

(RESEARCH ARTICLE)



## Formulation and evaluation of oxymel containing fenugreek extract

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### Abstract

**Introduction:** *Trigonella foenum-graecum* is medicinal plant of family Fabaceae, consists of bitter principles and traditionally used in treatment of diabetes and hepatoprotective. To mask its bitter taste, it could be formulated in honey based oral formulations like oxymel. Honey, as a saturated solution of various sugars, as per Ayurvedic system of medicine, could be consumed along with drug. This research attempt was aimed towards aqueous extraction of fenugreek seeds; formulation of oxymel by addition to honey and evaluation for different parameters. Oxymel was formulated as per procedure mentioned in United State Pharmacopeia for squill oxymel; and evaluated for pharmaceutical parameters those applied for oral syrups.

**Results:** The oxymel formulated was pale brownish with agreeable odour and sweet taste. It was pourable with viscosity of 80 CP measured at 100 rpm while density was found to be 1.47 g/cm<sup>3</sup>. There was also ease in cap opening of its container, also no crystallization of honey was observed. Its trigonelline content was found to be 3.6 µg/ml.

**Conclusion:** Alkaloids of fenugreek have significant pharmacological activities in human being, if administered orally. To mask their bitter taste and facilitate their increase in absorption, *Trigonella foenum-graecum* can successfully be formulated in honey based oral formulation of oxymel.

**Keywords:** *Trigonella foenum-graecum*; Trigonelline; Oral dosage form; Honey; Evaluation

### 1 Introduction

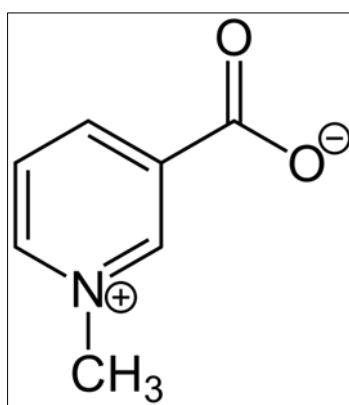
The incorporation of medicinal herbs in the dietary choices is renowned for maintaining the healthy life and avoiding several types of diet-related disorders including diabetes, cancer, hypertension, inflammation and cardiovascular diseases. Even on advancement in modern medicine, several diseases are still treated by medicine system based on medicinal plant, due to their enormous pharmacological activities and safety aspects. Fenugreek is one such plant with medicinal importance and place in human diet [1].

Fenugreek, *Trigonella foenum-graecum* (Fig.1) is a medicinal plant having botanical name belonging to family Fabaceae. It is edible to human as spice and therefore cultivated in various parts of world. Traditionally, it is consumed through diet and thereby prevents many disorders related to liver, metabolism and cardiovascular system. It is rich source of dietary fibres, so it avoids digestion related problems. Seeds are used in flatulence, dyspepsia, colic, diarrhoea, dysentery, liver enlargement, loss of appetite. Yadav and Baquer, in 2014 have summed up all the benefits and possible side effects of fenugreek [2]. Seeds exhibit these pharmacological actions due to various phytochemicals present in them. So far, phytochemicals of many classes were isolated or identified to be present in fenugreek seeds. Seeds contain trigonelline (fig.2), scopoletin, coumarin, fenugreekine, nicotinic acid, saponin, disogenin, gitogenin, neogitogenin, homoorientin, saponaretin, trigogenin, mucilaginous fiber [3] and various kaempferol derivatives. [4]

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**Figure 1** Fenugreek leafy vegetable and its seeds



**Figure 2** Structure of trigonelline

So far, various attempts have been made to formulate the fenugreek seed extract into suitable dosage form. Once, Jyoti et al. 2017 prepared herbal capsules for which a viscous sticky extract of fenugreek seeds was mixed with lactose and sodium starch glycolate, and filled in hard gelatin capsule (size 0) [5]. Then, Sultana, et al. 2020 formulated the fenugreek seed extract in syrup using sucrose, lactose and sodium benzoate, and evaluated for pH and specific gravity [6]. The present research work was aimed towards preparation of oral formulation of fenugreek seeds with edible ingredients and its evaluation for pharmaceutical parameters.

