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STUDY OF PRIMARY CONSTITUENTS IN TULSI OIL

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ABSTRACT
Tulsi, or Holy Basil (*Ocimum sanctum* L.), is renowned for its complex phytochemical profile, comprising a diverse array of bioactive compounds with significant medicinal properties, including antioxidant, antimicrobial, anti-inflammatory, and adaptogenic activities. The chemical composition varies depending on the plant part used, geographical origin, and extraction method, yet generally divides into volatile components (essential oil) and non-volatile phenolic compounds. Extracts, particularly those derived from leaves and stems, are rich in polyphenols, flavonoids, and triterpenoids. The volatile oil, primarily extracted from leaves via steam distillation, is heavily dominated by phenylpropanoids and terpenes. While eugenol is the defining active constituent of commercial tulsi oil responsible for its spicy aroma and antiseptic properties, the overall therapeutic efficacy of tulsi extract is attributed to a synergistic combination of eugenol, rosmarinic acid, and flavonoids like orientin and vicenin.

KEYWORDS: Tulsi, bioactive compounds, Essential oil, Eugenol, flavonoids.

INTRODUCTION
Aromatic plants have been used for centuries for their pleasant fragrances and therapeutic properties.[1] Aromatic plants, also known as herbs or fragrant plants, are a diverse group of plant species that are valued for their pleasant fragrances and essential oils. These plants have been used for thousands of years in various cultures for culinary, medicinal, cosmetic, and spiritual purposes. The importance and uses of aromatic plants are vast, ranging from flavoring foods to treating ailments and providing aromatherapy, due to various parts of the plant, such as leaves, flowers, seeds, and roots.[2]

Tulsi, also known as holy basil[3], is a widely used aromatic plant in traditional Indian medicine[4-5] due to its numerous therapeutic properties.[1] The essential oil extracted from tulsi leaves contains various volatile compounds, including terpenes and phenylpropanoids, eugenol, rosmarinic acid, apigenin, luteolin, and ursolic acid. Tulsi is also rich in essential oils that contribute to its aromatic properties which contribute to its characteristic aroma and flavor.[6]

METHODOLOGY
COLLECTION OF TULSI SAMPLE The sampling of tulsi leaves was collected from the local spice market of the city. 200 grams of tulsi leaves were taken for sampling purposes. The tulsi leaves were later grinded into smaller fragments for oil extraction purposes.

Figure 1: Actual image of the apparatus used during the extraction of tulsi oil.

EXTRACTION OF TULSI OIL

Clevenger Apparatus: Clevenger apparatus is a laboratory device used to carry out the extraction of essential oils from the plant materials. It works on the principle of steam distillation. The apparatus usually consists of a round bottom flask, a heating mantle with regulator to regulate temperature, water cooled condenser, water supply and a collection tube which has a knob fitted on it.

Extraction of Tulsi oil using Clevenger apparatus

The extraction of tulsi oil using Clevenger apparatus is done through following steps.

Preparation of the tulsi leaves sample: The collected sample of tulsi oil is crushed or grinded into smaller pieces. The process of crushing/grinding is very necessary as it increases the surface area of tulsi leaves for easy extraction. It helps to release the essential oil more effectively. For our research study we have took 200 grams of fresh tulsi sample.

Addition of water: Fill the round bottom flask with grinded tulsi leaves and then water is added to the sample. Before putting the sample, it is checked whether the round bottom flask is clean or not in order to reduce the chances of impurities in the sample. For a 200 grams sample of tulsi, approximately 1200ml of water is added to cover the plant material.

Setting up the apparatus: After mixing water in the tulsi sample the Clevenger apparatus is assembled. The round bottom flask is placed on the heating mantle and the condenser tube and collection tube are fitted above it. It is ensured that cold water supply is maintained in the cooling condenser tube.

Heat application: Once the assembly of apparatus is done, the heating mantle is turned on and the temperature is slightly increased. The mixture of tulsi leaves and water started to boil and evaporate after some time. The steam carries the extract of tulsi oil.

Collection of tulsi oil extract: The steam containing the tulsi oil extract starts evaporating, it rises and goes through the water cooled condenser tube and gets cooled and condenses. The condensed droplets of tulsi oil start accumulating on the collection tube of the apparatus.

Collection and storage of tulsi oil: In the collection tube, we obtain the mixture of tulsi oil and water. The oil droplets and water are immiscible due to their different densities. The tulsi oil obtained is collected carefully using the knob present in collection tube in air tight bottles in order to maintain their purity. The tulsi oil extracted from the above process must be stored in cold, dry and dark place.

Figure 2: Tulsi oil extracted using Clevenger apparatus.

GC-MS ANALYSIS OF TULSI OIL

GC-MS Instrumentation: Gas chromatography and mass spectrometry analysis of tulsi oil extract helps in identification and quantitative analysis of various organic compounds present in oil. Agilent 8890/5977B is the instrumentation used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis of tulsi oil. The compound identification was conducted using Mass hunter GCMS acquisition software by comparing the Wiley library data.

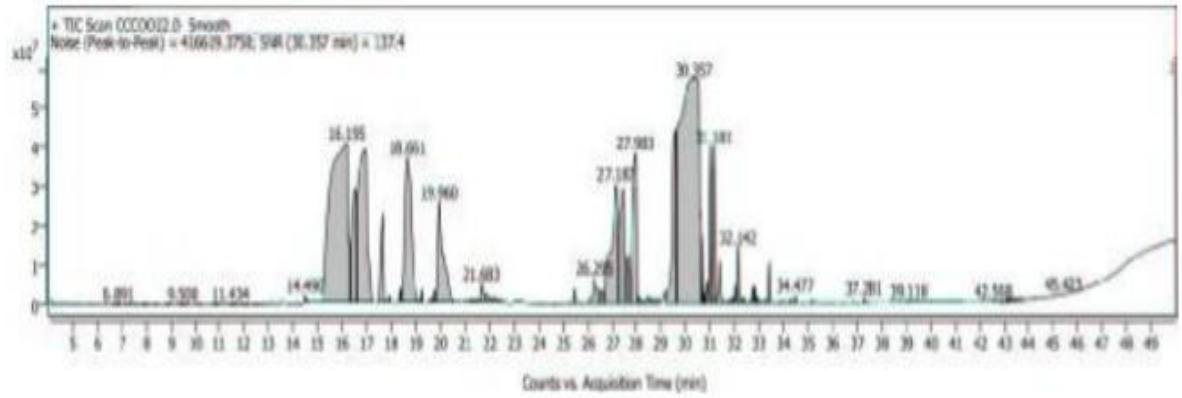
Procedure And Analysis: First of all the tulsi oil sample is prepared by dilution using suitable solvent such as hexane, ethanol etc. This is done to ensure that the sample vaporises properly when injected in the GC system. The prepared sample is injected to the GC system where it undergoes separation based on their different volatile nature. Compounds in the tulsi essential oil vaporise[7-9] and travel through the GC column at different rates which results in their separation. As the separated compounds elute from the gas chromatography column it enters to the mass spectrometer. In the mass spectrometer the compounds are bombarded by high energy electrons causing them to fragment into smaller ions.

The resulting mass spectrum provides information about the mass to charge ratio (m/z) of the compounds which is used further for identification of compounds. Compounds present in the tulsi essential oil are identified by comparing their retention times (RT) in the GC column and their mass spectra are compared from the reference databases present in the Wiley library data.

RESULT AND DISCUSSION

We got the data from GCMS it shows us the entire runtime of SNR 30.357 mins. Got 140 peaks, as shown in graph and readings.

