Profile of Volatile Organic Compounds from *Cuscuta campestris* Yunck

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Abstract
Cuscuta campestris, a holoparasitic plant reliant on host plants for sustenance, has been reported to harbor medicinally active phytochemicals. This study is aimed to evaluate the phytochemical profile, particularly VOCs, of *C. campestris* through gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).
Fresh and dried samples of twiners of *C. campestris* were analyzed using GC and GC-MS techniques. The results revealed notable differences in the profiles of volatile compounds between fresh and dried samples. The fresh samples showed minimal detection in GC-FID analysis while, GC-MS detected three compounds. Dried and powdered samples exhibited a more elaborate profile, with GC-FID detecting 29 peaks and GC-MS identifying 18 significant compounds, including hexanal, heptanone, and caryophyllene.
The study highlights the importance of VOCs in *C. campestris*, for standardization of plant material for medicinal and industrial applications. A reproducible analytical method for standardizing medicinal plant material based on fingerprints of volatiles is developed. This approach can aid in assessing plant quality and understanding the impact of environmental factors on the composition of volatiles. Furthermore, the variation in VOC profiles can indicate potential influence due to the host plant and environmental factors. This work provides a foundation for further investigations into phytochemical variations in medicinal plant materials using fingerprints of volatiles.

Keywords: Volatile organic compound (VOC), GC, GC-MS, *Cuscuta campestris*

1. Introduction:

Plant bioactives are produced mainly as secondary metabolites, which play a major role in plant adaptations to environmental changes. These secondary metabolites are often used as medicines after isolation from the plant sources [1]. Many of these metabolites are volatile and are less explored or evaluated for their medicinal use. Different groups of chemical compounds such as terpenes, alcohols, aldehydes, ketones, esters and heterocyclic compounds normally form the class of volatile organic compounds (VOC) [2].

Cuscuta campestris is a holoparasitic plant that has been reported to possess medicinally active phytochemicals. Being holoparasitic, the plant depends on the host plant for water, nutrients, and photosynthetic substances [3]. It also incorporates chemicals from its hosts along with other nutrition such as secondary metabolites [4][5]. The purpose of this research was to evaluate phytochemicals with special emphasis on volatile organic compounds present in *Cuscuta campestris* and generate a phyto-fingerprint of the volatiles.

Gas chromatography is one of the most widely used techniques of chromatography that is employed for analysis of volatile organic compounds [6]. Essential oils mainly consist of volatile and semi-volatile analytes that can be instantly identified and separated using GC-MS [7][8][9]. A combination of static head space sampling technique with Gas Chromatography (GC) and Mass Spectrometry (MS) is a great tool for analyzing volatiles. Therefore, sampling was performed using static head space technique and analysis was performed on GC and GC-MS.

2. Material and Method:

Fresh and healthy twiner with flowers of *Cuscuta campestris* were collected between November and December from its host plant from Lonavala region of Maharashtra, India. It was authenticated from Botanical Survey of India (BSI), Western Regional Center, Pune, India (Authentication no: BSI/WRC/100-1/Tech./2020/100). Fresh plant sample was collected in two batches. One batch was used for fresh plant sample analysis. This sample from batch one was washed thoroughly and kept for shade drying overnight; and was subjected to GC and GC-MS analysis the next day itself. The second batch of sample after washing and cleaning was shade dried thoroughly for a week. The dried plant raw material was powdered using mechanical grinder, sieved through BSS 85 mesh and stored in air tight containers at room temperature until further analysis. Fresh samples of twiners were collected from the same spot for replicate analysis. Static head space GC technique has significantly lower limits of detection which enable analytes to be determined at very low concentrations. The methodology of the static head space is mainly based on saturation of the extracting phase with analytes, which causes macro compounds to remain into the sample, which are relatively less volatile [10].

3. GC and GC-MS analysis:

Accurately weighed 1g fresh plant sample and powdered plant sample was taken and sealed in Borosil® head space GC vial using a crimping tool. The vials after sealing and crimping were heated in sand bath at 150°C for 15 minutes. 1mL of gas sample was aspirated using gas tight syringe immediately after 15 min of heating and was injected in the GC and GC-MS system for analysis. GC-MS analysis was supplemented with Wiley spectral library and National Institute of Standards and Technology (NIST 11) for identification of compounds.

