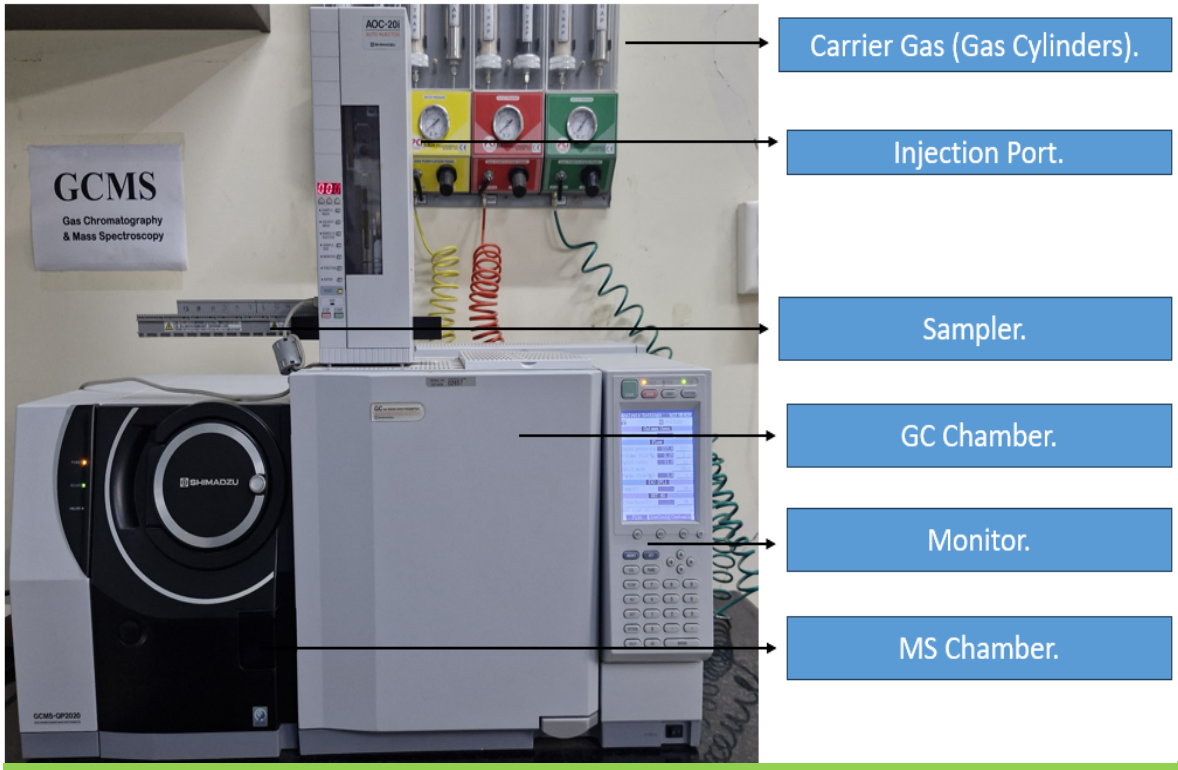


Fig: 3 **Gas chromatography-mass spectrometry (GC-MS)**
Location: Rani Durgavati University Jabalpur (M.P) campus

GC-MS Method.

For the sampler, rinse with solvent (pre-run) is set at 5 minutes and post run is setat 5 minutes. Rinse with sample set at 3 minutes and plunger (suction) speed isset at high, plunger speed (injection) is set at medium, viscosity comp. time is 0.2seconds, syringe injection speed is set at high, injection mode is set at normal.

For the GC, the injection temperature is set at 250°C with a split less injection mode and a sampling time of 1 minute. The column oven temperature is programmed with a three-stage gradient: starting at 50°C for 2 minutes, rampingup to 250°C at a rate of 20°C/min, and holding for 5 minutes. Then, it increases to 300°C at 10°C/min with a 10-minute hold. The carrier gas is set to a pressure of 117.6 kPa, with a total flow of 29.0 mL/min and a split ratio of 12.0. The column used is a SH-Rxi-5Sil MS with a length of 30.0 m, a thickness of 0.25 µm, and a diameter of 0.25 mm.

In the MS setup, the ion source temperature is 230°C and the interface temperature is 280°C, with a solvent cut time of 3 minutes. The system is set to scan continuously with a scan speed of 1666 across a range from 50.00 to 500.00m/z over a total run time of 32 minutes. This configuration ensures optimal resolution and sensitivity for the detection of compounds in the sample.

Fig: 4 **Overview of Instrument Setup**

1. Cytotoxicity Study Using MTT Assay

1.1 Isolation of Bacteria and Pure Culture

E. coli bacteria were isolated from a suitable source. The bacteria were grown onnutrient agar plates and incubated at 37°C for 24 hours. After the bacteria grew, individual colonies were picked and transferred to fresh media and nutrient brothto get pure cultures for further analysis

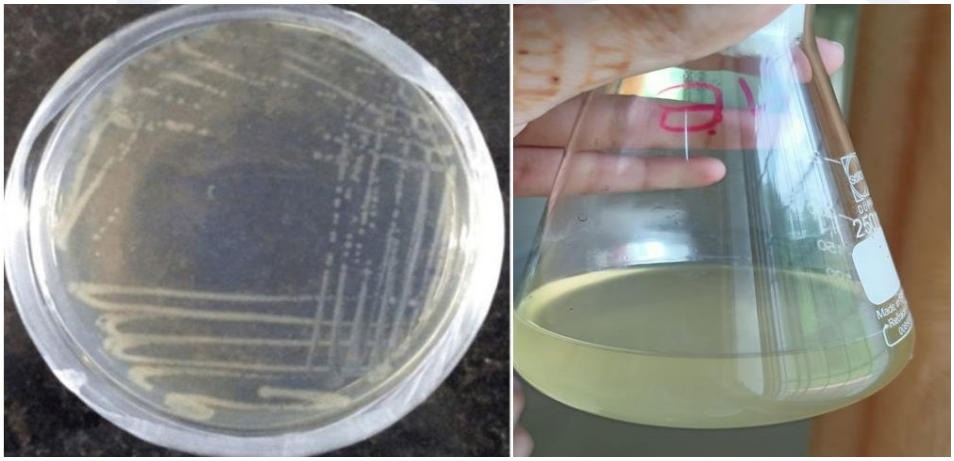


Fig: 5 **Isolation of Bacteria and Pure Culture**

1.2 Preparation of different concentration of antibiotics

Chloramphenicol antibiotic (250 mg) was bought from the market. A solution ofthe antibiotic with a final concentration of 1 µg/µl was prepared by diluting the stock solution (250 mg in 25 ml) using the serial dilution method.