Sample Chromatograms



SOFTWARE USED FOR COMPUTING GCMS DATA IS **MASS HUNTER**

Peak	Start	RT	End	Height	Area	Area %	Area Sum %	SNR
1	4.093	4.127	4.476	627494	6194345	0.20	0.06	1.5
2	4.476	4.574	5.340	117088	2514814	0.08	0.02	0.3
3	5.632	5.792	5.998	67735	519999	0.02	0.00	0.2
4	6.674	6.765	6.817	72179	291499	0.01	0.00	0.2
5	6.817	6.891	6.960	146433	633407	0.02	0.01	0.4
6	7.166	7.212	7.246	15730	43017	0.00	0.00	0.0
7	7.309	7.360	7.408	35848	107512	0.00	0.00	0.1
8	7.515	7.555	7.624	15744	48553	0.00	0.00	0.0
9	7.790	7.915	8.110	47582	353128	0.01	0.00	0.1
10	8.322	8.385	8.488	8507	44905	0.00	0.00	0.0
11	8.774	8.825	8.963	36876	145750	0.00	0.00	0.1
12	9.349	9.380	9.409	9315	23044	0.00	0.00	0.0
13	9.409	9.500	9.682	169017	936966	0.03	0.01	0.4
14	10.099	10.147	10.248	41467	165683	0.01	0.00	0.1
15	10.691	10.828	10.982	50689	318832	0.01	0.00	0.1
16	11.374	11.434	11.703	137153	784680	0.03	0.01	0.3
17	11.892	12.041	12.161	22518	137096	0.00	0.00	0.1
18	12.349	12.401	12.510	210750	1031417	0.03	0.01	0.5
19	12.510	12.567	12.643	55606	292473	0.01	0.00	0.1
20	12.676	12.785	12.968	245597	1833964	0.06	0.02	0.6
21	13.010	13.059	13.088	195351	635199	0.02	0.01	0.5
22	13.088	13.140	13.241	364854	1714814	0.06	0.02	0.9
23	13.260	13.294	13.386	50285	242692	0.01	0.00	0.1
24	13.475	13.523	13.595	400756	1535545	0.05	0.01	1.0
25	13.746	13.769	13.912	20953	311318	0.01	0.00	0.1
26	13.992	14.044	14.095	140721	485100	0.02	0.00	0.3
27	14.124	14.204	14.284	88290	480792	0.02	0.00	0.2
28	14.435	14.490	14.564	1662685	6635527	0.22	0.06	4.0
29	14.564	14.610	14.696	251712	1146436	0.04	0.01	0.6
30	14.817	14.942	14.987	68208	375671	0.01	0.00	0.2
31	15.058	16.195	16.315	40477441	2009096313	65.86	18.74	97.2
32	16.315	16.505	16.579	28597862	370904622	12.16	3.46	68.6
33	16.579	16.962	17.249	38883617	1001368978	32.83	9.34	93.3
34	17.481	17.654	17.812	21852266	156739042	5.14	1.46	52.5
35	17.846	17.912	17.977	1489446	6033737	0.20	0.06	3.6
36	18.043	18.083	18.129	147496	433854	0.01	0.00	0.4
37	18.152	18.192	18.215	55162	130758	0.00	0.00	0.1
38	18.298	18.370	18.415	3261060	13582326	0.45	0.13	7.8
39	18.415	18.661	19.102	36872707	678840710	22.25	6.33	88.5
40	19.102	19.148	19.176	343976	1116551	0.04	0.01	0.8
41	19.176	19.239	19.295	2977640	9738347	0.32	0.09	7.1
42	19.509	19.600	19.668	639804	4205939	0.14	0.04	1.5
43	19.668	19.783	19.823	2722673	15868136	0.52	0.15	6.5
44	19.823	19.960	20.595	25272736	381857625	12.52	3.56	60.7
45	20.653	20.704	20.761	156050	590809	0.02	0.01	0.4
46	20.902	21.070	21.147	372727	1958257	0.06	0.02	0.9
47	21.179	21.282	21.391	601185	4694204	0.15	0.04	1.4
48	21.391	21.454	21.482	510727	2138887	0.07	0.02	1.2
49	21.482	21.511	21.580	516938	2134423	0.07	0.02	1.2
50	21.580	21.683	21.860	4433560	33439276	1.10	0.31	10.6