Instrumental parameters for GC:

- Instrument specification : GC 2014
- Column details : Rtx®-1 (30meter, 0.25mm ID and 0.25µm particle size)
- Column oven temperature : 220°C
- Injection type : Split mode (ratio – 100)
- Injection port temperature : 200°C
- Detector : Flame Ionization Detector (FID)
- Detector Temperature : 250°C

Instrumental parameters for GC-MS:

- Instrument specification : GCMS-QP 2010 Ultra
- Injection type : Split mode (ratio – 90)
- Injection port temperature : 200°C
- Column Details : Rtx®-5MS (30meter, 0.25mm ID and 0.25µm particle size)
- Column oven temperature : Ramping program

rate	temperature	Hold time
--	50°C	3 min
5.00	200°C	7 min
0.00	250°C	15 min

- Interface temperature : 280°C

4. Discussion / Results:

Current research work is focused on characterizing the volatile organic bioactive compounds from *Cuscuta campestris.* No peaks were detected after analysis of fresh plant sample using GC-FID. GC-MS analysis of the fresh plant sample showed three peaks and is tabulated in table 1 and represented in figure 1.

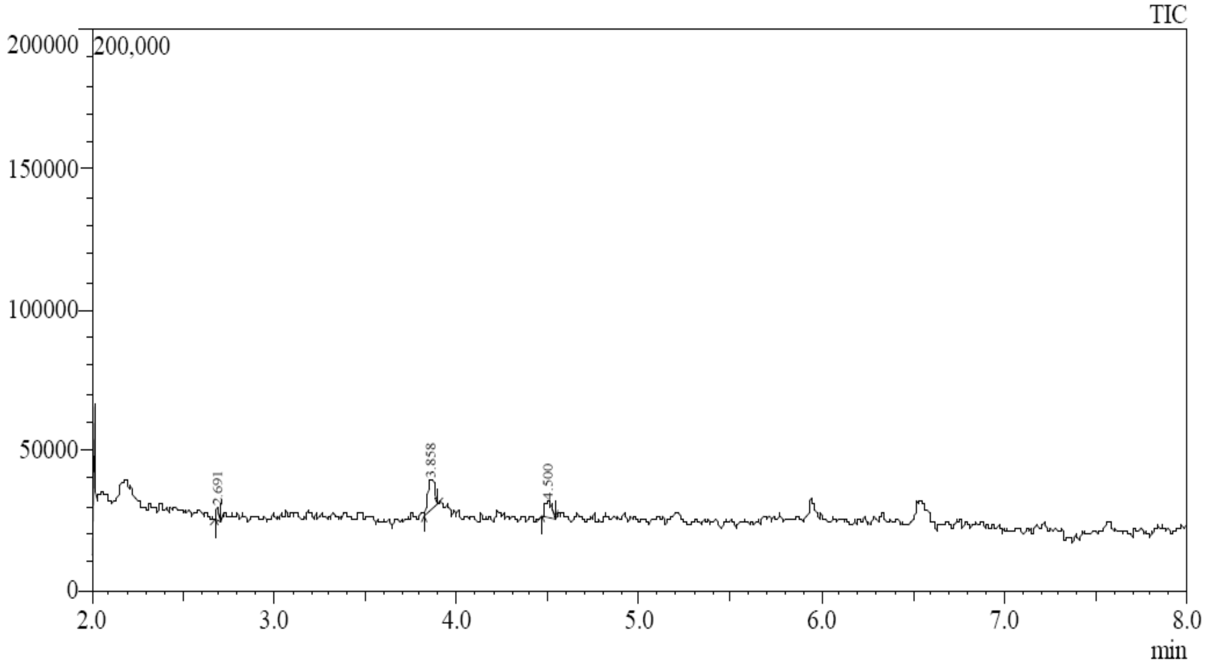

Figure 1: GC-MS spectrogram of *Cuscuta campestris* fresh plant sample

Table 1: Identification of compounds from *Cuscuta campestris* fresh plant sample

Peak no.	R. Time	Area	Name
1	2.691	4704	Chloro-difluoro-acetamide
2	3.858	30316	1,3,5,7-Cyclooctatetraene
3	4.500	14363	Cyclohexene, 3-bromo

Three compounds were detected in fresh plant sample after analysis using GC-MS. Detected compounds were, chloro-difluoro-acetamide, 1,3,5,7-Cyclooctatetraene and Cyclohexene, 3-bromo which are important phytochemicals. Chloroacetamide is used as preservative in cosmetics and household products. It is also used as a plasticizer and industrial solvent. 1,3,5,7-Cyclooctatetraene is a precursor of many fatty acids. Cyclohexene is an important compound used in synthesis of waterproof coating and oil extractions. Dried and powdered plant material was evaluated using GC for qualitative analysis and GCMS was employed for identification of the volatile organic compounds. Qualitative estimation of the plant raw material by GC has shown presence of 29 peaks which are represented in table 2 and the chromatogram is represented in figure 2.