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## 2 Material and methods

### 2.1 Collection of plant material and extraction

Fenugreek seeds were purchased from local market. Seeds were washed, dried and crushed to coarse powder. About 5 gm of powder was extracted using mixture of 27 ml ethanol, 40 ml water and 33 ml vinegar with heating for 30 mins on water-bath. After 24 hrs, extract was filtered and filtrate was used for quantification and preparation of oxymel.

### 2.2 Quantification of trigonelline in fenugreek seed extract

Total 10 ml extract was dried and subjected to HPTLC-densitometric analysis. About 10  $\mu$ l sample were spotted on pre-coated Silica gel aluminium plate which was stationary phase. The plate was allowed to run in mobile phase n-propanol-methanol-water-ammonia (10 : 1.5: 15: 0.25 v/v) and scanned at 270 nm. Instrument gave the graph of retention factor vs area under curve (AUC).

### 2.3 Preparation of oxymel containing fenugreek seed extract

About 50 ml of TF seed extract was added to 50 ml of honey under continuous homogenization for 30 mins. The orange flavour was added at the last and homogenised again for 10 mins.

## 2.4 Evaluation of oxymel

The resultant oxymel formulation was evaluated for various pharmaceutical parameters, those described for oral liquid dosage forms like syrup and squill oxymel USP.

## 2.5 Organoleptic evaluation

It included simple inspection of colour, odour and taste.

### 2.5.1 pH

The pH of oxymel was determined using pH meter, Equip-tronics model EQ-5.4.

### 2.5.2 Viscosity

Viscosity of oxymel was determined using Brookfield viscometer with spindle no. 4 at 100 rpm on normal room temperature.

### 2.5.3 Density

Density as weight per ml was determined using pycnometer.

### 2.5.4 Crystallization evaluation

To evaluation of possible crystallization, the oxymel was placed in refrigerator for a period of a week and then examined for precipitation.

### 2.5.5 Cap locking

To evaluate cap locking, oxymel was filled in container, capped and placed in inverted position for a week. Then, ease in cap opening was checked.

### 2.5.6 Assay for acetic acid:

About 20 ml of oxymel was diluted with 20 ml of carbon dioxide free water and titrated with 1 M sodium hydroxide, using phenolphthalein solution as indicator. Acetic acid content was determined on the basis of equivalence of each ml of 1 M sodium hydroxide to 60.05 mg of acetic acid. Acetic acid content of vinegar was also determined.

## 2.6 Quantification of trigonelline/ content determination

The trigonelline content in extract and oxymel formulation were determined by HPTLC analysis as described by Laila et al. 2019[7]. About 5 gm of seed powder was extracted by simple maceration using 40 ml water and added with 33 ml vinegar and 27 ml ethanol for 24 hrs with occasional shaking and boiling for 10 mins. From this 100 ml of extract, about 20 ml was dried and subjected to HPTLC by dissolving in 1 ml ethanol and 1 ml of distilled water. About 10 µl of this sample was spotted on precoated silica gel aluminium plate 60 F254 (10 cm ×10 cm) having 250 µm thickness using CAMAG Linomat 5 sample applicator. Then, plate was allowed to run in chamber saturated for 10 min by mobile phase n-propanol: methanol: ammonia water (10: 1.5: 15: 0.25 v/v) for 8 cm. Then, plate was dried by dryer. Then, plate was undertaken for densitometric scanning at 270 nm which was operated by software WINCATS 1.4.2.

Firstly, trigonelline content of *fenugreek* seed extracted with mixture of vinegar, ethanol and water was determined and it was added to honey to form oxymel. Then, trigonelline content of oxymel was determined.

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## 3 Results

### 3.1 Preparation of oxymel and its organoleptic properties

The oxymel, formulated by novel procedure, was pale brownish in colour with agreeable odour and sweet taste. It was pourable from one container to another.

#### 3.1.1 pH

The pH of oxymel was found to be 5.