Summary of Dilution Procedure

Stock Antibiotic Concentration	Distilled Water	Drug Amount
50%	0.5ml	0.5
40%	0.4ml	0.6
30%	0.3 ml	0.7
20%	0.2ml	0.8
10%	0.1ml	0.9
5%	0.05ml	0.95

1.3 E. coli sample preparation

E. coli was cultured in nutrient broth. A 1.5 mL aliquot of the culture was centrifuged at 4000 RPM for 5 minutes to pellet the cells. The pellet was washedtwice with 200 µL of PBS, followed by resuspension in 200 µL of PBS.

1.4 MTT assay protocol

The MTT assay was conducted to assess the cytotoxicity of the antibiotic on E. Coli. 100 µL of the E. Coli suspension was added to each well of a 96-well plate.Subsequently, 10 µL of the prepared antibiotic solutions were added to each well.

Negative Control: PBS + Cells (no antibiotic)

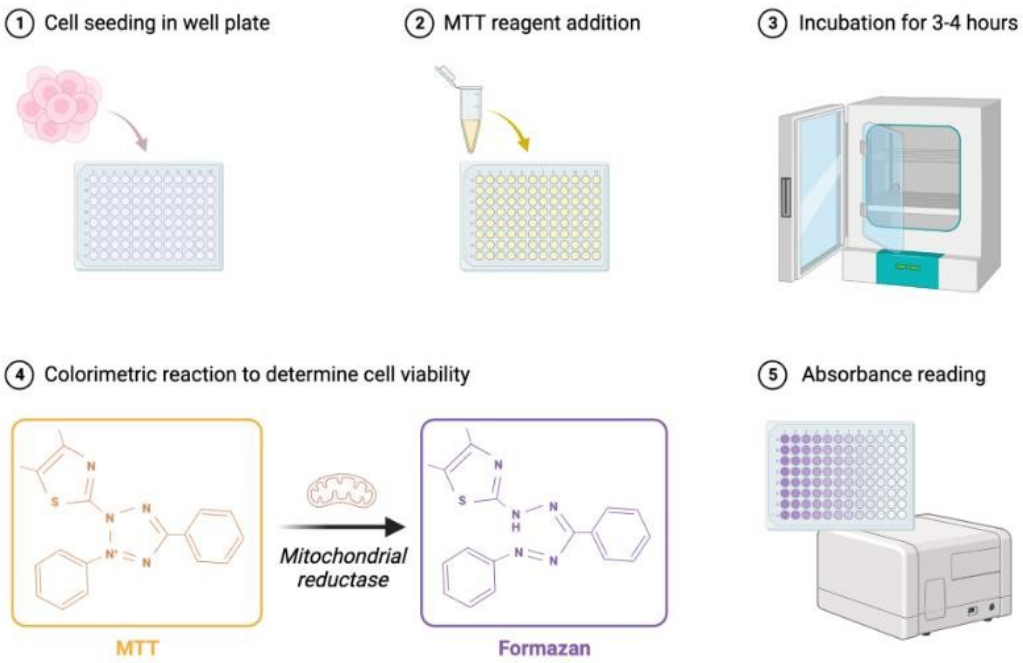
Positive Control: PBS + Antibiotic (no cells)

Experimental Groups: Cells + Varying concentrations of Antibiotic

The plate was incubated at 37°C for 24 hours. After incubation, 20 µL of MTT reagent (0.5 mg/mL) was added to each well and incubated for an additional hour.Then, 100 µL of a solubilizing reagent was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm using a microplate reader.

Cytotoxicity Assay *E. coli* was cultured and treated with varying concentrations (5%-50%) of chloramphenicol. Plant extracts were also tested. 100 µL bacterial suspension was incubated with 10 µL antibiotic or plant extract in 96-well plates. Post 24-hour incubation at 37°C, 20 µL of 0.5 mg/mL MTT reagent was added. After 1 hour, 100 µL solubilizer was added, and absorbance was measured at 570 nm.

Overall Experiment of Cytotoxicity Assay



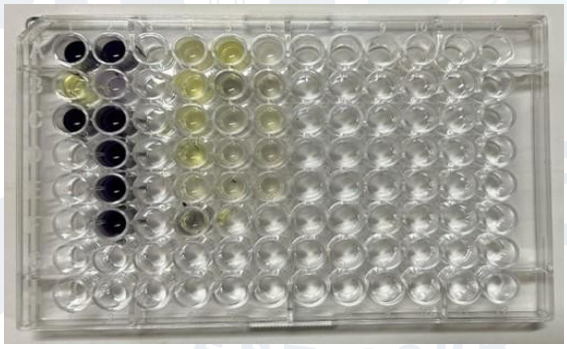
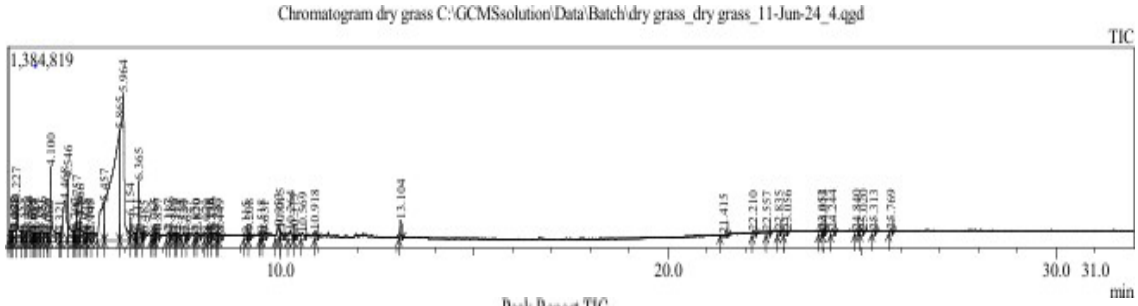