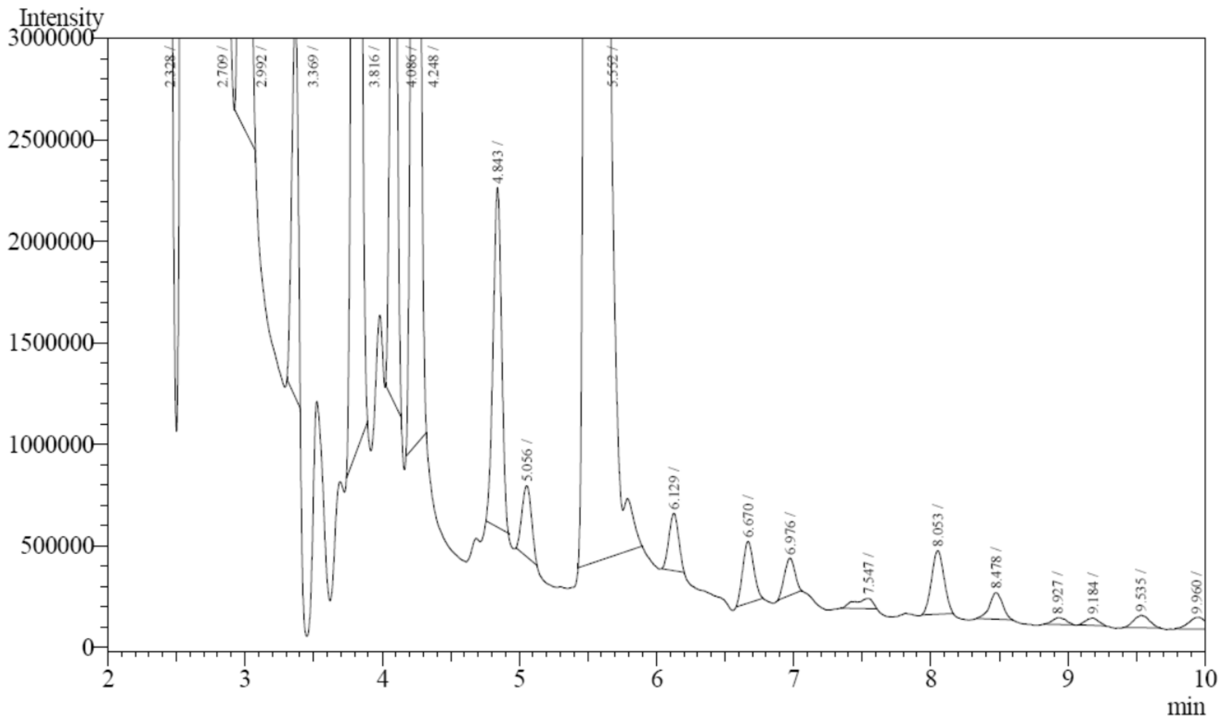

Figure 2: GC-FID chromatogram of *Cuscuta campestris* powdered sample

Table 2: Qualitative estimation of *Cuscuta campestris* powdered sample using gas chromatography

Peak	Retention Time	Area
1	1.817	15703812355
2	1.963	18925495271
3	2.328	6400073097
4	2.709	1040220970
5	2.992	17927619
6	3.369	5909541
7	3.816	43836134
8	4.086	12096769
9	4.248	30137512
10	4.843	7112771
11	5.056	1683910
12	5.552	1879410054

13	6.129	1366692
14	6.670	1673534
15	6.976	965622
16	7.547	465586
17	8.053	1969421
18	8.478	862412
19	8.927	237718
20	9.184	227943
21	9.535	479377
22	9.960	507641
23	10.639	1137663
24	12.486	173032
25	13.026	316132
26	16.249	1134946
27	17.296	640888
28	22.987	1203316
29	24.716	34935610

After qualitative detection of the volatiles the identification of VOC was carried out by static head space GC-MS. The results showed presence of phenols, acids and flavonoids in the sample like Eucalyptol, Hexanal, beta.-Myrcene, Carene, Butanoic acid and Caryophyllene which are medicinally important compounds. The chromatogram and the identified compounds are shown in figure 3 and table 3.