Peak	Start	RT	End	Height	Area	Area %	Area Sum %	SNR
51	21.860	21.911	22.106	1755605	16664470	0.55	0.16	4.2
52	22.106	22.215	22.556	812651	12226873	0.40	0.11	2.0
53	22.655	22.684	22.762	210295	903868	0.03	0.01	0.5
54	23.038	23.067	23.096	48150	125007	0.00	0.00	0.1
55	23.096	23.130	23.167	82373	223702	0.01	0.00	0.2
56	23.205	23.239	23.436	73091	692702	0.02	0.01	0.2
57	23.927	23.983	24.091	297579	1222187	0.04	0.01	0.7
58	24.116	24.177	24.254	98677	418048	0.01	0.00	0.2
59	24.603	24.652	24.698	36436	149934	0.00	0.00	0.1
60	24.698	24.738	24.787	33205	122983	0.00	0.00	0.1
61	24.881	24.956	24.996	39221	151713	0.00	0.00	0.1
62	25.179	25.265	25.362	436269	1952068	0.06	0.02	1.0
63	25.368	25.471	25.619	3587024	14278753	0.47	0.13	8.6
64	25.619	25.654	25.734	75017	432028	0.01	0.00	0.2
65	25.734	25.797	25.905	115811	754703	0.02	0.01	0.3
66	25.991	26.295	26.506	6403902	82572217	2.71	0.77	15.4
67	26.506	26.586	26.684	3257012	31566848	1.03	0.29	7.8
68	26.684	27.187	27.273	30302301	570449574	18.70	5.32	72.7
69	27.273	27.468	27.559	28899434	374477417	12.28	3.49	69.4
70	27.559	27.639	27.736	12075048	101355216	3.32	0.95	29.0
71	27.736	27.983	28.068	38278433	453975448	14.88	4.23	91.9
72	28.068	28.086	28.183	1778872	7976895	0.26	0.07	4.3
73	28.183	28.240	28.337	764216	5006353	0.16	0.05	1.8
74	28.337	28.469	28.555	1764283	14095074	0.46	0.13	4.2
75	28.555	28.641	28.761	1217714	12009643	0.39	0.11	2.9
76	28.761	28.864	28.984	1171818	11495769	0.38	0.11	2.8
77	29.201	29.613	29.642	44083591	500605133	16.41	4.67	105.8
78	29.642	30.357	30.649	57242539	3050560316	100.00	28.46	137.4
79	30.649	30.666	30.723	16097195	41028657	1.34	0.38	38.6
80	30.723	30.741	30.786	3107901	9185724	0.30	0.09	7.5
81	30.786	30.912	30.947	5324741	30843095	1.01	0.29	12.8
82	30.947	31.032	31.090	39779713	174592300	5.72	1.63	95.5
83	31.090	31.181	31.250	39844648	179859780	5.90	1.68	95.6
84	31.250	31.387	31.519	10180176	50674611	1.66	0.47	24.4
85	31.519	31.565	31.673	523500	3252929	0.11	0.03	1.3
86	31.673	31.759	31.862	966994	7103635	0.23	0.07	2.3
87	31.862	31.925	31.959	1510013	6205005	0.20	0.06	3.6
88	31.959	32.045	32.080	4367093	18755337	0.61	0.17	10.5
89	32.080	32.142	32.251	14206440	55658696	1.82	0.52	34.1
90	32.251	32.308	32.348	1190813	4823181	0.16	0.04	2.9
91	32.348	32.394	32.463	1304696	5407181	0.18	0.05	3.1
92	32.463	32.549	32.600	416956	2517609	0.08	0.02	1.0
93	32.600	32.755	32.789	4116707	19444147	0.64	0.18	9.9
94	32.789	32.818	32.875	4033072	14826233	0.49	0.14	9.7
95	32.875	32.915	33.018	1671585	9094724	0.30	0.08	4.0
96	33.018	33.058	33.167	614510	3148893	0.10	0.03	1.5
97	33.167	33.258	33.321	985107	4604769	0.15	0.04	2.4
98	33.321	33.418	33.596	9899904	39509610	1.30	0.37	23.8
99	33.619	33.716	33.785	131602	686711	0.02	0.01	0.3
100	33.785	33.865	33.928	677492	2567753	0.08	0.02	1.6
101	33.928	34.036	34.117	550359	2746774	0.09	0.03	1.3
102	34.128	34.294	34.403	969933	5987782	0.20	0.06	2.3
103	34.403	34.477	34.635	1835450	7443214	0.24	0.07	4.4
104	34.695	34.757	34.809	147542	526570	0.02	0.00	0.4
105	34.809	34.878	34.920	43769	223852	0.01	0.00	0.1
106	34.982	35.072	35.101	50428	281284	0.01	0.00	0.1
107	35.101	35.175	35.241	833985	3057244	0.10	0.03	2.0
108	35.321	35.387	35.511	60496	350118	0.01	0.00	0.1
109	35.572	35.627	35.684	62034	250462	0.01	0.00	0.1
110	35.684	35.747	35.833	121218	525832	0.02	0.00	0.3
111	35.833	35.890	35.942	30915	139127	0.00	0.00	0.1
112	35.942	35.993	36.028	36236	131253	0.00	0.00	0.1
113	36.028	36.079	36.158	85469	349187	0.01	0.00	0.2
114	36.269	36.337	36.377	79844	306262	0.01	0.00	0.2
115	36.377	36.417	36.474	62769	265613	0.01	0.00	0.2
116	36.474	36.526	36.594	58554	219642	0.01	0.00	0.1
117	36.737	36.875	37.155	292735	2262271	0.07	0.02	0.7
118	37.213	37.281	37.350	1090766	3950673	0.13	0.04	2.6
119	37.350	37.407	37.526	300339	1219320	0.04	0.01	0.7
120	37.561	37.601	37.636	16360	41841	0.00	0.00	0.0
121	37.730	37.773	37.842	38793	124494	0.00	0.00	0.1
122	38.330	38.425	38.540	80795	447698	0.01	0.00	0.2
123	38.540	38.700	38.763	32415	281023	0.01	0.00	0.1
124	38.763	38.889	38.929	109503	655624	0.02	0.01	0.3
125	38.929	39.003	39.049	208303	1128749	0.04	0.01	0.5
126	39.049	39.118	39.186	333309	1971112	0.06	0.02	0.8
127	39.186	39.261	39.558	205521	2960581	0.10	0.03	0.5
128	39.558	39.633	39.701	177391	925286	0.03	0.01	0.4
129	39.701	39.787	39.873	97208	731022	0.02	0.01	0.2
130	39.873	40.102	40.211	110218	1534333	0.05	0.01	0.3
131	40.211	40.268	40.422	66380	652384	0.02	0.01	0.2
132	40.422	40.554	40.720	71961	806834	0.03	0.01	0.2
133	40.720	40.840	40.949	42470	392145	0.01	0.00	0.1
134	40.949	41.206	41.344	31102	410753	0.01	0.00	0.1
135	42.063	42.110	42.213	22627	97060	0.00	0.00	0.1
136	42.494	42.568	42.665	42501	210261	0.01	0.00	0.1
137	44.187	44.245	44.279	58195	180446	0.01	0.00	0.1
138	45.006	45.069	45.103	75435	251730	0.01	0.00	0.2
139	45.372	45.423	45.452	85969	245075	0.01	0.00	0.2
140	49.915	49.967	49.983	105670	315689	0.01	0.00	0.3

Noise Type	Signal Type	Noise Multiplier	Noise	Start	End
Peak To Peak	Height	1.0	416619	42.757	43.757

As we see that we got 140 peaks but most of the compound has less then 0.1% so we will eliminate those compounds, and forms a new table of compound which has mainly the average sum % more then 0.50%. The table is given below with reference from above table, formatted in descending order of Average Sum %.

NO	Peak No.	Start	RT	End	Height	Area	Area %	Area Sum %	SNR
1	78	29.642	30.357	30.649	57242539	3050560316	100.00	28.46	137.4
2	31	15.058	16.195	16.315	40477441	2009096313	65.86	18.74	97.2
3	33	16.579	16.962	17.249	38883617	1001368978	32.83	9.34	93.3
4	39	18.415	18.661	19.102	36872707	678840710	22.25	6.33	88.5
5	68	26.684	27.187	27.273	30302301	570449574	18.70	5.32	72.7
6	77	29.201	29.613	29.642	44083591	500605133	16.41	4.67	105.8
7	71	27.736	27.983	28.068	38278433	453975448	14.88	4.23	91.9
8	44	19.823	19.960	20.595	25272736	381857625	12.52	3.56	60.7
9	69	27.273	27.468	27.559	28899434	374477417	12.28	3.49	69.4
10	32	16.315	16.505	16.579	28597862	370904622	12.16	3.46	68.6
11	84	31.090	31.181	31.250	39844648	179859780	5.90	1.68	95.6
12	83	30.947	31.032	31.090	39779713	174592300	5.72	1.63	95.5
13	34	17.481	17.654	17.812	21852266	156739042	5.14	1.46	52.5
14	70	27.559	27.639	27.736	12075048	101355216	3.32	0.95	29.0
15	67	25.991	26.295	26.506	6403902	82572217	2.71	0.77	15.4
16	89	32.080	32.142	32.251	14206440	55658696	1.82	0.52	34.1
17	74	28.337	28.469	28.555	1764283	14095074	0.46	0.13	4.2
18	76	28.761	28.864	28.984	1171818	11495769	0.38	0.11	2.8

These 18 peaks carry approx. 95% of the oil constituent, the top 5 peaks carry approx. 70 % of the compound. And some of the peaks are ionized form of same of the compound. and the rest of the peaks covered very less area and and their % composition is less then 0.1%, so we can say that these peaks shows presence of either trace compounds or impurities in extract of tulsi.

On summarizing all 140 peaks we got 18 major peaks which carry over 94% of the sample, tulsi oil is given below.

S.no.	Compound found in GCMS done by MassHunter Soft.	AREA SUM%
1.	Eugenol ; C10H12O2	28.46
2.	Caryophyllene;C15H24	18.74
3.	Caryophyllene;C15H24	9.34
4.	Caryophyllene;C15H24	6.33
5.	Methyleugenol; C11H14O2	5.32
6.	Eugenol ; C10H12O2	4.67
7.	14-Hydroxycaryophyllene; C15H24O	4.23
8.	Humulene;C14H24	3.56
9.	Methyleugenol; C11H14O2	3.49
10.	Caryophyllene;C15H24	3.46
11.	Eugenol ; C10H12O2	1.68
12.	(2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-; C15H26O T*	1.63
13.	Caryophyllene;C15H24	1.46
14.	Camphor	0.95
15.	beta.-Acorenol; C15H26O	0.77
16.	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol; C15H24O T*	0.52
17.	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-; C15H26O T*	0.13
18.	5-Methoxy-10,10-dimethyl-6- methylenebicyclo[7.2.0]undecan-2-one; C15H24O2 T*	0.11

T* Denotes the trace compounds, having higher percentage composition then 0.1%.