Fig: 6 **Experimental Setup**

2. Screening of bioactive compounds of plants using GC-MS

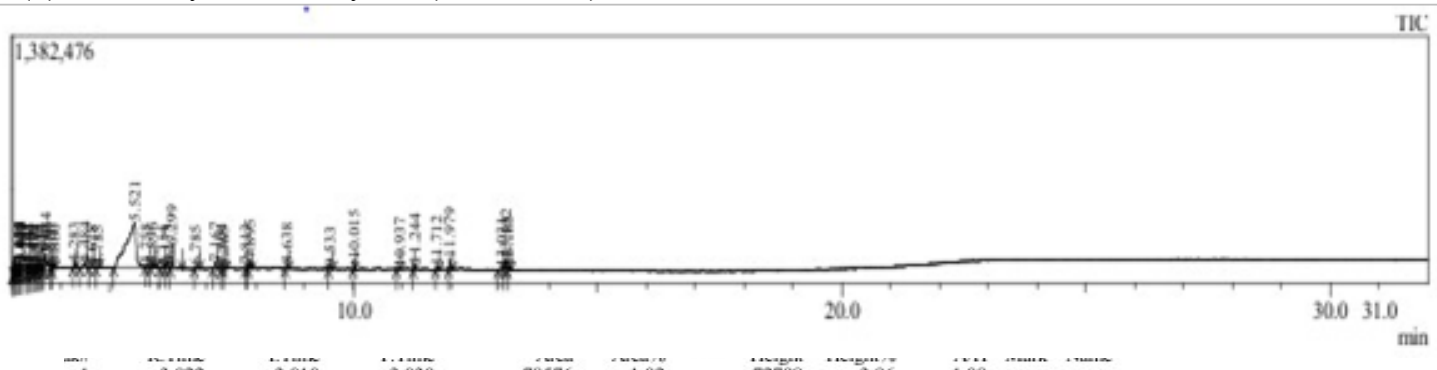
(a) Plant 1 *Cynodon dactylon* (Dry Durva)



Peak Report TIC

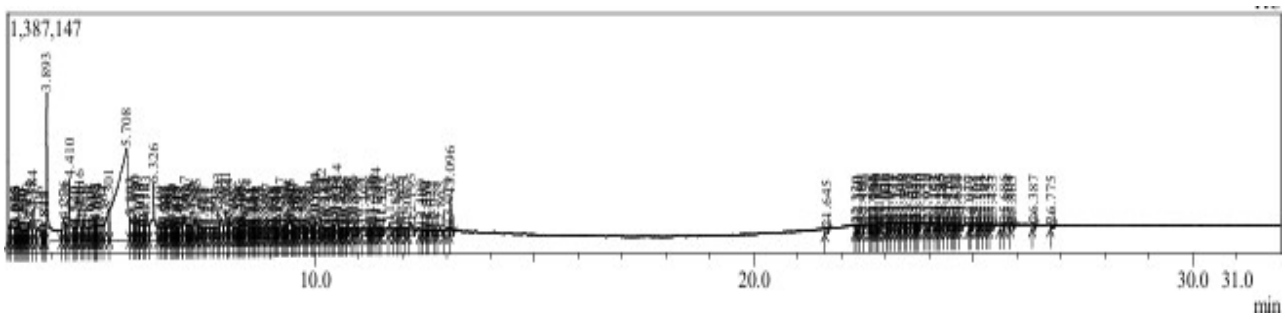
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.022	3.010	3.065	231913	0.57	77304	0.99	3.00		
2	3.085	3.065	3.115	203090	0.50	69175	0.88	2.94	V	
3	3.170	3.115	3.200	356176	0.88	84073	1.07	4.24	V	2-Propanone, 1-hydroxy-
4	3.227	3.200	3.345	901814	2.23	280238	3.58	3.22	V	Silanediol, dimethyl-
5	3.355	3.345	3.385	154320	0.38	65276	0.83	2.36	V	
6	3.465	3.415	3.490	285901	0.71	64846	0.83	4.41	V	
7	3.534	3.490	3.590	405688	1.00	82837	1.06	4.90	V	Propylene Glycol
8	3.605	3.590	3.640	185692	0.46	64075	0.82	2.90	V	
9	3.655	3.640	3.690	174802	0.43	60957	0.78	2.87	V	
10	3.717	3.690	3.760	241960	0.60	61765	0.79	3.92	V	Pentanedioic acid
11	3.797	3.760	3.830	237821	0.59	61471	0.78	3.87	V	2,3-Butanediol
12	3.856	3.830	3.905	275381	0.68	73332	0.94	3.76	V	2,3-Butanediol
13	3.956	3.905	4.005	344543	0.85	62811	0.80	5.49	V	Acetic acid, TBDMS derivative
14	4.060	4.005	4.075	211307	0.52	51152	0.65	4.13	V	1-Octadecanamine, N-methyl-
15	4.100	4.075	4.240	1174420	2.91	511308	6.53	2.30	V	1,3-Propanediol
16	4.321	4.305	4.365	182141	0.45	53987	0.69	3.37	V	4-Pentenoic acid
17	4.468	4.365	4.525	1658288	4.10	278471	3.55	5.95	V	Lactic acid
18	4.546	4.525	4.680	1117658	2.77	428954	5.47	2.61	V	2-Amino-6-methylbenzoic acid
19	4.707	4.680	4.725	178729	0.44	68037	0.87	2.63	V	
20	4.757	4.725	4.785	440381	1.09	225016	2.87	1.96	V	Butyrolactone
21	4.828	4.785	4.845	288925	0.72	84464	1.08	3.42	V	
22	4.866	4.845	4.965	574141	1.42	161514	2.06	3.55	V	1-Piperidinepropanenitrile
23	4.975	4.965	5.015	169053	0.42	61663	0.79	2.74	V	2(5H)-Furanone, 5-methyl-
24	5.060	5.015	5.105	288570	0.71	54632	0.70	5.28	V	
25	5.145	5.135	5.195	191998	0.48	60487	0.77	3.17	V	
26	5.457	5.285	5.475	2059727	5.10	265446	3.39	7.76	V	
27	5.865	5.475	5.875	11468912	28.38	764369	9.75	15.00	V	Pantolactone
28	5.964	5.875	6.130	6354498	15.73	1018533	13.00	6.24	V	
29	6.154	6.130	6.250	605614	1.50	166806	2.13	3.63	V	2-Pyrrolidinone
30	6.311	6.285	6.335	173814	0.43	63523	0.81	2.74	V	1,2,3-Propanetriol, 1-acetate
31	6.365	6.335	6.445	755577	1.87	410937	5.24	1.84	V	1,3-Dioxane
32	6.485	6.445	6.505	167990	0.42	47621	0.61	3.53	V	
33	6.712	6.670	6.740	164394	0.41	43072	0.55	3.82	V	Cyclotrisiloxane, hexamethyl-
34	6.765	6.740	6.795	162993	0.40	63874	0.82	2.55	V	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro
35	6.837	6.795	6.885	275462	0.68	62092	0.79	4.44	V	Benzoic acid
36	7.117	7.080	7.165	234340	0.58	56771	0.72	4.13	V	
37	7.186	7.165	7.235	181140	0.45	65614	0.84	2.76	V	Cyclotetrasiloxane, octamethyl-
38	7.310	7.285	7.355	199958	0.49	57659	0.74	3.47	V	Benzofuran, 2,3-dihydro-
39	7.374	7.355	7.420	167467	0.41	53327	0.68	3.14	V	m-Guaiacol
40	7.520	7.460	7.585	307153	0.76	50867	0.65	6.04	V	Benzeneacetic acid
41	7.627	7.585	7.655	185322	0.46	50045	0.64	3.70	V	2,5-Monomethylene-l-rhamnitol
42	7.820	7.785	7.855	189755	0.47	54592	0.70	3.48	V	2-Propenal, 3-phenyl-

