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Research Article

# Comparative study of the chemical composition of *Trigonella foenum-graecum* L. essential oil

Vanya Nalbantova[1], Niko Benbassat[1], Cédric Delattre[2,3], Velislava Todorova[1], Yoana Georgieva[1], Paolina Lukova[1], Stanislava Ivanova[1], Kalin Ivanov[1], Diana Karcheva-Bahchevanska[1]

1 *Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*
2 *Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, F-63000 Clermont-Ferrand, France*
3 *Institut Universitaire de France (IUF), 1 Rue Descartes, 75005 Paris, France*

Corresponding author: Vanya Nalbantova (vanya.nalbantova@mu-plovdiv.bg)

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## Abstract

Fenugreek is a well-known aromatic plant. Its leaves and seeds are commonly used for their many health benefits and the species is cultivated worldwide. For the first time, essential oils (EOs) from seeds of the Bulgarian and Indian populations were obtained by secondary distillation of hydrolat to extract the residual essential oil therein. They were analyzed by gas chromatography-mass spectrometry and the chemical composition of the two samples was compared. Thirty-six compounds were identified and although the results showed some differences in the component composition of the two essential oils, the major constituents identified in both oils were cubenol, γ-n-amylbutyrolactone and palmitic acid. Moreover, oxygenated sesquiterpenes were the predominant fraction in Bulgarian and Indian seeds oils. Information is limited regarding menthol and γ-n-amylbutyrolactone, which were found in the present study in both oils. The performed analysis confirmed the presence of phytol in fenugreek seeds regardless of the extraction method of the essential oil.

## Keywords

cubenol, essential oil, fenugreek, GC-MS, *Trigonella foenum-graecum*

## Introduction

*Trigonella foenum-graecum* L. (Fenugreek) is an annual dicotyledonous aromatic plant which belongs to Fabaceae family. It is a medicinal plant also known as "Methi", indigenous to the Mediterranean, South and Central Asia, North Africa and North America and cultivated in Australia, China, Egypt, Turkey. It is widely used as a condiment in food preparations and it is a constituent of the Indian spicy 'curry' (Srinivasan 2006; Basu et al. 2018; Ebrahimghochi et al. 2018; Rashid et al. 2018).

Fenugreek is a widely known forage crop with a leaf structure specific to legumes. The aerial part of the plant reaches a height of 45 cm. There are single pods containing 20 seeds, 5 mm long, hard and yellowish brown or golden brown with a waxy endosperm above the yellow embryo. The seeds have a characteristic rhomboid outline, pungent odor and slightly bitter-sweet taste (Mandal and DebMandal 2016; Basu et al. 2018).

Its leaves and seeds are widely used throughout the world. The seeds can be used as an antidiabetic agent due to their hypoglycemic properties and can reduce plasma cholesterol levels. This makes them a good alternative treatment for obesity by improving glucose tolerance and weight loss. In addition, they are a good treatment option for constipation (Khorshidian et al. 2016). Along with these

benefits, special porridges have been prepared from them in the past for female weight gain (Sulieman et al. 2007).

A previous randomized controlled trial showed good results with the administration of *Trigonella foenum-graecum* seeds syrup in mild asthma treatment. Fenugreek honey-based syrup may improve the quality of life of asthmatic patients. It is appropriate as a pulmonary tonic and can improve pulmonary secretion (Emtiazy et al. 2018).

The seeds have beneficial effects on digestion, bile, stomach and pancreas. The extracts have been reported to have antiulcer effects due to their influence on mucosal glycoproteins and secretory function (Srinivasan 2006).