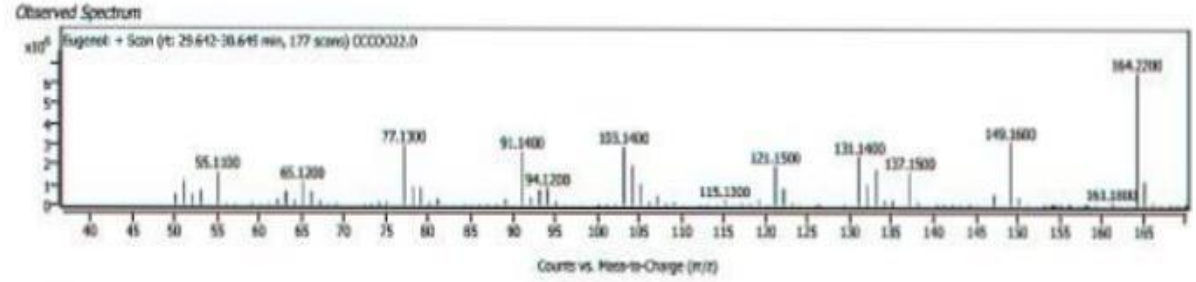
On Summerizing the peaks, we get % composition of the compound.

S.no	Compound Name	Percentage Composition
1	Eugenol ; C10H12O2	34.81
2	Caryophyllene;C15H24	37.87
3	Methyleugenol; C11H14O2	8.81
4	14-Hydroxycaryophyllene; C15H24O	4.23
5	Humulene;C14H24	3.56
6	Camphor	0.95
7	beta.-Acorenol; C15H26O	0.77

The list of compounds obtained from GC-MS analysis of Tulsi essential oil is represented in table above. Now we will discuss in detail about the observed chromatograms of these organic compounds. The chromatogram obtained from the GC-MS analysis of sample oil is given below.

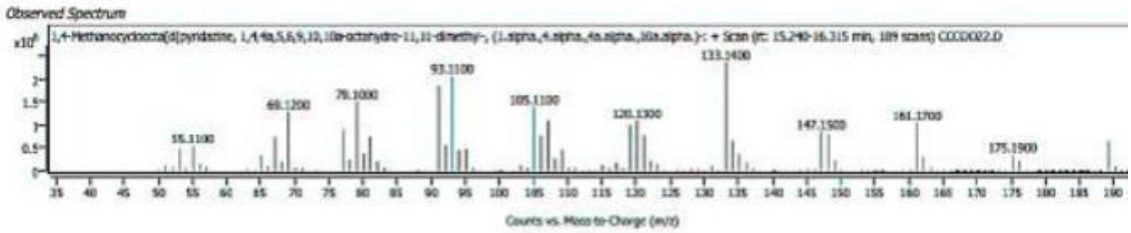
1. + Scan (rt: 29.642-30.649 min) Peak 78 from + TIC Scan Smo (Eugenol; C10H12O2)

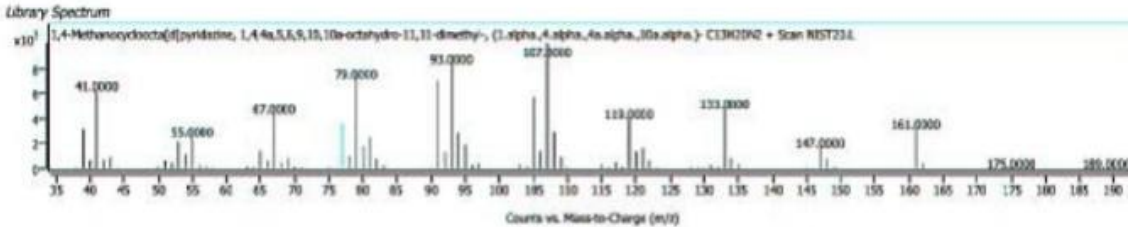
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Eugenol	C10H12O2	97-53-0	74.85	74.85	NIST20.L



2. + Scan (rt: 15.240-16.315 min) Peak 31 from + TIC Scan Smo (Caryophyllene;C15H24)

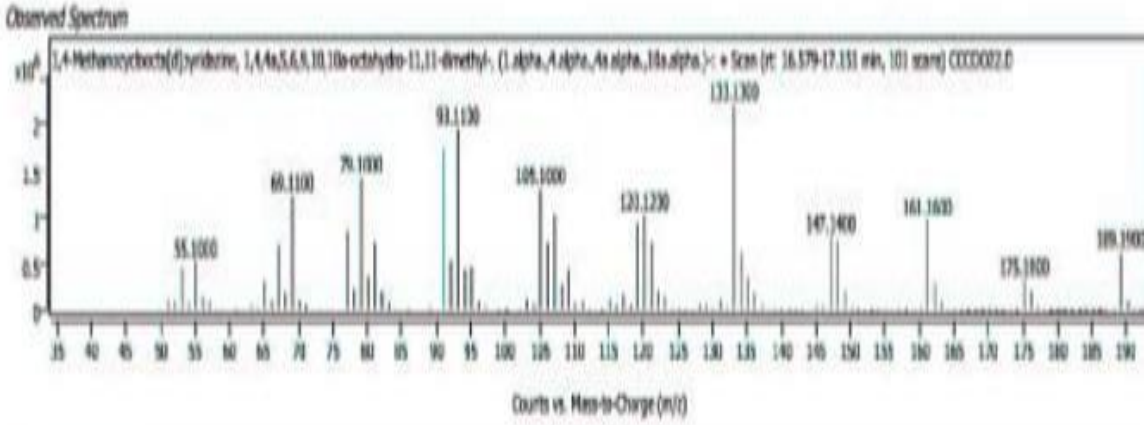
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Caryophyllene	C15H24		82.83	82.83	NIST20.L

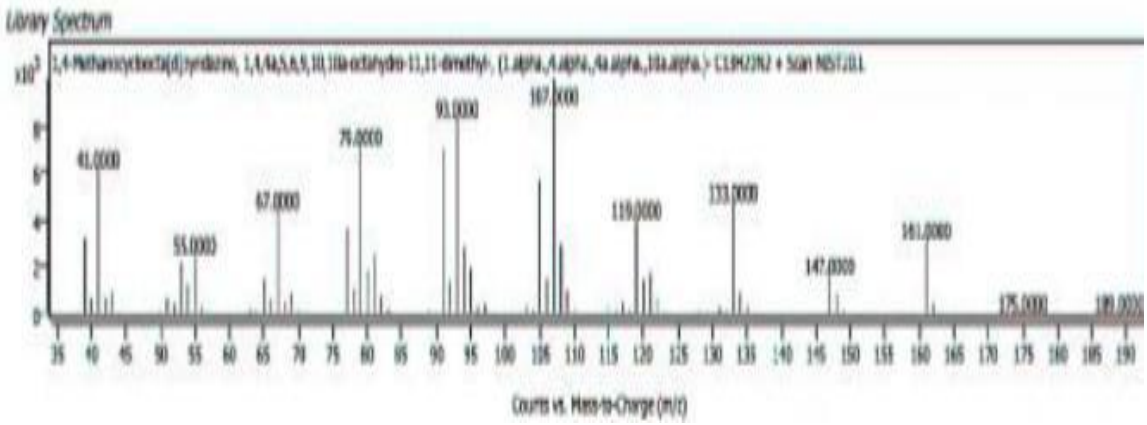




3+ Scan (rt: 16.579-17.151 min)Peak 33 from + TIC Scan Smo (Caryophyllene;C15H24)