(b) Plant 1 *Cynodon dactylon* (Wet Durva)



1	3.022	3.010	3.030	78576	1.02	72798	3.96	1.08		
2	3.055	3.030	3.070	162382	2.10	68615	3.73	2.37	V	
3	3.105	3.070	3.120	193023	2.50	65001	3.53	2.97	V	1-Propanol, 2-amino-, (.+/-.)-
4	3.140	3.120	3.155	127969	1.66	61300	3.33	2.09	V	
5	3.171	3.155	3.215	211415	2.74	62605	3.40	3.38	V	Pentanoic acid
6	3.251	3.215	3.285	218921	2.84	53357	2.90	4.10	V	2-Propanol, 1-methoxy-
7	3.295	3.285	3.325	113226	1.47	48645	2.64	2.33	V	
8	3.341	3.325	3.390	176424	2.29	50687	2.75	3.48	V	2,3-Butanediol
9	3.400	3.390	3.410	48425	0.63	40875	2.22	1.18	V	
10	3.425	3.410	3.460	117357	1.52	40067	2.18	2.93	V	
11	3.494	3.460	3.505	104624	1.36	39492	2.15	2.65	V	
12	3.515	3.505	3.560	112447	1.46	37787	2.05	2.98	V	
13	3.605	3.560	3.620	108705	1.41	30293	1.65	3.59	V	
14	3.640	3.620	3.665	68343	0.89	27576	1.50	2.48	V	
15	3.684	3.665	3.785	325574	4.22	94175	5.12	3.46	V	1,3-Propanediol
16	3.800	3.785	3.830	62401	0.81	25141	1.37	2.48	V	
17	3.860	3.830	3.890	62344	0.81	19210	1.04	3.25	V	
18	4.283	4.255	4.325	54427	0.71	26232	1.43	2.07	V	2-Amino-6-methylbenzoic acid
19	4.484	4.390	4.540	101571	1.32	28831	1.57	3.52	V	Butyrolactone
20	4.618	4.575	4.675	38753	0.50	13416	0.73	2.89	V	3-Isoxazolamine, 5-methyl-
21	4.785	4.725	4.805	38474	0.50	9994	0.54	3.85	V	
22	5.521	5.090	5.980	3823270	49.54	258475	14.04	14.79	SV	Glycerin
23	5.758	5.735	5.840	53145	0.69	15886	0.86	3.35	T	
24	5.896	5.840	5.935	49833	0.65	22180	1.21	2.25	TV	1,2-Benzenediol, 3,5-bis(1,1-dimethylethy
25	6.114	6.030	6.135	43924	0.57	13433	0.73	3.27		2-Pyrrolidinone
26	6.173	6.135	6.240	70063	0.91	18754	1.02	3.74	V	2,4-Dihydroxybenzaldehyde, 2TMS deriva
27	6.299	6.240	6.485	213348	2.76	125992	6.85	1.69	SV	1,3-Dioxane
28	6.785	6.740	6.850	48929	0.63	16364	0.89	2.99	V	Camphor
29	7.167	7.115	7.220	82438	1.07	31416	1.71	2.62	V	Estragole
30	7.304	7.275	7.345	51799	0.67	22172	1.20	2.34	V	Nordazepam, TMS derivative
31	7.365	7.345	7.405	33383	0.43	10562	0.57	3.16	V	m-Guaiacol
32	7.813	7.785	7.840	36782	0.48	24423	1.33	1.51		2-Propenal, 3-phenyl-
33	7.895	7.840	7.920	89836	1.16	50155	2.73	1.79	V	Estragole
34	8.638	8.595	8.680	51919	0.67	29399	1.60	1.77	V	1-Tridecene
35	9.533	9.485	9.575	31879	0.41	7914	0.43	4.03	V	
36	10.015	9.990	10.040	69163	0.90	55235	3.00	1.25	V	1-Heptadecene
37	10.937	10.875	10.970	42870	0.56	10181	0.55	4.21	V	Hexanoic acid, 2-ethyl-, methyl ester
38	11.244	11.220	11.270	45943	0.60	33332	1.81	1.38	V	1-Nonadecene
39	11.712	11.680	11.765	45319	0.59	19875	1.08	2.28	V	
40	11.979	11.940	12.030	112934	1.46	66368	3.61	1.70	V	Hexadecanoic acid, methyl ester
41	13.031	12.955	13.075	45803	0.59	13250	0.72	3.46	V	9,12,15-Octadecatrienoic acid, methyl este
42	13.102	13.075	13.150	106004	1.37	58149	3.16	1.82	V	Phytol
43	7.879	7.855	7.960	275439	0.68	55787	0.71	4.94	V	2H-Pyran-2-one, tetrahydro-6-propyl-
44	8.079	8.050	8.130	202189	0.50	51648	0.66	3.91	V	4-Hydroxy-3,5,5-trimethylcyclohex-2-eno
45	8.198	8.155	8.215	178366	0.44	56202	0.72	3.17	V	2,5-Monomethylene-l-rhamnitol
46	8.230	8.215	8.305	252099	0.62	56974	0.73	4.42	V	12-Crown-4
47	8.387	8.365	8.420	172022	0.43	70281	0.90	2.45	V	Cyclotrisiloxane, hexamethyl-
48	8.449	8.420	8.515	220591	0.55	51919	0.66	4.25	V	Heptanoic acid, anhydride
49	9.115	9.060	9.160	209801	0.52	38862	0.50	5.40	V	
50	9.208	9.185	9.250	150225	0.37	53297	0.68	2.82	V	1,3-Dioxolane, 2-ethyl-4-methyl-
51	9.518	9.480	9.545	161075	0.40	48558	0.62	3.32	V	Cyclotetrasiloxane, octamethyl-
52	9.631	9.545	9.645	252339	0.62	47134	0.60	5.35	V	2-Propanone, 1-(4-hydroxy-3-methoxyphe
53	9.953	9.885	9.995	410965	1.02	90860	1.16	4.52	V	.alpha.-D-Glucopyranoside, methyl
54	10.015	9.995	10.125	362093	0.90	71769	0.92	5.05	V	
55	10.264	10.205	10.350	378932	0.94	59308	0.76	6.39	V	.alpha.-D-Galactopyranoside, methyl
56	10.375	10.350	10.425	150876	0.37	36228	0.46	4.16	V	2,5-Piperazinedione, 3-methyl-6-(1-methyl
57	10.569	10.535	10.660	268134	0.66	45964	0.59	5.83	V	Barbituric acid, 2-thio-
58	10.918	10.890	10.980	214361	0.53	62789	0.80	3.41	V	1,3-Dioxolane, 2-ethyl-4-methyl-
59	13.104	13.065	13.160	269726	0.67	133625	1.71	2.02	V	Phytol
60	21.415	21.340	21.540	185134	0.46	15817	0.20	11.70	V	
61	22.210	22.165	22.265	175227	0.43	31144	0.40	5.63	V	
62	22.557	22.525	22.635	195447	0.48	30638	0.39	6.38	V	
63	22.835	22.815	22.900	165896	0.41	33470	0.43	4.96	V	
64	23.056	22.975	23.070	191670	0.47	35027	0.45	5.47	V	
65	23.952	23.875	23.980	210573	0.52	35335	0.45	5.96	V	
66	24.015	23.980	24.060	165427	0.41	35499	0.45	4.66	V	
67	24.244	24.190	24.300	211064	0.52	33323	0.43	6.33	V	
68	24.840	24.805	24.905	172175	0.43	29389	0.38	5.86	V	
69	25.020	24.945	25.040	156621	0.39	28229	0.36	5.55	V	
70	25.313	25.250	25.350	150094	0.37	26552	0.34	5.65	V	
71	25.769	25.685	25.800	167984	0.42	27263	0.35	6.16	V	Cyclotetrasiloxane, octamethyl-
				40405373	100.00	7835884	100.00			

