Limonene dominates the Phytochemistry of Trigonella foenum-graceum in Nature

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Abstract: Many plant families have some unique medicinal properties due to the presence of special chemical molecules in different parts of plants also known as "phytofoods." Himalaya is one of the greatest mountain chains in the world which not only supports a vast variety of plants and animals but also the great diversity of traditional phytofoods. Many of these phytofoods are very vital to humans in different diseases also. In this paper, we have crucially analyzed some of the major volatile compounds and anti-microbial activities of *Trigonella foenum*-graceum. By the help of our gc-ms data, we are reporting the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%) in this plant. [Kamal Kishore Pande, Lata Pande, Bharat Pande, Atul Pujari, Pankaj Sah and Stuti Sah. Limonene Dominates the Phytochemistry of Trigonella foenum-graceum. Nature and Science 2011;9(5):17-20]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Phytofoods, Herbal medicine, Himalaya, Limonene

1. Introduction:

Many plants of the Central Himalayan region have found use as indigenous medicines. Different parts of these plants have different properties and researchers are analyzing the chemical composition of the plant part which is traditionally known to have medicinal properties and used in food in daily lives. Such edible parts of the medicinal plant are commonly known as *phytofoods*. One such important Indian Himalayan phytofood is *Trigonella foenum*-graceum.

Earlier literature reports have revealed the presence of biologically active and novel compounds in these foods. In this paper we have thoroughly analyzed the chemical composition and anti-microbial activity of volatile compounds found in *Trigonella foenum*-graceum.

2. Materials and Methods

The essential oil was obtained by steam distillation of fresh plant material (500 g) using a copper still fitted with spiral glass condensers. The distillate was saturated with NaCl and extracted with hexane. The hexane extract was dried using anhydrous sodium sulphate and the solvent was removed with a rotovap at reduced pressure and at 25 °C to yield oil. The gc-ms analysis was performed in Agilent 6890 series equipped with MSD 19091S-433 system. It is

known that there is a selective loss of low boiling terpenoids by conventional hydro-distillation technique. Headspace-gc is therefore more suitable for quantification of highly volatile compounds than conventional hydro distillation technique.

For the Dynamic Headspace, 1.0 gm of sample was taken in 20 ml headspace vial. Headspace is created and these vapours are injected in GC equipped with MSD. Headspace components were identified by matching their mass spectra with those in NIST 05 MS library search and by comparing with literature reports and GC retention indices (RI).

Determination of antimicrobial activity (minimum inhibitory concentration MIC) of oil extracts by two-fold serial dilution method. The test organisms; bacterial and fungal cultures (*Staphylococcus aureus* (S.a), *Escherichia coli* (E.c), *Cndida albicans* (C.a) and *Cryptococcus neoformans* (C.n.) were obtained from Central Drug Research Institute, Lucknow and maintained as well as experimented in the Microbiology laboratory, Department of Biotechnology, M.B. Govt. P.G. College, Haldwani (Nainital).

Trigonella foenum-graecum:

Trigonella foenum-graecum seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an anti diabetic effect. The seed and leaves

are anti-cholesterolemic, anti-inflammatory, antitumor and hypoglycaemic. The main components of the oil were found to be [delta]-cadinene (27.6%), []-cadinol (12.1%). GC analysis of oil identified palmitic acid, linoleic acid oleic acid and stearic acid. Headspace analysis shows prominent presence of (hexanal, 2methyl-2-butenal, 3-octen-2-one). *Trigonella foenumgraecum* was evaluated for their antifungal and antibacterial activity against *Aspergillus niger, and Staphylococcus aureus*.

3. Result and discussion:

Trigonella foenum

Present gc-ms study also reveals the presence of fatty acid. The volatile component Palmidrol (28.72%) which is an antiviral, antiinflammatory and nonsteroidal analgesic is as major bioactive constituent. Present dynamic headspace gc-ms of *trigonella foenum* claim the dominance of limonene (82.30%), d-carvone (12.97%) and n-caproaldehyde (1.87%) which are being reported first time.