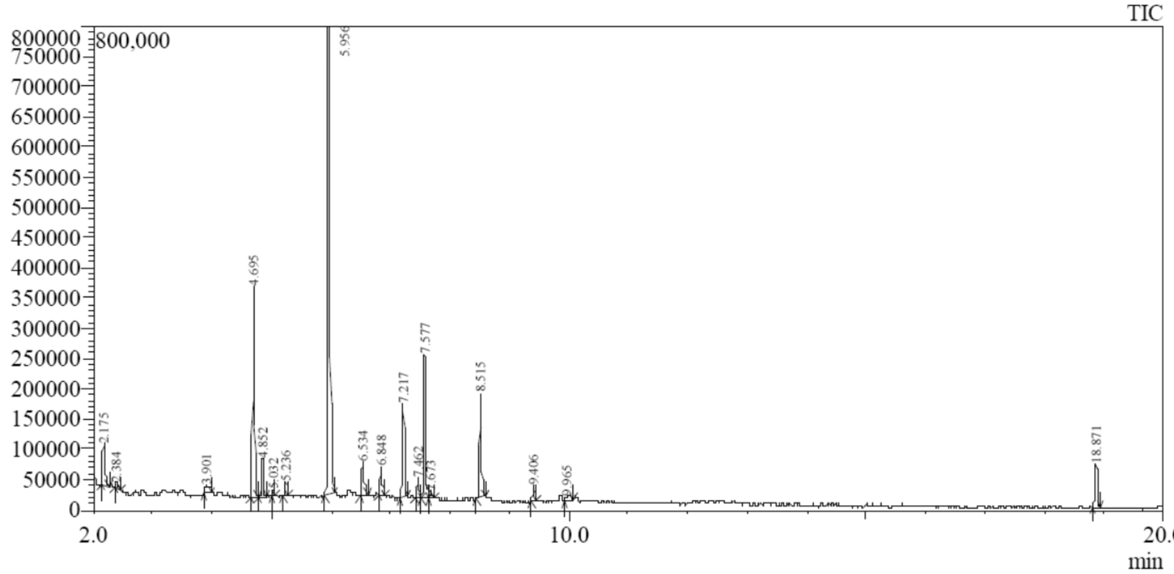

Figure 3: GCMS spectrogram of *Cuscuta campestris* powder

Table 3: Identification of compounds from *Cuscuta campestris* powder

Peak no.	Retention Time	Area	Name
1	2.175	205074	Hexanal
2	2.384	39006	1H-Pyrrole, 1-ethyl
3	3.901	64106	2-Heptanone

4	4.695	863913	Bicyclo hex-2-ene, 2-methyl-5-(1-methylethyl)
5	4.852	166067	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
6	5.032	23477	1,6-Octadiene, 5,7-dimethyl-, (R)
7	5.236	64754	1H-Pyrrole, 1-butyl-
8	5.956	4140268	beta.-Phellandrene
9	6.534	175983	beta.-Myrcene
10	6.848	121932	alpha.-Phellandrene
11	7.217	397562	Carene
12	7.462	87479	Cymene
13	7.577	636538	Cyclohexene, 1-methy1-5-(1-methylethenyl)-, (R)
14	7.673	35026	Eucalyptol
15	8.515	447436	gamma.-Terpinene
16	9.406	64448	Carene
17	9.965	44619	Butanoic acid, 2-methyl-, 2-methylbutyl ester
18	18.871	201579	Caryophyllene

In current research work, VOC were extracted using static headspace method of sampling which is simple and fast procedure for extracting the volatile fraction of phytochemicals [10]. VOC profile of both fresh plant sample and dried plant sample were analyzed with the same analytical condition using GC and GC-MS. Fresh plant sample of *C. campestris* did not show any peak in GC-FID analysis. On the other hand, three compounds were detected in GC-MS analysis. The dried and powdered sample of the same plant material showed 29 volatiles in GC-FID analysis while GC-MS analysis have identified 18 important volatiles such as hexanal, heptanone, careen, cymene, eucalyptol and, butanoic acid etc. The variations observed in VOC profile of the fresh plant sample and dried plant sample are interesting. Drying and powdering probably enables release of VOCs that are in bound form within cells. The VOC profile is reproducible and repeatable for the same plant sample. The VOC profile can be evaluated to understand the host-parasite association better.

5. Conclusion:

To understand the quality of plant material and standardize its usage in sustainable manner; use of fingerprint of volatiles from the plant raw material has been reported in this work. Current research work focused on characterizing the volatiles from *Cuscuta campestris* plant using GC and GC-MS techniques. Medicinally and industrially important, volatile bioactive compounds were identified from the plant material. 29 Volatile compounds were detected by using GC in dried powder of *Cuscuta campestris*. GC-MS analysis for the same identified 18 bioactives that are of significant value. A simple, reliable and reproducible analytical method has been reported for standardization of medicinal plant material using the fingerprint of volatiles. Same method can be used as a standardization technique for other plants with volatiles, like aromatic plants. The variation in the VOC profile can provide important quality related evaluation for better use of plant material and also understand the influence of microenvironment on the volatiles. Fresh plant samples have shown lesser volatiles as compared to the dried powdered plant sample in both GC and GC-MS analysis.

VOC profile in *Cuscuta campestris* can vary due to variation in the host plant because *Cuscuta campestris* is a holo-parasitic plant and nutrients absorbed from the host plant can influence the VOC profile. Many of the volatile organic compounds are secondary metabolites; and synthesis of these metabolites is significantly influenced by several environmental factors. Climatic changes, altitudinal variations where the plant is grown, latitude, precipitation, wind speed, air quality, humidity and aridity etc are major influencing factors. Based on these points, evaluations can be made further for phytochemical variations using the method developed in this research work for several other plant raw materials.

6. References:

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