(c) Plant 2 Sarpunkha (*Tephrosia purpurea*)



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.065	3.010	3.075	339389	0.61	89824	0.59	3.78		
2	3.093	3.075	3.155	423931	0.76	93797	0.61	4.52	V	Ethoxy(dimethyl)isopropylsilane
3	3.175	3.155	3.200	223812	0.40	83986	0.55	2.66	V	
4	3.219	3.200	3.235	177808	0.32	88488	0.58	2.01	V	
5	3.252	3.235	3.290	279803	0.50	97767	0.64	2.86	V	Propanoic acid, 2-methyl-
6	3.310	3.290	3.330	176920	0.32	75261	0.49	2.35	V	Tetrahydro-1,3-oxazine-2-thione
7	3.350	3.330	3.375	192246	0.35	71970	0.47	2.67	V	
8	3.400	3.375	3.425	209430	0.38	70784	0.46	2.96	V	
9	3.474	3.425	3.490	336395	0.60	115106	0.75	2.92	V	Butanoic acid
10	3.512	3.490	3.565	384191	0.69	131333	0.86	2.93	V	2,3-Butanediol
11	3.584	3.565	3.665	548872	0.99	186544	1.22	2.94	V	2,3-Butanediol
12	3.706	3.665	3.795	510522	0.92	84864	0.55	6.02	V	Acetic acid, TBDMS derivative
13	3.814	3.795	3.830	119367	0.21	59310	0.39	2.01	V	
14	3.845	3.830	3.865	120063	0.22	58012	0.38	2.07	V	
15	3.893	3.865	4.225	2515671	4.52	861395	5.61	2.92	SV	1,3-Propanediol
16	4.276	4.225	4.320	518501	0.93	119350	0.78	4.34	V	Propylene Glycol
17	4.330	4.320	4.385	349482	0.63	97687	0.64	3.58	V	Butanoic acid, 2-methyl-
18	4.410	4.385	4.490	817729	1.47	364141	2.37	2.25	V	2-Amino-6-methylbenzoic acid
19	4.520	4.490	4.545	265003	0.48	80937	0.53	3.27	V	
20	4.560	4.545	4.585	193395	0.35	80999	0.53	2.39	V	Acetamide, 2,2,2-trichloro-
21	4.616	4.585	4.660	499300	0.90	180303	1.17	2.77	V	Butyrolactone
22	4.670	4.660	4.710	242336	0.44	81016	0.53	2.99	V	
23	4.737	4.710	4.790	431838	0.78	114772	0.75	3.76	V	1,3-Cyclopentanedione
24	4.830	4.790	4.855	304666	0.55	79403	0.52	3.84	V	
25	4.910	4.855	4.920	310776	0.56	84496	0.55	3.68	V	
26	4.933	4.920	4.970	247742	0.45	84975	0.55	2.92	V	2-Propenamide
27	4.987	4.970	5.005	192504	0.35	102869	0.67	1.87	V	Ethyl(dimethyl)ethoxysilane
28	5.024	5.005	5.040	193260	0.35	99562	0.65	1.94	V	CH3C(O)CH2CH2OH
29	5.059	5.040	5.105	355767	0.64	95739	0.62	3.72	V	2,5-Dihydroxybenzaldehyde, 2TMS deriv
30	5.164	5.105	5.185	408640	0.73	89456	0.58	4.57	V	
31	5.301	5.185	5.320	1090031	1.96	184197	1.20	5.92	V	Di-trimethylsilyl peroxide
32	5.708	5.320	5.785	9003341	16.18	532610	3.47	16.90	V	Glycerin
33	5.803	5.785	5.870	559424	1.01	130445	0.85	4.29	V	Pantolactone
34	5.890	5.870	5.910	280904	0.50	142482	0.93	1.97	V	Acetic acid, TBDMS derivative
35	5.930	5.910	5.990	530420	0.95	144682	0.94	3.67	V	Cyclotrisiloxane, hexamethyl-
36	6.010	5.990	6.020	167852	0.30	94029	0.61	1.79	V	
37	6.033	6.020	6.080	324098	0.58	92946	0.61	3.49	V	
38	6.112	6.080	6.125	244159	0.44	94775	0.62	2.58	V	2-Butenal, 3-methyl-, dimethylhydrazone
39	6.163	6.125	6.220	633185	1.14	143474	0.93	4.41	V	2,5-Dihydroxybenzaldehyde, 2TMS deriv
40	6.326	6.220	6.430	1440886	2.59	322218	2.10	4.47	V	1,3-Dioxane
41	6.449	6.430	6.490	270437	0.49	80662	0.53	3.35	V	Cyclohexanone, 2,6-dimethyl-
42	6.523	6.490	6.560	310739	0.56	80819	0.53	3.84	V	Cyclopentasiloxane, decamethyl-