### 3.1.2 Viscosity

Viscosity, as resistance to flow, of oxymel was determined and it was found to be 80 CP at 100 rpm.

### 3.1.3 Density

Density was found to be 1.47g/cm<sup>3</sup>.

### 3.1.4 Crystallization

It was also observed that oxymel sample did not get crystallized even after placed in refrigerator for a week.

### 3.1.5 Cap locking

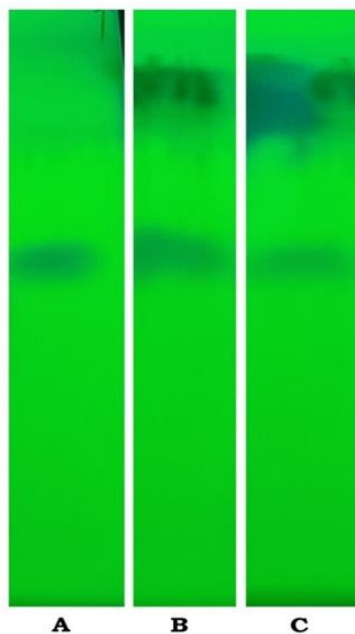
There was also ease in cap opening after placing in inverted position for a period of a week.

### 3.1.6 Assay for acetic acid:

The content of acetic acid content of fenugreek seed extract was found to be 39 mg/ml while that of oxymel formulation was found to be 7.5 mg /ml

## 3.2 Quantification of trigonelline

On the HPTLC fingerprint, R<sub>f</sub> value of spot was found corresponding to what was mentioned in the literature (R<sub>f</sub>, 0.57) (Fig.3). Area under curve (AUC) of peak at R<sub>f</sub> value 0.57 for extract was 22118 (Fig. 4) while that for oxymel was 17581.3 (Fig.5). On putting these values in equation  $y = 2225 + 4.16 x$  and performing calculations, the trigonelline content of fenugreek seed extract was found to be 23.9 µg/ml while that of oxymel was found to be 3.6 µg/ml.