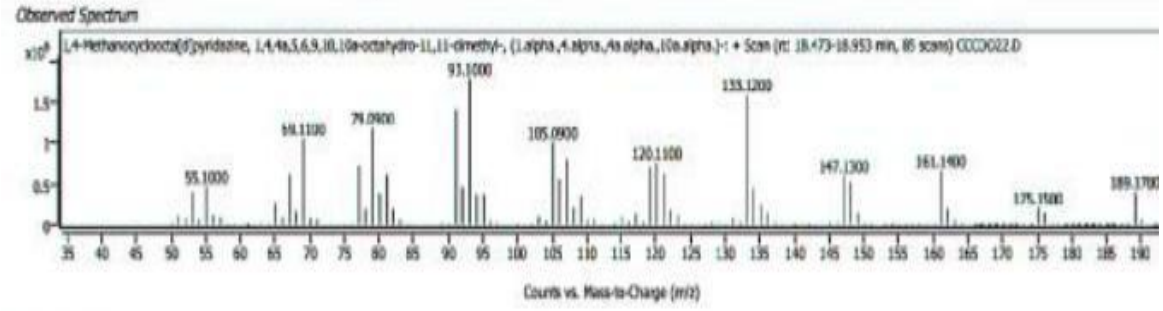
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Caryophyllene	C15H24	1000221-85-9	83.13	83.13	NIST20.L

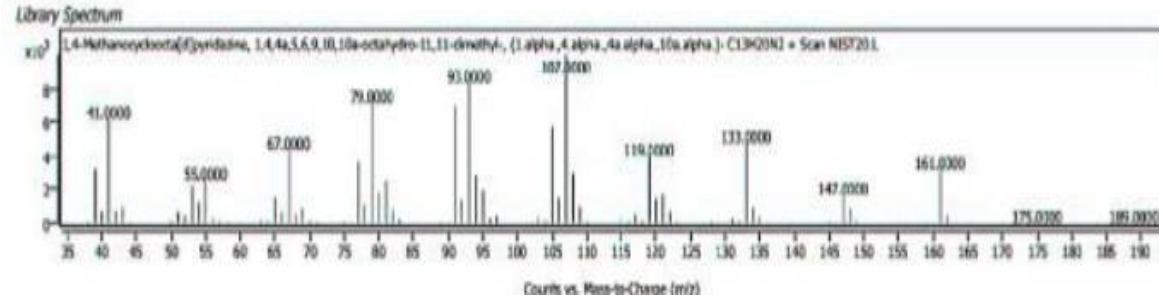




4. + Scan (rt: 18.473-18.953 min) Peak 39 from + TIC Scan Smo TIC Scan Smo (Caryophyllene;C15H24)

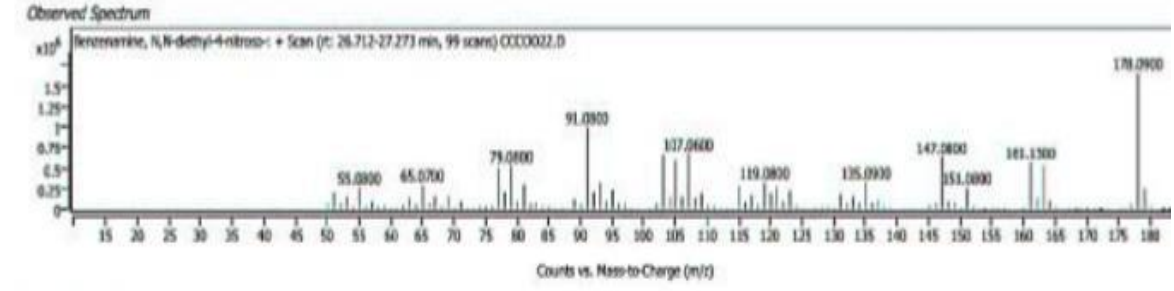
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Caryophyllene	C15H24		84.55	84.55	NIST20.L

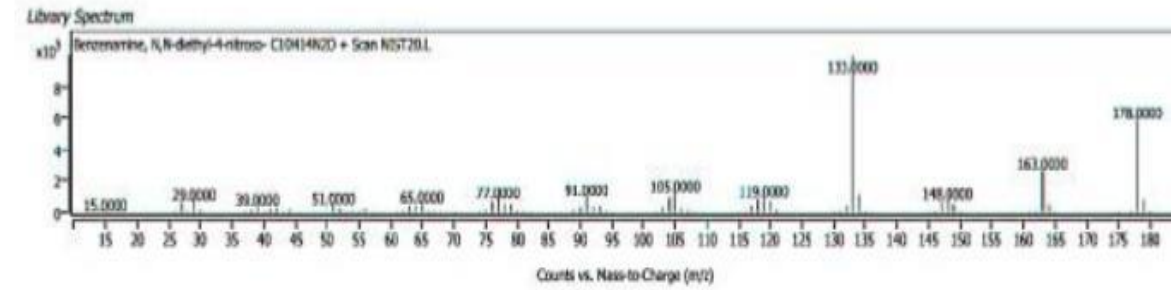




5. + Scan (rt: 26.712-27.273 min)Peak 68 from + TIC Scan Smo (methyl Eugenol)

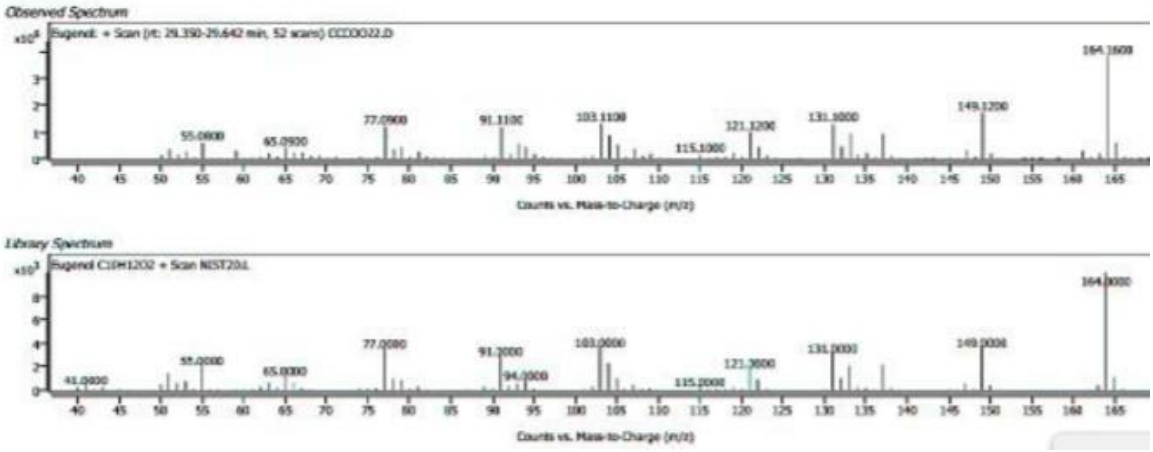
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
methyl Eugenol	C10H14N2O	120-22-9	64.47	64.47	NIST20.L