43	6.580	6.560	6.590	117680	0.21	66450	0.43	1.77	V	Succinimide
44	6.608	6.590	6.635	181741	0.33	69833	0.45	2.60	V	2-Propanamine, N-methyl-N-nitroso-
45	6.686	6.635	6.710	331730	0.60	87318	0.57	3.80	V	Cyclotrisiloxane, hexamethyl-
46	6.731	6.710	6.755	216680	0.39	95449	0.62	2.27	V	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydro>
47	6.780	6.755	6.800	211643	0.38	83762	0.55	2.53	V	Camphor
48	6.815	6.800	6.835	161270	0.29	80984	0.53	1.99	V	Benzoic acid
49	6.845	6.835	6.870	151586	0.27	75308	0.49	2.01	V	
50	6.897	6.870	6.905	146221	0.26	71365	0.46	2.05	V	
51	6.948	6.905	6.970	284202	0.51	75848	0.49	3.75	V	
52	7.017	6.970	7.065	536550	0.96	133764	0.87	4.01	V	Cyclohexasiloxane, dodecamethyl-
53	7.099	7.065	7.115	246034	0.44	88183	0.57	2.79	V	
54	7.136	7.115	7.150	170275	0.31	83238	0.54	2.05	V	
55	7.170	7.150	7.200	232508	0.42	87902	0.57	2.65	V	Cyclotetrasiloxane, octamethyl-
56	7.247	7.200	7.275	393326	0.71	108596	0.71	3.62	V	6,6,8,8-Tetramethyl-2,5,7,9,12-pentaoxa-6
57	7.295	7.275	7.330	312763	0.56	119824	0.78	2.61	V	Nordazepam, TMS derivative
58	7.355	7.330	7.405	318874	0.57	80344	0.52	3.97	V	m-Guaiacol
59	7.418	7.405	7.435	110536	0.20	64546	0.42	1.71	V	Piperazine, 2-methyl-
60	7.501	7.460	7.525	238839	0.43	68007	0.44	3.51	V	Propanedioic acid, phenyl-
61	7.585	7.525	7.610	301795	0.54	60427	0.39	4.99	V	
62	7.665	7.635	7.690	202578	0.36	65356	0.43	3.10	V	Benzaldehyde, 4-methoxy-
63	7.733	7.690	7.750	232566	0.42	68120	0.44	3.41	V	
64	7.803	7.775	7.840	375954	0.68	149712	0.98	2.51	V	2-Propenal, 3-phenyl-
65	7.867	7.840	7.915	412336	0.74	118828	0.77	3.47	V	2H-Pyran-2-one, tetrahydro-6-propyl-
66	7.971	7.915	8.005	500241	0.90	158165	1.03	3.16	V	Dodecanedioic acid, 2TBDMS derivative
67	8.034	8.005	8.060	312086	0.56	122217	0.80	2.55	V	1,3,5-Benzetriol, 3TMS derivative
68	8.082	8.060	8.150	413978	0.74	98267	0.64	4.21	V	Dodecanedioic acid, 2TBDMS derivative
69	8.167	8.150	8.200	186942	0.34	65192	0.42	2.87	V	Tetradecanal
70	8.228	8.200	8.250	181260	0.33	62271	0.41	2.91	V	
71	8.276	8.250	8.285	140702	0.25	74222	0.48	1.90	V	
72	8.301	8.285	8.325	177515	0.32	77795	0.51	2.28	V	
73	8.345	8.325	8.355	138384	0.25	80263	0.52	1.72	V	1,3,5-Benzetriol, 3TMS derivative
74	8.375	8.355	8.405	263428	0.47	112206	0.73	2.35	V	1,3,5-Benzetriol, 3TMS derivative
75	8.434	8.405	8.485	379430	0.68	110769	0.72	3.43	V	1,3,5-Benzetriol, 3TMS derivative
76	8.515	8.485	8.525	140909	0.25	59827	0.39	2.36	V	Benzene, 1-(1,1-dimethylethyl)-3-ethyl-5-(
77	8.554	8.525	8.580	195777	0.35	62549	0.41	3.13	V	
78	8.603	8.580	8.620	136695	0.25	58883	0.38	2.32	V	
79	8.649	8.620	8.660	139818	0.25	60914	0.40	2.30	V	
80	8.688	8.660	8.715	213194	0.38	69988	0.46	3.05	V	1H-Indole, 6-methyl-
81	8.725	8.715	8.755	142297	0.26	62787	0.41	2.27	V	
82	8.810	8.785	8.835	160138	0.29	53870	0.35	2.97	V	
83	8.887	8.860	8.915	229407	0.41	83728	0.55	2.74	V	Cycloheptasiloxane, tetradecamethyl-
84	8.929	8.915	8.950	121805	0.22	59836	0.39	2.04	V	
85	9.038	8.975	9.055	270984	0.49	57819	0.38	4.69	V	
86	9.080	9.055	9.090	124021	0.22	62406	0.41	1.99	V	d-Mannitol, 1-O-heptyl-
87	9.103	9.090	9.125	123747	0.22	65926	0.43	1.88	V	Cycloheptasiloxane, tetradecamethyl-
88	9.144	9.125	9.170	143434	0.26	59039	0.38	2.43	V	Docosane
89	9.197	9.170	9.230	272940	0.49	119155	0.78	2.29	V	3,8-Dioxa-2,9-disiladecane, 2,2,9,9-tetram
90	9.290	9.260	9.305	142435	0.26	56085	0.37	2.54	V	
91	9.326	9.305	9.345	158803	0.29	79518	0.52	2.00	V	Dodecanedioic acid, 2TBDMS derivative
92	9.369	9.345	9.390	195860	0.35	94691	0.62	2.07	V	Benzamide, N-(3-nitrophenyl)-2,6-difluor
93	9.425	9.390	9.465	373965	0.67	109616	0.71	3.41	V	Dodecanedioic acid, 2TBDMS derivative
94	9.480	9.465	9.495	137017	0.25	81864	0.53	1.67	V	Citric acid, 4TBDMS derivative
95	9.508	9.495	9.550	217606	0.39	78099	0.51	2.79	V	Cyclooctasiloxane, hexadecamethyl-
96	9.563	9.550	9.585	120591	0.22	58830	0.38	2.05	V	1,2-Ethanediamine, N-(2-aminoethyl)-
97	9.596	9.585	9.650	209497	0.38	58481	0.38	3.58	V	1,3-Benzodioxole, 4-methoxy-6-(2-propen
98	9.686	9.650	9.740	301861	0.54	62654	0.41	4.82	V	
99	9.757	9.740	9.780	125554	0.23	54241	0.35	2.31	V	
100	9.799	9.780	9.815	107209	0.19	52075	0.34	2.06	V	
101	9.880	9.845	9.925	335196	0.60	94815	0.62	3.54	V	2,2,4-Trimethyl-1,3-pentanediol diisobutyr
102	9.953	9.925	9.975	174875	0.31	61580	0.40	2.84	V	Hexadecane, 1-iodo-
103	10.009	9.975	10.020	196201	0.35	88969	0.58	2.21	V	Cetene
104	10.034	10.020	10.110	363135	0.65	83647	0.54	4.34	V	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
105	10.142	10.110	10.165	249389	0.45	112817	0.73	2.21	V	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
106	10.183	10.165	10.215	169078	0.30	64105	0.42	2.64	V	Cyclooctasiloxane, hexadecamethyl-
107	10.245	10.215	10.295	244096	0.44	56659	0.37	4.31	V	
108	10.318	10.295	10.335	120357	0.22	54241	0.35	2.22	V	
109	10.382	10.335	10.395	194610	0.35	64075	0.42	3.04	V	Benzophenone
110	10.417	10.395	10.450	211304	0.38	71563	0.47	2.95	V	Cyclooctasiloxane, hexadecamethyl-
111	10.514	10.450	10.535	438254	0.79	135711	0.88	3.23	V	Androsta-3,5-dien-3-ol, 17-acetyl-3-O-(t-b
112	10.545	10.535	10.570	127119	0.23	68770	0.45	1.85	V	.tau.-Cadinol
113	10.597	10.570	10.625	177738	0.32	56504	0.37	3.15	V	
114	10.666	10.625	10.690	205123	0.37	56629	0.37	3.62	V	
115	10.706	10.690	10.725	100888	0.18	49201	0.32	2.05	V	
116	10.747	10.725	10.775	168411	0.30	66886	0.44	2.52	V	Coprost-25-en-16,22-epoxy-3.alpha.-ol
117	10.852	10.835	10.875	111943	0.20	50417	0.33	2.22	V	
118	10.899	10.875	10.960	280664	0.50	69847	0.45	4.02	V	
119	10.978	10.960	10.995	104026	0.19	53489	0.35	1.94	V	
120	11.103	11.060	11.180	337797	0.61	60502	0.39	5.58	V	Cyclononasiloxane, octadecamethyl-
121	11.236	11.215	11.260	146663	0.26	71354	0.46	2.06	V	1-Nonadecene
122	11.273	11.260	11.300	100880	0.18	45571	0.30	2.21	V	