These numerous pharmacological activities are attributed to various bioactive compounds present in the composition of fenugreek seeds. Of the primary metabolites, such are fatty acids (linoleic and palmitic acid), which are of considerable importance to the health of the human body (Akbari et al. 2019). The seeds also contain carbohydrates and in particular soluble galactomannans with antioxidant and wound healing efficiency (Ktari et al. 2017; Feki et al. 2019). Secondary metabolites such as steroidal sapogenins (diosgenin, tigogenin, neotigogenin and yamogenin) are also part of the seed composition. Their content can reduce the level of serum cholesterol by increasing its bile excretion (Zameer et al. 2018). Diosgenin is associated with therapeutic effects, such as anticarcinogenic and hepatoprotective (Syed et al. 2020). Furthermore, there are reports that its content can be increased by treating fenugreek with ascorbic acid (Pundarikakshudu and Bhavsar 1990). A significant decrease in liver glycogen content and serum insulin level is due to the flavonoids contained such as quercetin, apigenin, luteolin, vitexin (Basu et al. 2018; Jiang et al. 2018). Fenugreek seeds are also a source of alkaloids such as trigonelline, choline, gentianine, neurin, (Mandal and DebMandal 2016) which have been shown to exhibit anti-inflammatory and antinociceptive effects (Mandegary et al. 2012). In addition, they contain essential oil (EO) (Ahmadiani et al. 2004; Naimi et al. 2022) with antibacterial activity (Moniruzzaman et al. 2015).

The composition of the essential oil can be very different depending on the climate and geography where the plants grow, their genetics, extraction method, part of the plant used etc.

Moreover, data on the chemical composition of fenugreek essential oil obtained by redistillation is limited. The aim of the present study was to investigate the component composition of fenugreek essential oil and to compare Bulgarian and Indian seed essential oil obtained by secondary distillation of hydrolat.

# Materials and methods

## Plant material

Indian and Bulgarian seeds were purchased from a local market in Bulgaria.

## Chemicals and reagents

Hexane was purchased from Merck KGaA (Darmstadt, Germany). It was used to isolate and dilute essential oils. Hydrocarbons were used to determine retention indices (RI). They were purchased from Merck KGaA (Darmstadt, Germany).

## Isolation of essential oils

The two essential oils were isolated using the same distillation method. Bulgarian and Indian fenugreek seeds (400 g) were pulverized and subjected to water distillation for 4 hours in a field distillation unit. The resulting aromatic distillation waters were redistilled in Clevenger type apparatus by hydrodistillation for 4 hours. The obtained essential oil samples were dried over anhydrous sodium sulphate and stored in dark vials at 4 °C until future analyses.

## Chromatography analysis

Fenugreek essential oils were analyzed using Gas chromatography – mass spectrometry (GC-MS). The both of analyses were carried out using Bruker Scion 436-GC SQ MS, Bremen, Germany, equipped with Bruker BR-5ms fused silica capillary column (0.25 μm film thickness and 15 m × 0.25 mm). The oven temperature was initially held at 50 °C for 1 min and then increased to 270 at 5 °C/min. The temperatures of detector and injector were 300 and 250 °C, respectively. Volume of the injection was 1 μL, split/split-less with a split ratio 1:20. The carrier gas was helium with a flow rate 1 mL/min. Fenugreek essential oils compounds were compared with retention indices with Wiley NIST11 Mass Spectral Library and literature evidence.

# Results and discussion

The extracted essential oils from Bulgarian and Indian fenugreek seeds have a pungent and specific flavor and they were analyzed using GC-MS. In table 1. are presented the identified chemical compounds of both essential oils with their retention indices, formulas and relative percentages of the total in Bulgarian and Indian population.

Thirty-six components in the essential oil from Bulgarian and Indian seeds are shown, representing 59.67% and 53.10% of the total identified compounds, respectively. The essential oil of Bulgarian fenugreek seeds appeared rich in compounds of oxygenated monoterpenes (6.54%), oxygenated sesquiterpenes (29.80%) and sesquiterpene hydrocarbons (0.31%). Among the major identified compounds are cubenol (29.88%), docosane (10.41%), benzyl benzoate (3.00%), palmitic acid (2.75%), *p*-cymen-7-ol (2.67%), *γ*-n-amylbutyrolactone (1.78%), *p*-cumic aldehyde (1.93%) and phytol (1.14%). Oxygenated sesquiterpenes constitute the major fraction in both essential oils. In the EO of Indian fenugreek seeds they account for 39.89% of the total identified mixture. In this EO, again