Result and discussion: Chemical Investigation *Trigonella foenum - graceum (Family:Fabacease)* GC-MS Analysis of Essential Oil

The seeds of *Trigonella foenum* yielded 0.2% by weight of *yellow* oil with a pleasant aroma. GC and GC-MS analysis of the essential oil from the seeds of

Trigonella foenum revealed the presence of seven major compounds and all of them were identified by comparing their mass spectra with MS library. The oil is rich in Fatty acids. The fatty acids, monoterpinene hydrocarbon, pthlates and oxygenated monoterpene amounted to 53.19%, 21.92%, 15.03% and 9.85% respectively. The major constituents are 28.72% palmidrol, 24.47% octanamide n-(2-hydroxyethyl), 15.03% dioctyl phthalate, 14.58% d-limonene, 9.85% 1-carvone, 4.31% o-cymene and 3.03% -terpinene. (**Table 1, Figure 1**).

3.1 Dynamic Headspace GC-MS Analysis

The seeds of fresh Trigonella foenum graceum were shade dried and crushed to powder. One gram of powdered material is taken for dynamic headspace gc-ms analysis. Head space (hs) gc-ms of Trigonella foenum - graceum revealed the presence of seven volatile organic components and all of them were identified by comparing their mass spectra with ms library. The dominant component of the oil is a monoterpene hydrocarbon. The monoterpene hydrocarbons, oxygenated monoterpenes, aliphatic aldehyde, hetrocyclic compounds and alcohols amounted to 82.57%, 13.34%, 1.87%, 1.82% and 0.4% respectively. The major constituents are 82.30% limonene, 12.97% d-carvone, 1.87% n-caproaldehyde, 1.82% 2-amylfuran and 0.40% 2,3-butanediol. (Table 2, Figure 2).

Pk	RT	Area%	Common Name	Method
1	12.305	4.31	o-cymene	a,b
2	12.41	14.58	d-limonene	a,b
3	13.453	3.03	-terpinene	a,b
4	19.299	9.85	1-carvone	a,b
5	39.222	28.72	palmidrol	a,b
6	42.725	24.47	octanamide, n-(2-hydroxyethyl)-	a,b
7	45.965	15.03	dioctyl phthalate	a,b

 Table 1: Chemical composition (%) of essential oil of Trigonella foenum – graceum (Seed)

a = Retention Index; b = MS (GC-MS) Library

Abundance



Figure 1: GC of essential oil of Trigonella foenum – graceum (Seed).

Table 2: Chemical composit	ion (%) of <i>Trigonell</i>	a foenum– graceum	(seed) by dynamic hs/gc-ms.

Pk	RT	Area %	Common Name	Method of Identification
3.	4.576	1.87	n-caproaldehyde	a,b
4.	4.966	0.40	2,3-butanediol	a,b
5.	10.725	1.82	2-amylfuran	a,b
6.	12.258	82.30	limonene	a,b
7.	12.951	0.27	- terpinene	a,b
8.	17.329	0.37	trans-dihydrocarvone	a,b
9.	18.838	12.97	d-carvone	a,b

a = Retention Index; b = MS (GC-MS) Library



Figure 2: Dynamic headspace gc of essential oil of Trigonella foenum - graceum (seed).

4. Discussion

Mazza, G. et al. has reported over 50 volatile compounds in *Trigonella foenum*^[1]. Pritee wagh observed gc analysis of oil and identified hexadecanoic acid methyl ester (palmitic acid), 9, 12-octadecadienoic acid methyl ester (linoleic acid) 9-octadecenoic acid methyl ester (oleic acid) and octadecanoic acid methyl ester (stearic acid)^[2]. Present gc-ms study also reveals the presence of fatty acid. The volatile component palmidrol, (28.72%) which is an antiviral, antiinflammatory and non steroidal analgesic is as major bioactive constituent. Mazza, G. et al. reported prominent presence of carbonyl compounds in headspace analysis ^[3]. In present headspace dynamic gc-ms of Trigonella foenum claim the dominance of limonene (82.30%), d-carvone (12.97%) and ncaproaldehyde (1.87%), which are being reported first time in the seeds of Trigonella foenum.

3/17/2011

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