123	11.349	11.300	11.370	205889	0.37	67361	0.44	3.06	V	Cyclooctasiloxane, hexadecamethyl-
124	11.394	11.370	11.450	304124	0.55	116799	0.76	2.60	V	Cyclooctasiloxane, hexadecamethyl-
125	11.489	11.450	11.505	157870	0.28	56495	0.37	2.79	V	Eicosen-1-ol, cis-9-
126	11.521	11.505	11.575	183894	0.33	56230	0.37	3.27	V	Digitoxin
127	11.702	11.670	11.765	292657	0.53	73330	0.48	3.99	V	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydr
128	11.823	11.800	11.875	179401	0.32	41541	0.27	4.32	V	
129	11.925	11.875	11.935	164543	0.30	56168	0.37	2.93	V	
130	11.971	11.935	12.025	274440	0.49	71421	0.47	3.84	V	Hexadecanoic acid, methyl ester
131	12.087	12.065	12.130	157815	0.28	44664	0.29	3.53	V	
132	12.195	12.130	12.270	432738	0.78	74080	0.48	5.84	V	
133	12.400	12.385	12.440	115324	0.21	39270	0.26	2.94	V	Nonacosane
134	12.492	12.465	12.525	112134	0.20	34103	0.22	3.29	V	
135	12.550	12.525	12.590	105197	0.19	29842	0.19	3.53	V	
136	12.622	12.590	12.680	134315	0.24	27068	0.18	4.96	V	
137	12.731	12.710	12.785	120435	0.22	34418	0.22	3.50	V	Cyclooctasiloxane, hexadecamethyl-
138	12.865	12.785	12.915	185992	0.33	25929	0.17	7.17	V	
139	13.029	12.955	13.065	209399	0.38	48030	0.31	4.36	V	9,12,15-Octadecatrienoic acid, ethyl ester,
140	13.096	13.065	13.160	477472	0.86	230004	1.50	2.08	V	Phytol
141	21.645	21.615	21.695	133382	0.24	28227	0.18	4.73	V	
142	22.320	22.300	22.345	115371	0.21	43993	0.29	2.62	V	
143	22.360	22.345	22.385	109543	0.20	46374	0.30	2.36	V	Pentasiloxane, dodecamethyl-
144	22.460	22.415	22.490	213559	0.38	49205	0.32	4.34	V	Dihydroxymaleic acid
145	22.550	22.490	22.615	356247	0.64	49384	0.32	7.21	V	
146	22.668	22.640	22.680	117073	0.21	50168	0.33	2.33	V	
147	22.720	22.680	22.740	175212	0.31	50030	0.33	3.50	V	
148	22.750	22.740	22.795	161018	0.29	49693	0.32	3.24	V	Cyclotetrasiloxane, octamethyl-
149	22.820	22.795	22.830	104177	0.19	50653	0.33	2.06	V	
150	22.842	22.830	22.890	180338	0.32	51574	0.34	3.50	V	
151	22.990	22.950	23.020	206484	0.37	49667	0.32	4.16	V	N,N-Dimethylacetamide
152	23.046	23.020	23.055	106640	0.19	51947	0.34	2.05	V	Cyclotetrasiloxane, octamethyl-
153	23.092	23.055	23.115	180914	0.33	51641	0.34	3.50	V	
154	23.171	23.115	23.185	205866	0.37	50610	0.33	4.07	V	Hydrazinecarbothioamide, N-ethyl-
155	23.235	23.210	23.270	178167	0.32	50994	0.33	3.49	V	Cyclotrisiloxane, hexamethyl-
156	23.306	23.295	23.330	106588	0.19	51608	0.34	2.07	V	
157	23.408	23.330	23.425	286148	0.51	51288	0.33	5.58	V	Cyclotrisiloxane, hexamethyl-
158	23.475	23.425	23.485	182320	0.33	52508	0.34	3.47	V	
159	23.505	23.485	23.560	230927	0.41	54130	0.35	4.27	V	
160	23.621	23.560	23.645	247623	0.44	50406	0.33	4.91	V	
161	23.685	23.645	23.700	155370	0.28	48671	0.32	3.19	V	
162	23.720	23.700	23.745	132811	0.24	50750	0.33	2.62	V	
163	23.770	23.745	23.785	118581	0.21	51369	0.33	2.31	V	
164	23.920	23.880	23.945	172727	0.31	44935	0.29	3.84	V	
165	23.974	23.945	23.995	135456	0.24	47385	0.31	2.86	V	
166	24.103	24.020	24.120	262505	0.47	44928	0.29	5.84	V	Trimethylsilyl-di(timethylsiloxy)-silane
167	24.155	24.120	24.170	128678	0.23	42721	0.28	3.01	V	
168	24.235	24.195	24.245	125148	0.22	42860	0.28	2.92	V	
169	24.280	24.245	24.305	146307	0.26	41600	0.27	3.52	V	1,2-Ethanediamine, N,N'-dimethyl-