**Table 1.** Identified compounds of essential oils of Bulgarian and Indian fenugreek seeds.

| № | Compound | RI | Formula | % of Total in BP | % of Total in IP |
|---|---|---|---|---|---|
| 1. | 2-Methylbutyric acid | 889 | $C_5H_{10}O_2$ | 0.15 | nd |
| 2. | 3-Octen-2-one | 1037 | $C_8H_{14}O$ | tr | nd |
| 3. | 3,5-Octadien-2-one | 1055 | $C_8H_{12}O$ | tr | 0.47 |
| 4. | *β*-Linalool | 1055 | $C_{10}H_{18}O$ | 0.32 | nd |
| 5. | Isophorone | 1083 | $C_9H_{14}O$ | 0.25 | 0.20 |
| 6. | Camphor | 1111 | $C_{10}H_{16}O$ | 0.44 | nd |
| 7. | Menthol | 1149 | $C_{10}H_{20}O$ | 0.94 | 1.06 |
| 8. | *p*-Cumic aldehyde | 1221 | $C_{10}H_{12}O$ | 1.19 | nd |
| 9. | Carvone | 1223 | $C_{10}H_{14}O$ | nd | tr |
| 10. | Nerol | 1254 | $C_{10}H_{18}O$ | 0.16 | nd |
| 11. | 1-Decanol | 1263 | $C_{10}H_{22}O$ | nd | tr |
| 12. | *p*-Cymen-7-ol | 1279 | $C_{10}H_{14}O$ | 2.66 | tr |
| 13. | Carvacrol | 1283 | $C_{10}H_{14}O$ | 0.81 | tr |
| 14. | Thymol | 1291 | $C_{10}H_{14}O$ | 0.21 | tr |
| 15. | 2,4-Decadienal | 1310 | $C_{10}H_{16}O$ | nd | tr |
| 16. | Methyl anthranilate | 1330 | $C_8H_9NO_2$ | 0.33 | nd |
| 17. | Eugenol | 1345 | $C_{10}H_{12}O_2$ | 0.20 | 0.19 |
| 18. | *γ*-n-Amylbutyrolactone | 1353 | $C_9H_{16}O_2$ | 1.78 | 3.59 |
| 19. | Methyleugenol | 1402 | $C_{11}H_{14}O_2$ | 0.21 | nd |
| 20. | *α*-Ionone | 1422 | $C_{13}H_{20}O$ | 0.24 | 0.17 |
| 21. | Geranylacetone | 1453 | $C_{13}H_{22}O$ | 0.28 | 0.45 |
| 22. | *γ*-Cadinene | 1520 | $C_{15}H_{24}$ | 0.31 | nd |
| 23. | Lauric acid | 1560 | $C_{12}H_{24}O_2$ | 0.97 | 2.04 |
| 24. | Cubenol | 1616 | $C_{15}H_{26}O$ | 29.88 | 38.17 |
| 25. | longipinocarveol | 1624 | $C_{15}H_{24}O$ | 0.72 | 0.91 |
| 26. | Ledene oxide | 1653 | $C_{15}H_{24}O$ | nd | 1.05 |
| 27. | *α*-Bisabolol | 1684 | $C_{15}H_{26}O$ | nd | 0.67 |
| 28. | Octanal, 2-(phenylmethylene)- | 1739 | $C_{15}H_{20}O$ | nd | 0.55 |
| 29. | Benzyl benzoate | 1771 | $C_{14}H_{12}O_2$ | 3.00 | nd |
| 30. | Tetradecanoic acid | 1764 | $C_{14}H_{28}O_2$ | 0.40 | nd |
| 31. | *n*-Cetyl alcohol | 1866 | $C_{16}H_{34}O$ | nd | 0.82 |
| 32. | 1-Octadecanol | 2033 | $C_{18}H_{38}O$ | nd | 0.82 |
| 33. | Phytol | 2051 | $C_{20}H_{40}O$ | 1.14 | 0.29 |
| 34. | Docosane | 2198 | $C_{22}H_{46}$ | 10.41 | nd |
| 35. | Palmitic acid | 1939 | $C_{16}H_{32}O_2$ | 2.75 | 0.90 |
| 36. | Pentadecanoic acid | 1866 | $C_{15}H_{30}O_2$ | nd | 0.77 |