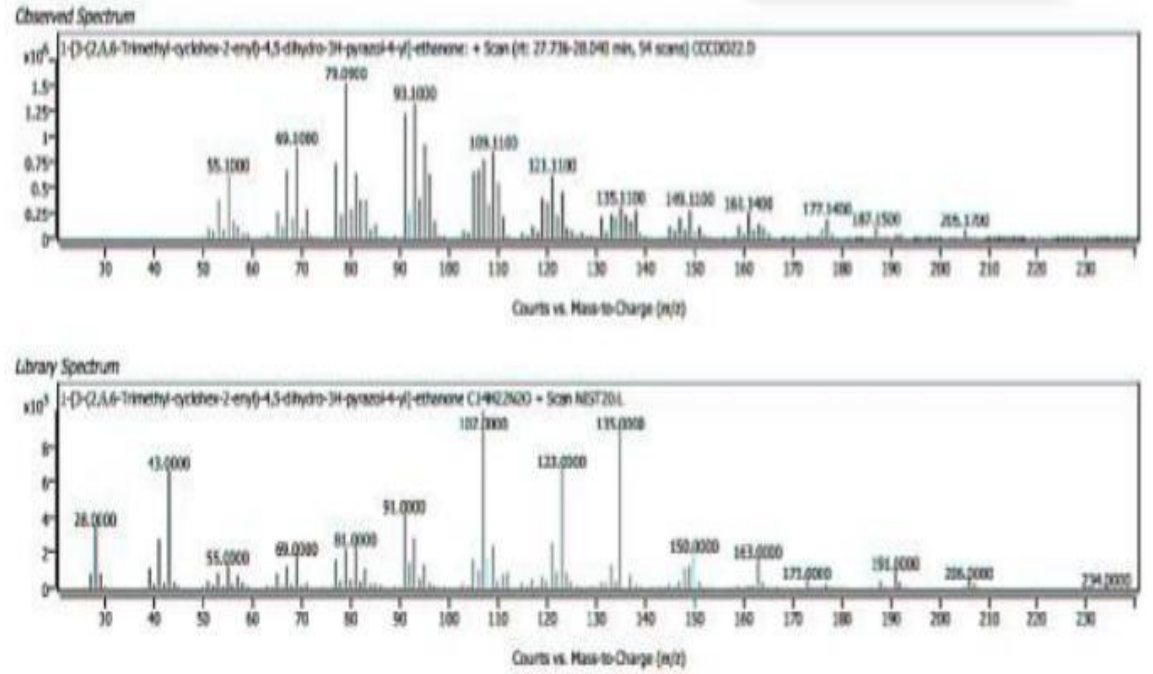
6. + Scan (rt: 29.350-29.642 min) Peak 77 from + TIC Scan Smo (Eugenol; C10H12O2)

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Eugenol	C10H12O2	97-53-0	74.06	74.06	NIST20.L



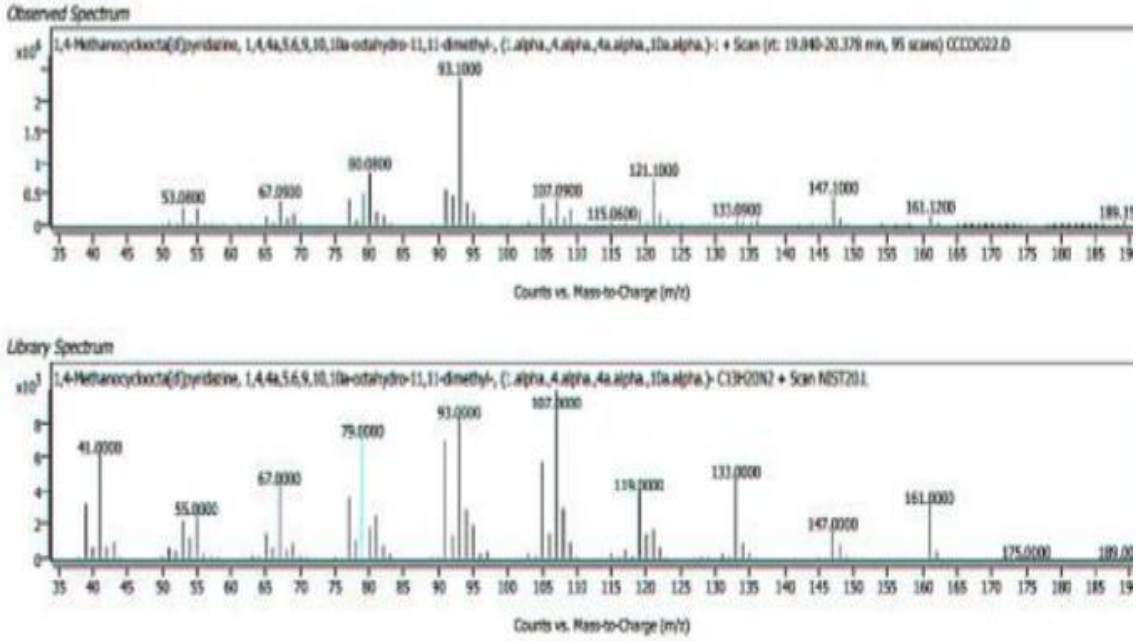
7. + Scan (rt: 27.736-28.040 min) Peak 71 from + TIC Scan Smo (14-Hydroxycaryophyllene; C15H24O)

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
14-Hydroxycaryophyllene; C15H24O -	C15H24O		74.67	74.67	NIST20.L



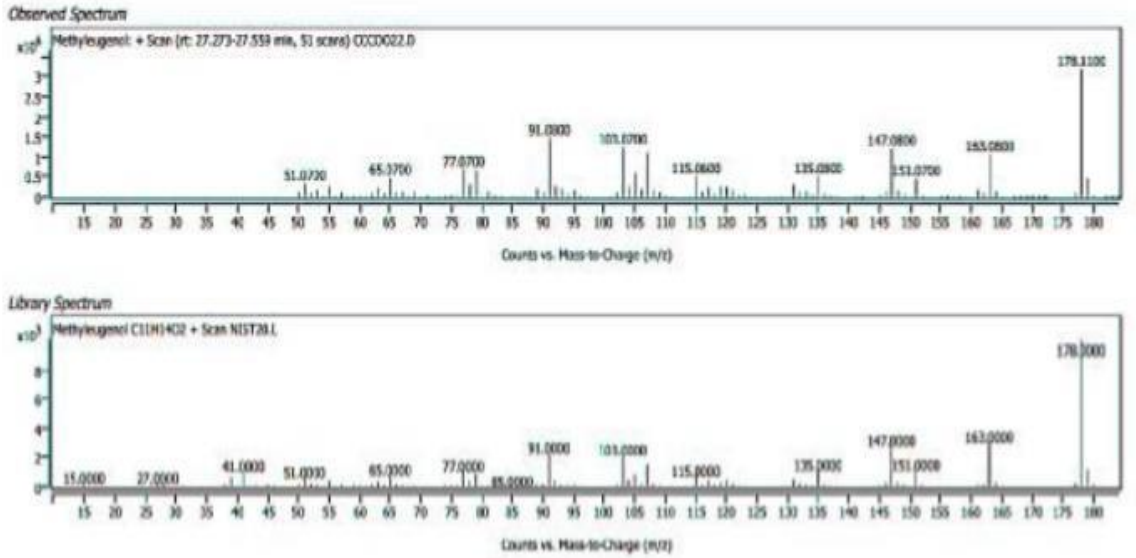
8. + Scan (rt: 19.840-20.378 min) Peak 44 from + TIC Scan Smo (Humulene;C14H24)

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Humulene	C14H24		77.41	77.41	NIST20.L