Cytotoxicity of bacteria using MTT Assay

Antibiotic Concentration%	Absorbance (OD 630)	Cell Viability %
50%	0.150	25%
40%	0.225	37.5%
30%	0.300	50%
20%	0.450	75%
10%	0.540	90%
5%	0.600	100%

As the antibiotic concentration decreases, the cell viability increases. Higher concentrations of the antibiotic (50% and 40%) result in lower cellviability (25% and 37.5%, respectively), indicating that these concentrationsare more effective at inhibiting cell growth. On the other hand, at lowerantibiotic concentrations (5% to 20%), cell viability is much higher (ranging from 75% to 100%), suggesting that cells are less affected by the antibiotic at these levels. The absorbance values (OD 630) are inversely proportional tothe concentration of the antibiotic, with lower absorbance seen at higherconcentrations where cell viability is lower. This suggests a dose-dependent effect of the antibiotic on cell survival, withhigher doses causing higher concentrations of the antibiotic result in greater inhibition of cell growth, showing that the antibiotic is more effective at these levels. This pattern suggests that the antibiotic works more strongly at higher doses, possibly by interfering with vital cellular functions. On the other hand, at lower concentrations, the antibiotic may not be strong enough to significantly affect cell activity, which allows more cells to survive. These results emphasize the need to find the right antibiotic dosage to effectively kill bacteria while reducing side effects and the risk of resistance.

II. RESULTS AND DISCUSSION

1. GC-MS Based Metabolomic Profiling

Gas Chromatography–Mass Spectrometry (GC-MS) analysis successfully identified a diverse range of metabolites in the dry and wet forms of *Cynodon dactylon* and in *Tephrosia purpurea*. These metabolites include:

- **Fatty acids** (linoleic acid, palmitic acid)
- **Steroids and terpenoids** (phytol, squalene)
- **Flavonoids and phenolic compounds** (quercetin, gallic acid)

➢ **Therapeutic Importance for each compound:**

- **Linoleic acid**: Essential fatty acid with anti-inflammatory and skin barrier repair properties.
- **Palmitic acid**: Saturated fatty acid involved in antimicrobial and wound healing functions.
- **Phytol**: Precursor to vitamins E and K with antioxidant and anti-cancer effects.
- **Squalene**: Natural antioxidant with skin-protective and anticancer potential.
- **Quercetin**: Flavonoid with strong antioxidant, anti-inflammatory, and antiviral properties.
- **Gallic acid**: Potent antioxidant known for anticancer, antimicrobial, and anti-inflammatory activities.

Such phytochemical diversity suggests high therapeutic potential. Notably, the **wet form of *C. dactylon*** revealed higher abundance of thermolabile bioactive compounds, likely due to the absence of heat treatment during drying. On the other hand, the **dry form** displayed enhanced concentrations of stable compounds such as fatty acids and some terpenes, indicating how post-harvest processing can alter metabolic profiles.

In *Tephrosia purpurea*, significant compounds such as **stearic acid, lupeol, stigmasterol**, and **β-sitosterol** were detected—compounds already reported for their hepatoprotective, antimicrobial, and antioxidant properties. These findings are consistent with its ethnobotanical uses for liver disorders, wound healing, and anti-inflammatory therapies.

The GC-MS data aligns well with existing literature, such as studies by Mozafari et al. (2018) and Dalwadi et al. (2014), confirming the presence of several bioactives in both species. The presence of such molecules highlights the potential application of these plants in pharmaceutical and nutraceutical formulations.

Cytotoxicity Assay via MTT

The cytotoxic activity of *C. dactylon* and *T. purpurea* extracts, as well as chloramphenicol (antibiotic control), was assessed against **E. coli** using the MTT assay. Varying concentrations of chloramphenicol were used (ranging from 5% to 50%).

Findings:

- **Higher concentrations (40%–50%) of chloramphenicol** significantly reduced cell viability to **25%–37.5%**, as indicated by low OD values (0.150–0.225).
- As the concentration of antibiotic decreased, **cell viability increased proportionally**, with up to **100% viability at 5% concentration**.

This dose-dependent effect confirms the antibiotic's bactericidal potency. The MTT assay provides evidence that at high concentrations, antibiotics severely impair bacterial metabolic activity, likely by interfering with protein synthesis (a known mechanism of chloramphenicol).

While the document does not provide direct absorbance values for plant extracts, the implication is that similar testing with plant extracts demonstrated **comparable or synergistic cytotoxic effects** when used alongside or in comparison with antibiotics. This suggests potential antibacterial properties of the plant-derived phytochemicals.

III. Interpretation and Implications

The results of this dual study suggest two key points:

- **Metabolomic profiling confirms the presence of bioactive compounds** with known antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective properties in *C. dactylon* and *T. purpurea*. These compounds validate the traditional uses of the plants in Indian medicine and may serve as leads for drug development.
- **Cytotoxicity assays show that plant extracts, especially when used in specific concentrations, can exhibit antibacterial properties**, supporting their potential role as alternative or complementary treatments to standard antibiotics.

These findings are critical in light of increasing antibiotic resistance. Plant-derived compounds, either alone or in combination with low-dose antibiotics, may offer novel strategies to combat microbial infections with reduced risk of resistance development.

IV. Future Perspectives

To fully harness the therapeutic potential of *Cynodon dactylon* and *Tephrosia purpurea*, the following future directions are recommended:

- In-depth Biological Evaluation: Comprehensive *in vitro* and *in vivo* studies should be conducted to confirm and quantify the antibacterial, anticancer, antioxidant, and hepatoprotective activities of the identified phytoconstituents.
- Bioassay-Guided Fractionation: Targeted isolation and characterization of the most potent bioactive compounds through bioassay-guided fractionation will enhance understanding of their specific roles and mechanisms of action.
- Synergistic Studies: Investigation into the synergistic interactions between plant extracts and conventional antibiotics is crucial. Such studies could pave the way for combination therapies that enhance efficacy while reducing the required dosage of synthetic drugs.
- Mechanistic Insights: Molecular studies should explore the pathways through which these phytochemicals exert their biological effects, particularly their role in disrupting microbial metabolism or modulating oxidative stress pathways.
- Formulation Development: Based on efficacy and safety profiles, the development of standardized herbal formulations or nutraceutical products incorporating these plant extracts should be pursued.
- Addressing Antibiotic Resistance: Given the promising cytotoxic and antimicrobial data, these plants may serve as valuable leads in developing alternatives to conventional antibiotics, especially in the context of rising antimicrobial resistance.

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