**nd** – Not detected; **tr** – Traces (the amount is less than 0.05%); **% of Total in BP** – % of Total in Bulgarian population; **% of Total in IP** – % of Total in Indian population.

the prominent component is cubenol (38.17%). The remaining oxygenated sesquiterpenes are ledene oxide (1.05%) and *α*-bisabolol (0.67%). Other principal compounds identified in the seeds of Indian population include *γ*-n-amylbutyrolactone (3.59%), lauric acid (2.04%) and menthol (1.06%). In Fig. 1 and Fig. 2 are shown chromatograms of identified compounds of fenugreek isolated EOs from Bulgarian and Indian seeds, respectively.

Previous studies have reported high concentrations of cubenol in the aerial part as well as in the fenugreek seeds. However, its amount in seeds observed in the present study was higher (Ahmadiani et al. 2004; Naimi et al. 2022). Furthermore, its amount in EO of seeds obtained by secondary distillation was higher than that isolation by primary one (28.78%) (Naimi et al. 2022). Literature data indicate antifungal activity of cubenol (Takao et al. 2012) and its high content, especially in the Indian species (38.17%), may serve as a basis for future evaluation of the antifungal and antibacterial properties of the EO obtained from these species by secondary distillation of the hydrolat.

The monocyclic sesquiterpene *α*-bisabolol has been detected in previous studies (Ahmadiani et al. 2004; Mahmood et al. 2015) and our analyses confirmed its presence only in the EO obtained from Indian seeds. Regarding menthol and *γ*-n-amylbutyrolactone, data is limited, but these compounds were detected in both oils, and were higher in the EO from Indian fenugreek. Thymol, carvacrol and eugenol have been reported in previous studies and confirmed in the present analysis (Girardon et al. 1985; Riasat et al. 2017; Naimi et al. 2022). Their amounts were higher in Bulgarian seeds. Confirmation of the importance of the extraction method is also the absence of carvacrol in raw fenugreek seeds reported by Rajhi et al. They showed the absence of 3,5-octadien-2-one in raw seeds but its presence in roasted seeds. In the Indian EO it was at a higher concentration (0.47%) than in Bulgarian. In addition, methyleugenol is detected in Bulgarian EO (0.21%). It's found in higher quantities in sprouted, roasted and boiled seeds but is missing in raw seeds (Rajhi et al. 2022).