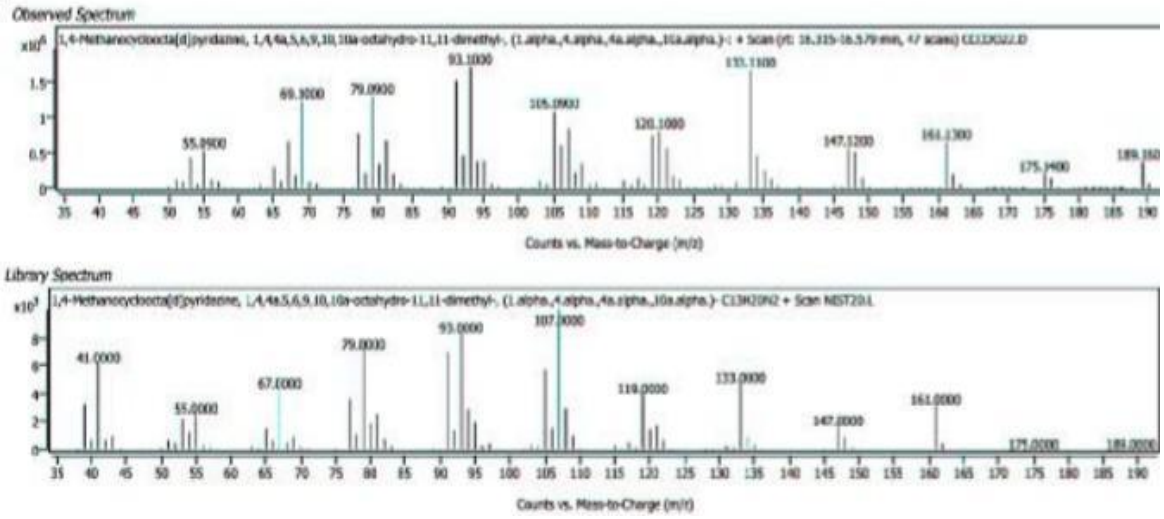
9. + Scan (rt: 27.273-27.559 min) Peak 69 from + TIC Scan Smo (Methyleugenol; C11H14O2)

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Methyleugenol	C11H14O2	93-15-2	74.07	74.07	NIST20.L



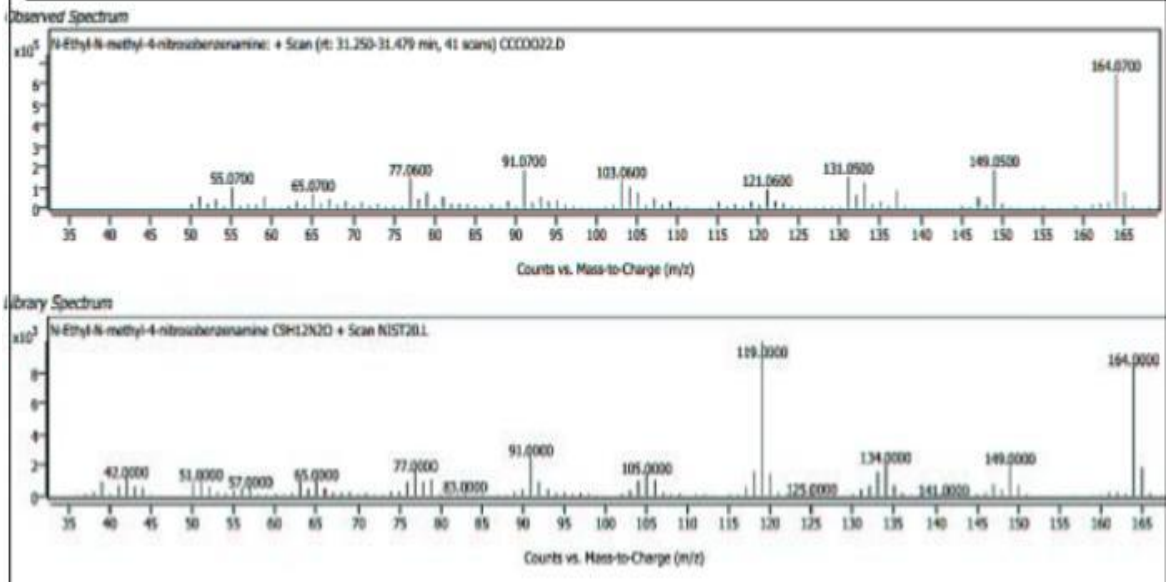
10.+ Scan (rt: 16.315-16.579 min) (Caryophyllene;C15H24) Peak 32 from + TIC Scan Smo

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Caryophyllene	C15H24	1000221-85-9	85.12	85.12	NIST20.L



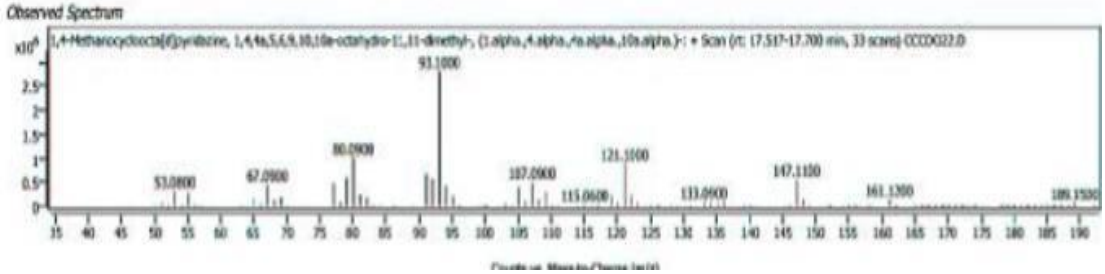
11.+ Scan (rt: 31.107-31.221 min) (Eugenol;C10H1202) Peak 84 from + TIC Scan Smo

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Eugenol;C10H1202	C10H12O2	473-15-4	74.60	74.60	NIST20.L



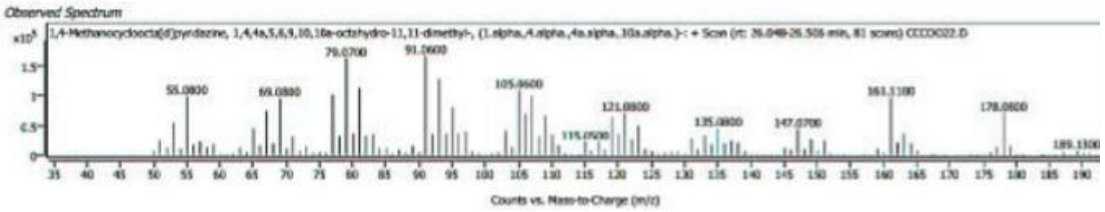
13. + Scan (rt: 17.517-17.700 min) Peak 34 from + TIC Scan Smo (Caryophyllene;C15H24)

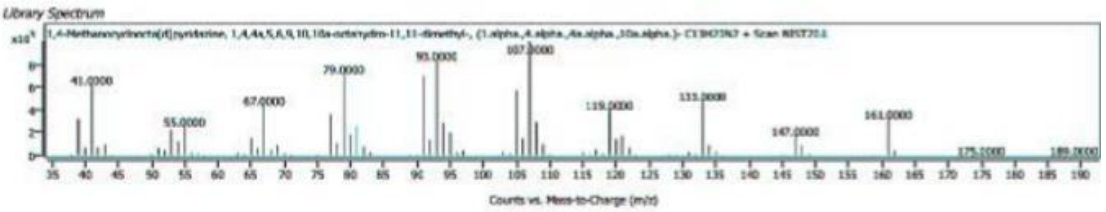
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
Caryophyllene	C15H24		76.73	76.73	NIST20.L



15. + Scan (rt: 26.048-26.506 min) Peak 67 from + TIC Scan Smo (beta.-Acorenol; C15H26O)