**Figure 3** HPTLC fingerprint of trigonelline, A; fenugreek seed extract, B; oxymel formulation, C

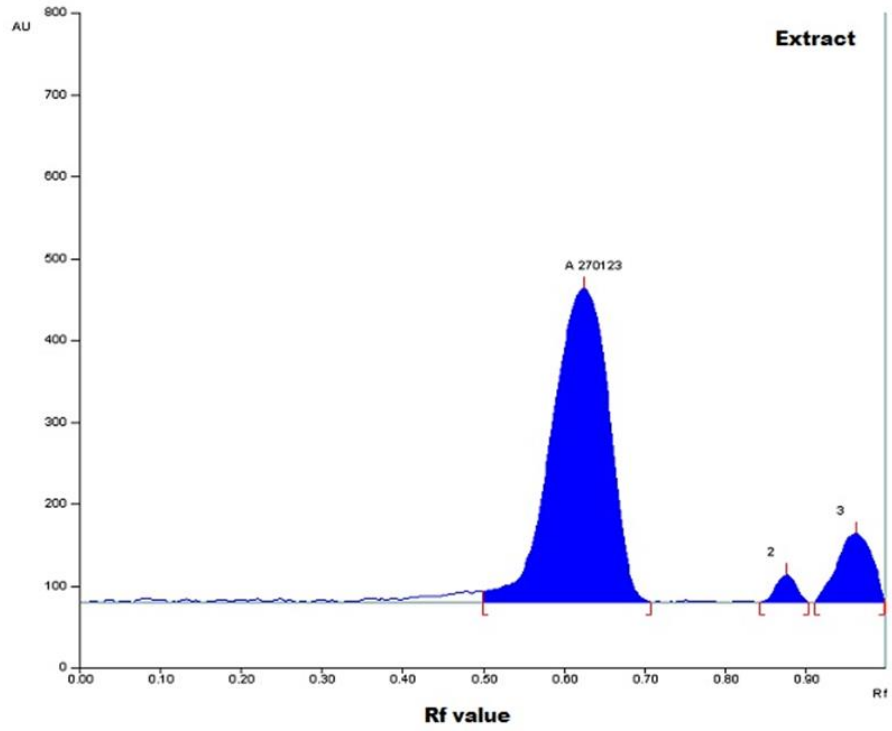


Figure 4 Trigonelline content of extract

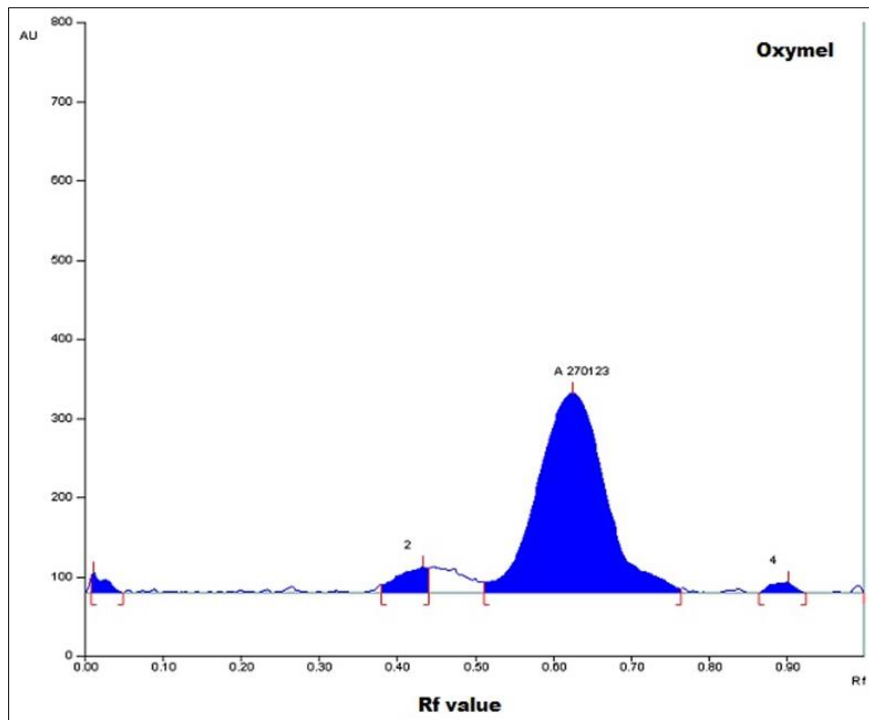


Figure 5 Trigonelline content of oxymel

## 4 Discussion

The decrease in acetic acid content from 39 mg/ml to 7.5 mg/ml indicated that during extraction of *fenugreek* seed powder, some quantity of acid got neutralised, which may be due to total alkaloidal and other alkaline contents of seeds.

Many pharmacologically active molecules get metabolised by phase I, mainly oxidation and phase II, conjugation with sulpho group reactions. However, both of these reactions are inhibited by flavonoids, mainly Galangin found in honey inhibits cytochrome P450-dependent mixed-function oxidases (CYPs) and sulphotransferases (SULTs) [8]. Hence, it could be concluded that combination of honey with active principles, decreases their metabolism at least up to some extent.

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## 5 Conclusion

The bitter principles and alkaloids of fenugreek could be extracted using hydroalcoholic solution of vinegar (33%). They exhibit significant pharmacological actions when administered orally. Brownish oxymel formulated with fenugreek extract has agreeable odour and sweet taste. Its pharmaceutical parameters evaluated were found to be in acceptable range. HPTLC analysis determined the trigonelline content in extract and oxymel. Combination of bitters and alkaloids with honey decreases their metabolism.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

### *No conflict of interest*

### *Authors Contribution*

- DM: Formulated oxymel and quantified trigonelline by HPTLC
- SC: Carried out extraction of seeds and preliminary tests
- SK: Performed evaluation parameters
- RE: Procured raw materials and chemicals
- SK: Drawn figures and diagrams
- SPP Selected topic and written manuscript

### *Author's Declaration*

All authors have approved the submission and publication

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