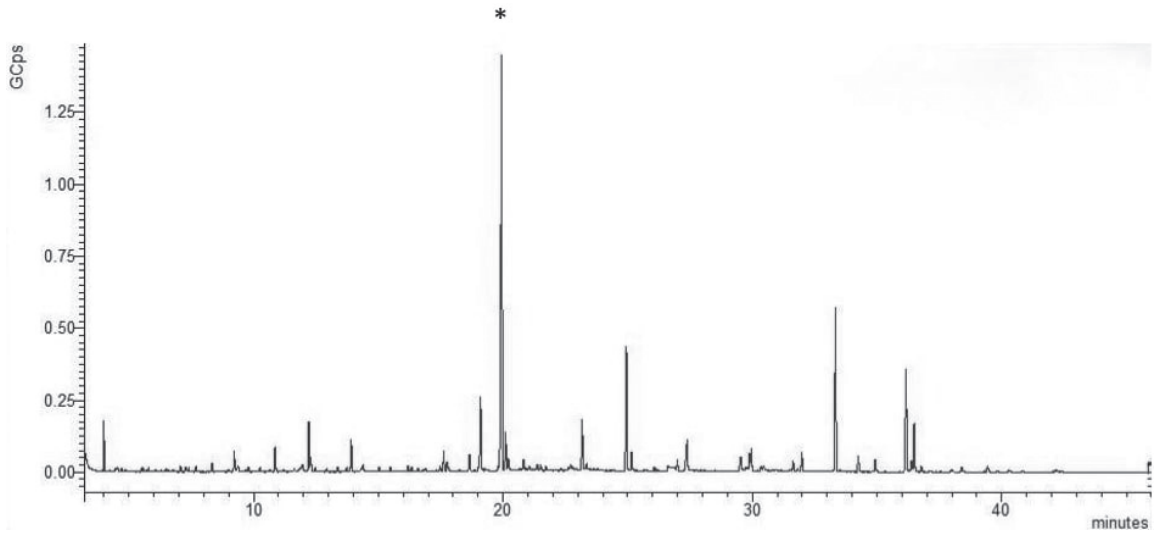


**Figure 1.** Chromatogram of identified compounds of essential oil of Bulgarian fenugreek seeds, where * is cubenol.

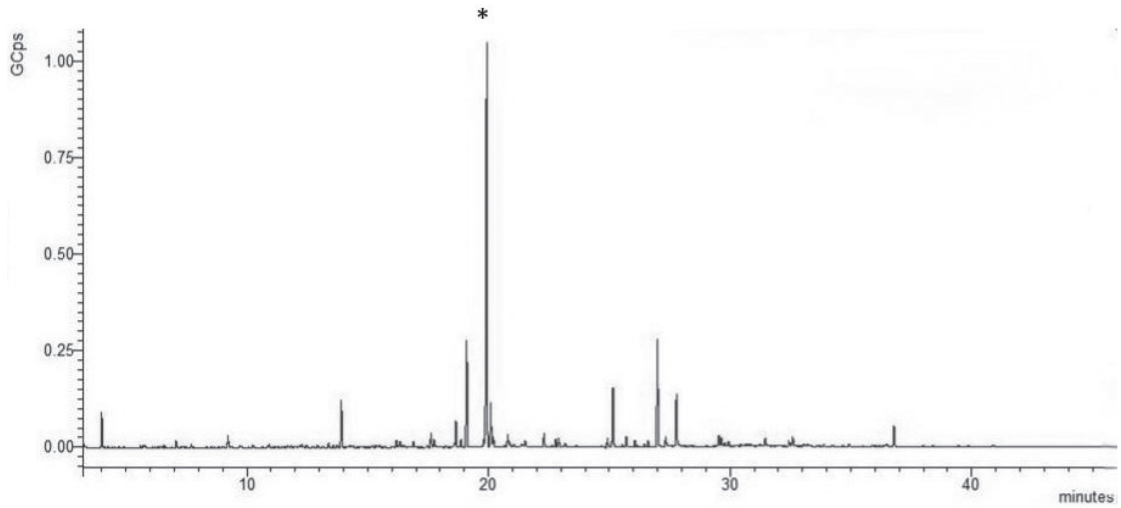


**Figure 2.** Chromatogram of identified compounds of essential oil of Indian fenugreek seeds, where * is cubenol.

Furthermore, one of the main ingredients responsible for the specific smell of fenugreek is the butenolide lactone – sotolone. It can leave odorous traces in human urine and sweat (Mebazaa et al. 2009). Sotolone has not been detected in Bulgarian fenugreek oil, nor in that of Indian fenugreek when secondary distillation of the hydrolat was used for their isolation. In addition, Mebazza reported only 0.01% concentration of $\alpha$-ionone in polydimethylsiloxane (PDMS) film, while in EO of Bulgarian fenugreek the amount was 0.24%.

The acyclic hydrogenated diterpene alcohol phytol was identified in both essential oils and detected in a higher amount in the sample isolated from Bulgarian species (1.14%). Weisany et al. reported lower concentrations in fenugreek leaves and shoots (0.51%) compared to that found from Bulgarian seeds. These authors also reported the presence of carvone, which in the present analysis was found only in Indian fenugreek seed oil, and at a very low concentration of less than 0.05% (Weisany et al. 2017). On the other hand, the amount of this monocyclic monoterpene identified in hexane seed extract was almost the same as that observed in the current investigation (Akbari et al. 2019).

## Conclusion

The present study was carried out to determine the chemical composition of essential oils, obtained by secondary distillation of distillation water, from Bulgarian and Indian fenugreek seeds. The main fraction in both oils is represented by oxygenated sesquiterpenes and the prominent component is cubenol. The data obtained from GC-MS analysis confirmed the presence of phytol in the EOs of the two fenugreek species. Despite the similarities in the component composition of the two oils, some differences were also observed due to different factors such as geographical origin, climate, plant part used, extraction method.

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