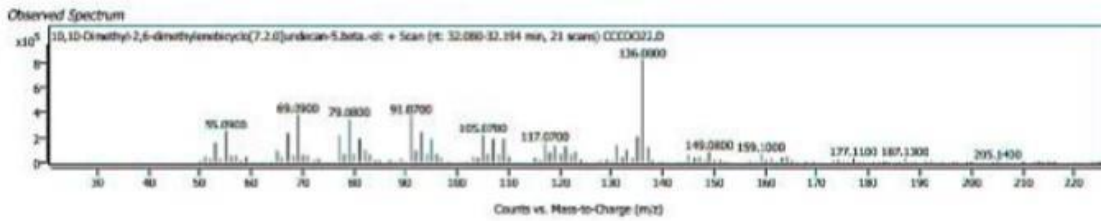
Name	Formula	CAS	Score	Score (Lib)	Lib/DB
beta.-Acorenol	C15H26O	1000221-85-9	72.50	72.50	NIST20.L

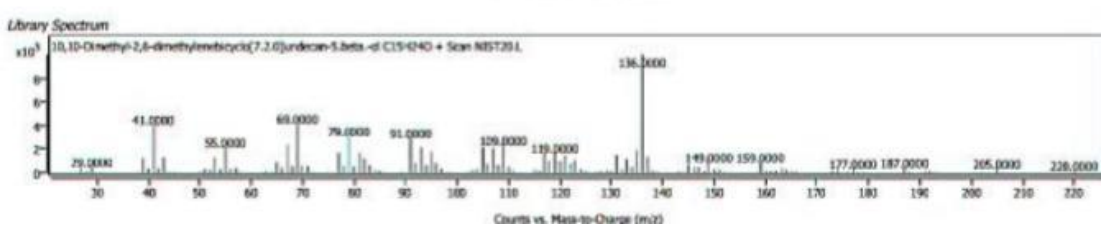




16. + Scan (rt: 32.080-32.194 min) Peak 89 from + TIC Scan Smo (10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol; C15H24O)

Name	Formula	CAS	Score	Score (Lib)	Lib/DB
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	C15H24O	19431-80-2	72.14	72.14	NIST20.L





PRIMARY CONSTITUENTS OF TULSI OIL

Tulsi oil, extracted from Ocimum sanctum (Holy Basil), contains a diverse array of primary constituents, each contributing to its therapeutic properties. Research conducted in India has extensively studied these constituents, shedding light on their chemical composition and pharmacological effects. Eugenol, the principal constituent of tulsi oil, constitutes approximately 70-80% of the oil and presents as a pale yellow liquid with a potent, spicy aroma. It boasts antiseptic, analgesic, and anti-inflammatory properties[10], as evidenced by research in India which highlights its effectiveness in relieving pain and inflammation associated with conditions such as arthritis and dental ailments.

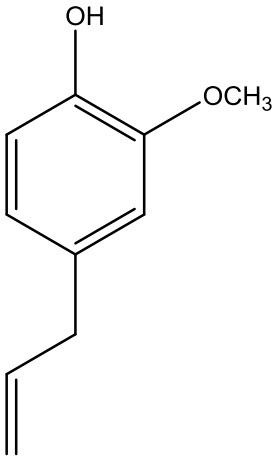


Figure 3: Structure of Eugenol.

Methyl eugenol, a methyl ether derivative of eugenol, enhances the aromatic profile of tulsi oil and enriches its fragrance. Research conducted in India indicates its potential as a natural insect repellent and antimicrobial agent.

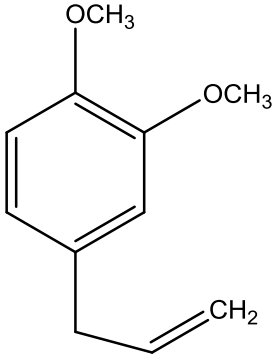


Figure 4: Structure of Caryophyllene.

Caryophyllene, a sesquiterpene hydrocarbon, exhibits notable anti-inflammatory and analgesic properties, rendering it valuable for pain management. Studies conducted in India have underscored its capacity to modulate the body's endocannabinoid system, suggesting its potential therapeutic efficacy in addressing pain and inflammation.

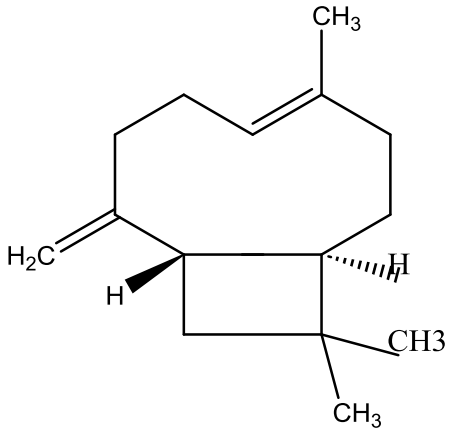


Figure 5: Structure of Caryophyllene.

We have discussed in detail about the major and minor components present in the extracted Tulsi essential oil, discussed about the structures of obtained compounds and have done quantitative analysis of Tulsi essential oil. Now we will study about the commercially available Tulsi oil and compare the results of GC-MS analysis of the extracted Tulsi oil with the commercial Tulsi oil.

COMPARISON WITH COMMERCIAL OIL CONSTITUENTS

The GC-MS analysis of tulsi (Ocimum sanctum) revealed the presence of two major compounds: eugenol (34.81%) and caryophyllene (37.87%). These concentrations indicate significant contributions to the chemical profile of tulsi. In a study of commercial Tulsi oil, the GC-MS chromatogram showed very sharp and clear peaks. This sharpness is due to the purification processes that commercial Tulsi oils undergo, resulting in minimal impurities. Both extracted Tulsi oil and commercial Tulsi oil share common compounds such as eugenol, caryophyllene, Methyl eugenol, Hydroxy caryophyllene, Humulene, Camphor, beta.-Acorenol. However, the eugenol content is significantly higher in commercial Tulsi oil compared to extracted Tulsi oil.

Sno.	Compound Name	% composition in commercial oil	% composition in Our Extracted oil Analysis
1	Eugenol ; C10H12O2	57.9±2.7	34.81
2	Caryophyllene;C15H24	15.32±3.1	37.87
3	Methyleugenol; C11H14O2	0.655	8.81
4	14-Hydroxycaryophyllene; C15H24O	3.3±0.05	4.23
5	Humulene;C14H24	0.9±0.05	3.56
6	Camphor	24.15	0.95
7	beta.-Acorenol; C15H26O	0.8-2.5%	0.77

CONCLUSION

Through a comprehensive quantitative analysis utilizing gas chromatography-mass spectroscopy (GC-MS), we have conducted a detailed analysis of the essential oil extracted from Tulsi (Ocimum sanctum) leaves. Employing the steam distillation method with the Clevenger apparatus, we obtained a pure Tulsi oil extract, which underwent rigorous analysis to elucidate its chemical composition.

The GC-MS analysis unveiled a rich array of constituents within Tulsi oil, highlighting key compounds such as eugenol, methyl eugenol, caryophyllene, among others. These compounds contribute not only to the characteristic fragrance but also to the therapeutic properties associated with Tulsi oil. The eugenol content of our tulsi oil sample is about 33.13% while some other studies conclude that the eugenol concentration of tulsi essential oil ranges from 60-70%. It is noteworthy that the concentration of eugenol, a predominant constituent in Tulsi oil, may vary depending on factors such as geographical location, cultivation practices, and extraction methods. Our findings contribute to the understanding of these variations and underscore the importance of standardization in Tulsi oil production. In conclusion, our study underscores the profound significance of Tulsi oil as a valuable natural resource with multifaceted applications. From traditional remedies to modern pharmaceutical formulations, Tulsi oil emerges as a potent agent with the potential to enhance health and well-being across diverse populations.

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